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Poster

365. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 365.01/A1

Topic: A.02. Postnatal Neurogenesis

Support: CONACyT Grant PN-2016-01-465
CONACyT Grant INFR-280414
CONACyT Fellowship Grant 660523

Title: Permanent whisker removal reduces the expression of calcium-related proteins, disrupts hippocampal neurogenesis, and affects spatial memory

Authors: *O. GONZALEZ-PEREZ, N. IBARRA-CASTANEDA, V. LOPEZ-VIRGEN, J. GUZMAN-MUNIZ, N. MOY-LOPEZ
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Abstract: Facial vibrissae, commonly known as whiskers, are the main sensitive tactile system in rodents. Whisker stimulation triggers neuronal activity that promotes neural plasticity in the barrel cortex and helps create spatial maps in the adult hippocampus. Therefore, the neuronal activity of the barrel cortex possibly regulates hippocampal neurogenesis. To assess whether tactile information from facial whiskers may modulate hippocampal neurogenesis, we permanently eliminated whiskers in CD1 male mice and analyzed the effects in cellular composition, molecular expression and memory processing in the adult hippocampus. Our data indicated that the permanent deprivation of whiskers reduced in 4-fold the expression of c-Fos (a calcium-dependent immediate early gene) in cornu ammonis subfields (CA1, CA2 and CA3) and dentate gyrus. A significant reduction in the expression of calcium-binding protein calbindin-D_{28k} was also observed in granule cells of the dentate gyrus. Notably, calbindin and c-Fos reduction was linked to a dramatic decrease in proliferation and cell survival of neural precursor cells in the subgranular zone, which ultimately reduced the number of NeuN+ mature neurons generated after whisker elimination. These abnormalities in the neurogenic process coincide with a significant impairment of spatial memory and navigation skills. This is the first evidence indicating that tactile inputs from vibrissal follicles strongly modify the expression of calcium-related proteins in the dentate gyrus, disrupt different aspects of hippocampal neurogenesis, and support the notion that spatial memory and navigation skills strongly require tactile information in hippocampus.

Disclosures: O. Gonzalez-Perez: None. **N. Ibarra-Castaneda:** None. **V. Lopez-Virgen:** None. **J. Guzman-Muniz:** None. **N. Moy-Lopez:** None.

Poster

365. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 365.02/A2

Topic: A.02. Postnatal Neurogenesis

Support: IoPPN Prize Studentship

Title: Nutrient-sensing pathways in ageing models of hippocampal progenitor cells

Authors: *C. DE LUCIA[1], T. MURPHY[1], C. STEVES[2], R. DOBSON[3], S. THURET[1]
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Abstract: Ageing is associated with changes in cellular and molecular processes including the alteration of stem cell pools. In particular, biological and functional changes observed in ageing neural stem cells (NSCs), are linked to age-related cognitive decline. Recently, the systemic environment has been shown to alter both NSC regulation and age-related cognitive decline. Interestingly, a well-documented and naturally occurring way of altering the composition of the systemic environment is through diet and nutrition. Studies have found an overabundance of nutrients to be detrimental for human and animal health; the presence of specific nutrients as well as the overall increase in calorie or protein intake was shown to overstimulate conserved molecular pathways and to reduce lifespan. Conversely, dietary restriction was found to be the most efficient way of extending an organism's lifespan. In this study, we examined nutrient sensing pathways in relation to their function on NSC and ageing. We focus on the Sirtuin, mTOR and Insulin / Insulin like growth factor-1 pathways. We used a human hippocampal progenitor cell line (HPCs) and employed pharmacological interventions and increasing passage number to induce, and measure ageing phenotypes. A semi-automated imaging platform was used for the quantification of NSC differentiation by using cellular markers for neuronal progenitors (DCX) and immature neurons (Map2) and machine learning was employed to measure morphological changes. Candidate gene selection was preformed through literature search and validated by qPCR to measure gene expression alterations within our model. Finally, we investigated the association of SNP variants within these candidate genes with lifestyle and cognition using 1633 participants from the adult longitudinal population-based TwinsUK cohort (n= 12 000). Our data suggests this novel ageing model induces changes in the expression levels of several key nutrient sensing genes (*FOXO3A, NAMPT, PTEN, GRB10* and *MTOR*) and alterations in neuronal morphology reminiscent of ageing phenotypes. We believe this assay has great potential to investigate NSC ageing and could be employed to validate existing pathways, uncover new targets and test novel therapies.

Disclosures: C. De Lucia: None. **T. Murphy:** None. **C. Steves:** None. **R. Dobson:** None. **S. Thuret:** None.

Poster

365. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 365.03/A3

Topic: A.02. Postnatal Neurogenesis

Title: Multiple neonatal anesthesia exposures suppress neuronal proliferation in the adult rodent hippocampus

Authors: *R. MAKARYUS[1], M. YU[1], K. CHEN[2], M. BOYD[2], G. ENIKOLOPOV[1]
[1]Stony Brook Med., Stony Brook, NY; [2]Stony Brook Univ., Stony Brook, NY

Abstract: Background: We recently found in rodents that multiple anesthesia exposures caused a greater decrease in a neuronal marker over the course of neuronal development compared to a single exposure of the same cumulative duration. This was further evidenced by more severe behavioral deficits in adult rodents with such exposures. Given the persistence of effects in adult rodents, we sought to investigate the effects of neonatal anesthesia on neurogenesis in the adult hippocampus. Methods: On postnatal day 5 (P5), rats were divided into three groups: single exposed (receiving 6h of sevoflurane exposure on P7), multiple exposed (receiving 2h of exposures on P5, 7, and 10), and unexposed. All were injected with IdU and CldU on P30 and P60, respectively. Behavioral testing was performed from P30-45 using open field testing (OFT), novel object recognition (NOR) testing, and Barnes Maze testing, with a small subset in each group not undergoing behavioral testing. Perfusion fixation was performed on P60 for histological analysis, staining for IdU, CldU, and Dcx. Results: The multiple exposed group had the most severe behavioral changes, specifically in NOR and OFT. Histological analysis of the dentate gyrus revealed a decrease in number of IdU-positive and CldU-positive cells in the multiple exposed group (Fig 1A). Of note, behavioral testing increased the number of double-labeled (IdU^+CldU^+) cells in the multiple exposed group, and the vast majority of these double labeled cells were not Dcx^+ (Fig 1B). Discussion: We have shown that the multiple exposed group had the greatest changes in behavior. This is directly correlated with a decrease in neuronal cell proliferation at P30 and P60, evidenced by the changes in IdU^+ and $CldU^+$ cell populations, respectively. Interestingly, most double labeled cells in the dentate gyrus were not Dcx^+, indicating that they were not neurons. The increase of these cells in multiple exposed with behavioral testing compared to those without behavioral testing may indicate an increase in glial cell proliferation related to the enriched environment provided by such testing.

Figure 1 – Effects on Neuronal Stem Cells

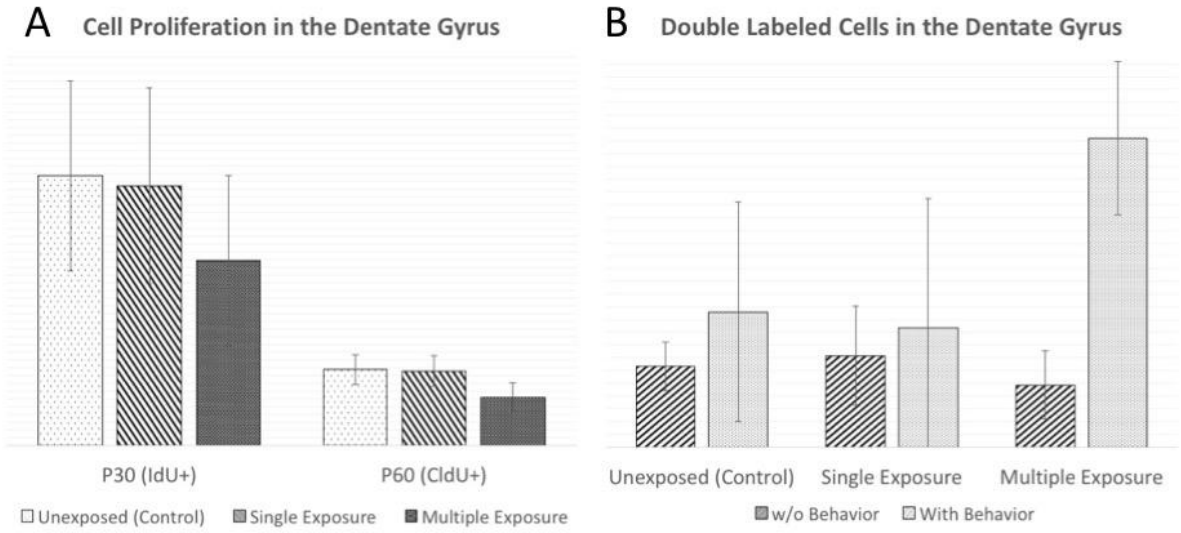

Fig 1. (A) Cell proliferation at P30 as determined by IdU+ cells, and P60 determined by CldU+ cells, demonstrating that multiple neonatal anesthesia exposures causes a decrease in cell proliferation even in the adult rodent brain. (B) Double labeled (IdU+,CldU+) cells are highest in the multiple exposed group that had behavioral testing. These were determined to be non-neuronal cells, possibly indicating an increase in glial cells in response to being in an enriched environment after neonatal anesthesia neurotoxicity.

Disclosures: R. Makaryus: None. **M. Yu:** None. **K. Chen:** None. **M. Boyd:** None. **G. Enikolopov:** None.

Poster

365. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 365.04/A4

Topic: A.02. Postnatal Neurogenesis

Support: NIH R01NS089770

Title: Age and experience-dependent hippocampal neurogenesis is regulated by sphingosine 1-phosphate

Authors: *A. P. CHEN[1,2], G. W. KIRSCHEN[2,3], J. SCHRANDT[3], A. AHAMAD[4], N. U. SCHWARTZ[1], L. M. OBEID[5], C. MAO[5], S. GE[4]
[1]Program in Neurosci., [2]Med. Scientist Training Program, [3]Mol. and Cell. Pharmacol. Program, [4]Neurobio. and Behavior, [5]Dept. of Medicine, Div. of Cancer Prevention, Stony Brook Med., State Univ. of New York At Stony Brook, Stony Brook, NY

Abstract: The rate of adult hippocampal neurogenesis is well known to decline rapidly with advancing age, and to increase in response to experiences such as environmental enrichment. Understanding the regulatory mechanisms controlling this process, which remain poorly characterized, may aid in the development of novel strategies to target age-related decline in hippocampal neurogenesis, and consequent impairments in memory function. Pathways related to cell growth and energy metabolism are likely important in the age-related neurogenesis decline, yet signaling pathways implicated have not been fully elucidated. Further, it is not known how neurogenesis may be regulated from cues originating from outside of the central nervous system. We hypothesized that bioactive lipid signaling through sphingosine 1-phosphate, important for proliferation and survival of non-neuronal cells throughout the body and shown to be present and active in the brain of adult mice, may influence adult hippocampal neurogenesis. We made the surprising discovery that environmental enrichment, known to potently increase adult hippocampal neurogenesis, sharply decreased circulating blood S1P levels. To test the causal relation between S1P and neurogenesis, we used two independent transgenic mouse models that exhibit limited S1P production, and found that both exhibited heightened neurogenesis. Together, our findings implicate the S1P pathway in basal and activity-dependent neurogenesis, and implicate a potential therapeutic target to promote or restore neurogenesis associated with aging or disease.

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Poster

365. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

Location: SDCC Halls B-H

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Program #/Poster #: 365.05/A5

Topic: A.02. Postnatal Neurogenesis

Support: Lebanese National Council for Scientific Research (LNCSR)

Title: Hippocampal neurogenesis induced by single or multiple sessions of thalamic electrical stimulation

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Abstract: Background: Deep brain stimulation (DBS) has provided substantial clinical benefit for a variety of movement disorders. It is still unknown how DBS alters neural activity to induce beneficial outcomes. One area of interest is to investigate a possible role for modulation of adult hippocampal neurogenesis in mediating DBS effects. The hippocampus is a structural and functional component of the limbic system and contains a neurogenic niche of neural stem/progenitor cells in the subgranular zone (SGZ) of the dentate gyrus (DG). It possesses extensive interconnections with the anteromedial thalamic nucleus (AMN) proposing that stimulation to the AMN conveys physiological fluctuations in the hippocampus and possibly elicits neurogenesis.
Objective: The aim of the study is to examine the effect of single as well as multiple sessions of AMN electrical stimulation in modulating stem/progenitor cell proliferation at early stages and track their fate at later stages.
Methods: Adult male Sprague-Dawley rats received either single or multiple unilateral electrical stimulation in the right AMN. Electrodes were implanted without current delivery in sham groups. Another control group, for selective site stimulation, received electrical stimulation in the ventral posterolateral thalamic nucleus (VPL) that has no established direct projections to the hippocampus. All
groups received 3 injections (66mg/Kg/injection) of 5'-bromo-2'-deoxyuridine (BrdU) and were euthanized on two time points. Early stages of neurogenesis were examined at 7 days post-surgery and the late stages after 4 weeks. The BrdU positive cells in the dentate gyrus (DG) of the hippocampus were counted. Double labeling of BrdU with NeuN was examined. The Exploratory Behavior test was performed using a Y-maze to detect for enriched hippocampal skills following stimulation.
Results: Single AMN electrical stimulation was marked by a 2-fold increase in proliferation of neural stem/progenitor cells in the ipsilateral DG at 7 days and translated into a 1.8-fold increase in hippocampal neurogenesis at 4 weeks. Multiple sessions of electrical simulation further increased hippocampal neurogenesis to 3-folds higher than the sham at 4 weeks. The Y-maze test showed that single electrical stimulation to the AMN enhanced exploratory behavior at 4 weeks while it was detected at the earlier 7 days-time point in multiple sessions of stimulation.
Conclusions: The current study presents an increase in hippocampal neurogenesis in response to single or multiple sessions of electrical stimulation. It also reveals a translational behavioral enhancement of hippocampal-related skills following stimulation.

Disclosures: F. Chamaa: None. **Z. Nahas:** None. **N.E. Saade:** None. **W. Abou-Kheir:** None.

Poster

365. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Topic: A.02. Postnatal Neurogenesis

Support: NSF GRFP
NIH IMSD-MERGE

Title: Mode of action of memantine on adult hippocampal neurogenesis

Authors: *S. ITAMAN[1], O. PODGORNY[1], G. N. ENIKOLOPOV[2]
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Abstract: Adult hippocampal neurogenesis can be altered by various endogenous and exogenous stimuli. Both an increase and a decrease in production of new neurons can arise as a result of several distinct scenarios, with the stimuli impacting various subpopulations of neural stem and progenitor cells and various steps of the stem cells' division/differentiation cascade. Memantine, a non-competitive NMDA receptor antagonist used clinically to treat symptoms of Alzheimer's disease, can increase the division of progenitors and production of new neurons in the adult brain. However, its basic mode of action is unknown. In particular, it is not known whether it activates stem cells, their progeny (or both), whether it targets division of neuronal progenitors or their elimination, and whether it induces symmetric division of stem cells, increases the number of divisions of already activated stem cells, or augments activation and recruitment of quiescent stem cells into the cell cycle. Conventional analysis methods of neurogenesis do not distinguish between these scenarios. We developed new experimental paradigms based on double- and triple S phase labeling and phenotyping stem and progenitor cells, which allow fine analysis of the division and fate of stem cells and their progeny. Our results provide a blueprint for the analysis of stem cell maintenance and division. These methods indicate that memantine induces increased production of new neurons in the hippocampus of adult mice by increasing the rate of de novo activation of hippocampal neural stem cells.

Disclosures: S. Itaman: None. **O. Podgorny:** None. **G.N. Enikolopov:** None.

Poster

365. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Program #/Poster #: 365.07/A7

Topic: A.02. Postnatal Neurogenesis

Support: R01HL104173
R01HL128546
R01HL139712
U54HD090257
Children's Heart Foundation

Title: Postnatal neurogenesis after cardiopulmonary bypass in a juvenile porcine model

Authors: C. LEONETTI[1], Z. DHARI[3], L. KOROTCOVA[2], *R. A. JONAS[2], N. ISHIBASHI[4]
[1]Ctr. for Neurosci. Res., [2]Children's Natl. Med. Ctr., Washington, DC; [3]Neurosci., Children's Natl. Hosp., Washington, DC; [4]Children's Natl. Heart Inst. and Ctr. for Neurosci. Res., Children’s Natl. Hlth. Syst., Washington, DC

Abstract: Impairments in higher-order cognitive and behavioral function are widely observed in children after cardiac surgery with cardiopulmonary bypass (CPB). The subventricular zone (SVZ) in the postnatal/adult brain is the region where most neural stem/progenitor cells (NSPC) originate. Recent studies suggest that the SVZ plays an important role in neocortical growth of the gyrencephalic frontal lobe during postnatal life. However, the effect of CPB-induced insults on postnatal SVZ neurogenesis is largely unexplored and poorly understood. The aim of our study is to determine SVZ neurogenic activity after CPB using a porcine survival model. Piglets were randomly assigned to control group and severe-CPB insult group (reproducing CPB-induced systemic inflammation and ischemia-reperfusion injury). To assess SVZ neurogenic activity, we performed immunohistochemistry and quantified NSPCs and neuroblasts with antibodies directed against Sox2, GFAP and doublecortin. The SVZ was divided into 3 tiers as previously described in the human SVZ and into ventral (V-SVZ) and dorsolateral regions (DL-SVZ). Severe-CPB resulted in a significant reduction of $GFAP^+$ processes length and $Sox2^+$ layer thickness in tier-1 of DL-SVZ. On the other hand, there was no difference in $Sox2^+$ cell number in other tiers. Moreover, the number of neuroblasts in the DL-SVZ was remarkably reduced in severe-CPB group. Recent studies have demonstrated that neuroblasts form migration chains moving tangentially through the SVZ and appear as clusters on coronal sections. Although there was no difference in the number of these clusters we found a significant decrease in their surface area after severe-CPB insult, indicating a disruption of neuroblast migration. Furthermore, 4 weeks after severe-CPB we continued to observe a significant reduction of $Sox2^+$ layer thickness in tier-1 of DL-SVZ. Collectively, our results demonstrate that severe-CPB

reduces neurogenesis up to 4 weeks after insult. Our preliminary data demonstrate prolonged impairments in the NSPC pool following severe-CPB insult. The impact is confined to the dorso-lateral region which is the most active part of the SVZ under normal conditions. Our results consistently showed reduced neurogenic activity and suggest disruption of neuroblast migration towards the frontal cortex after severe-CPB insult. The frontal cortex is the region responsible for a wide range of higher-order cognitive functions; our study therefore provides novel insights into cellular mechanisms underlying complex neurological impairments in children with congenital heart disease.

Disclosures: C. Leonetti: None. **Z. Dhari:** None. **L. Korotcova:** None. **R.A. Jonas:** None. **N. Ishibashi:** None.

Poster

365. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Topic: A.02. Postnatal Neurogenesis

Support: NIH/NIAAA R37AA007789 to JW
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Kids Brain Health Network 20R64153 to JW
CIHR MOP142308 to LAMG
NSERC CGSM to SLB
Aboriginal Graduate Fellowships to SLB

Title: Social isolation stress alters the expression of hippocampal neurogenesis in adolescent animals prenatally exposed to alcohol

Authors: *S. L. BAGLOT[1,2], P. UBI[1], E. MORGAN[1], S. E. LIEBLICH[2], W. YU[1], J. WEINBERG[1], L. A. M. GALEA[2]
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Abstract: Fetal alcohol spectrum disorders encompass a wide range of deficits following prenatal alcohol exposure (PAE), including central nervous system abnormalities. Of particular importance, PAE alters the development and functioning of the hippocampus. The hippocampus, a brain area sensitive to the teratogenic effects of alcohol, is unique because it possesses the ability to form new neurons (i.e. neurogenesis) into adulthood. Hippocampal neurogenesis has been implicated in stress regulation, emotional behaviour, and learning and memory. It is well known that both PAE and exposure to chronic stress produce changes in hippocampal neurogenesis across the lifespan. Further, many of the effects of PAE are altered or exacerbated

by chronic stress exposure. However, little research has looked at the combinatorial effects of PAE and chronic stress on hippocampal neurogenesis in adolescence. Adolescence is an extremely important maturation period that often involves exposure to chronic stress, especially social stressors. Animal models have shown that hippocampal neurogenesis is sensitive to social isolation stress in adulthood, but that these alterations are different in males and females. Thus, the goal of this study is to look at the unique and/or interactive effects of PAE and social isolation stress on hippocampal neurogenesis during late adolescence in male and female rats. Utilizing an animal model, male and female offspring from control, pair-fed, and ethanol exposed dams were examined in adolescence. Half the animals from each prenatal condition were exposed to social isolation stress from postnatal day 35 for 10 continuous days. On the last day of stress exposure animals were perfused and brains removed. Rat hippocampi were sectioned and immunohistochemically processed for the endogenous protein doublecortin (DCX), a marker for immature neurons. As expected, social isolation stress resulted in decreased density of DCX expression (reduced immature neurons) in the dorsal dentate gyrus in control animals. Interestingly, the effect of social isolation stress was absent in PAE animals. In fact, following social isolation stress, PAE animals actually expressed increased DCX density compared to controls. Analysis of cell morphology similarly revealed alterations following stress and PAE. Taken together, our results suggest that PAE animals respond differently to social isolation stress in adolescence. Importantly, these differential neurogenic changes may underlie some of the common cognitive, emotional, and behavioural deficits seen in animal models of PAE, as well as in individuals exposed to alcohol *in utero.*

Disclosures: S.L. Baglot: None. **P. Ubi:** None. **E. Morgan:** None. **S.E. Lieblich:** None. **W. Yu:** None. **J. Weinberg:** None. **L.A.M. Galea:** None.

Poster

365. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Program #/Poster #: 365.09/A9

Topic: A.02. Postnatal Neurogenesis

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Title: WM-CLICK: The effects of memantine on adult brain cell proliferation in 3D

Authors: *A. LAZUTKIN[1], S. SHUVAEV[2], R. KIRYANOV[4], K. ANOKHIN[5], A. KOULAKOV[3], G. N. ENIKOLOPOV[6]
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Abstract: 3D visualization of dividing cells in the whole brain could facilitate the analysis of neurogenesis, increase its accuracy, and reveal new functional patterns of stem cell division. We developed whole-mount click-reaction technique (WM-CLICK) which allows detecting the population of dividing cells using with 5-ethynyl-2'-deoxyuridine (EdU) and fluorescent azide, with and further reconstruction of the 3D maps of EdU-labeled cells in the whole brain. We now used WM-CLICK as well as our new algorithms for rapid processing and comparison of multiple brain samples to analyze the 3D patterns of cell division in the brains of adult animals which were treated with saline or memantine (50 mg/kg) daily for 5 days and analyzed 2 h after injecting EdU injection at the last day of experiment. The images of whole stained brain were registered to each other and then averaged group images were created and compared. This analysis revealed several areas of the adult brain where memantine had particularly strong effect on cell division, with high densities of EdU+ cells found in the CA and DG regions of the hippocampus, subcallosal zone, olfactory bulb, and piriform cortex.

Disclosures: S. Shuvaev: None. **R. Kiryanov:** None. **K. Anokhin:** None. **A. Koulakov:** None. **G.N. Enikolopov:** None.

Poster

365. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Program #/Poster #: 365.10/A10

Topic: A.02. Postnatal Neurogenesis

Title: Adult neurogenesis in turtles increases when they learn a visual discrim

Authors: *A. S. POWERS
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Abstract: Adult neurogenesis occurs throughout the telencephalon in turtles, and it is increased by exercise or an enriched environment, as in the hippocampus of mammals. Another factor that has been shown to influence adult neurogenesis in mammals is learning, and in this experiment we studied the effects of visual discrimination learning on adult neurogenesis in painted turtles (*Chrysemys* picta).
The experiment had 4 groups, which were run in a discrimination box with a response key and a food magazine that delivered beef baby food. All animals were trained to press the response key for food and then two groups were given 3 injections of BrdU (50 mg/kg) over one week. One group (Acquisition, n=6) was then trained to criterion on a visual discrimination between vertical

and horizontal lines while they continued to receive 6 more BrdU injections over the next two weeks. The second group (Control, n=5) was housed throughout this time in their home cage with no discrimination training. The other two groups were trained to criterion on the horizontal-vertical discrimination *before* being given BrdU injections for one week, and then they were tested on retention (Retention, n=6) or reversal of the discrimination they had learned (Reversal, n=5) while receiving 2 more weeks of BrdU injections. Turtles were euthanized 6 weeks after the first BrdU injection, and the brains were treated for BrdU immunohistochemistry. We counted the number of new cells in the telencephalon in each group.
Only the Acquisition group, which learned the discrimination while receiving BrdU, showed an increase in new cells in the brain. The other groups did not differ from the Controls. This finding suggests that, in turtles, neurogenesis is facilitated when animals have to learn a new task but not when they remember one already learned, even if the memory task requires them to learn a reversal of the previous learning. The result is consistent with studies in mammals that show an increase in neurogenesis when animals learn a new task and suggest that a facilitative role for learning in adult neurogenesis may have been present in the common ancestor of both groups, the stem amniotes.

Disclosures: A.S. Powers: None.

Poster

365. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Program #/Poster #: 365.11/A11

Topic: A.02. Postnatal Neurogenesis

Support: DST INSPIRE Fellowship IF10053

Title: Antioxidant (aox) mediated allteration incalbindin expression within cerebellar purkinje cells of rat pups exposed to sodium arsenite

Authors: *P. DHAR[1], P. KUMAR[2], P. KAUSHAL[3]
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Abstract: Calbindin D28k (CB), a member of calcium-binding protein (CaBP) superfamily, plays important role in regulation of calcium homeostasis in the cells. In the cerebellum, most of the Purkinje cells express calbindin-D28k as well as parvalbumin, whereas basket, stellate and Golgi cells express parvalbumin alone. Purkinje cells play a crucial role in motor learning especially during the developmental period. Heavy metal toxicity could reduce expression of these CaBPs, thereby inducing aberrations in the developing cells. Arsenic toxicity is a matter of global concern and consumption of water contaminated with *iAs* is the major source of exposure

to *iAs*. Morbidity and mortality induced by *iAs* exposure is prevalent in endemic areas all over the globe.
We studied Calbindin D28k expression in the cerebellum of rat pups subjected to sodium arsenite ($NaAsO_2$) exposure. Also, any modulation in the expression of these proteins, following anti-oxidant (AOX) supplementation, was determined.
Mother-reared Wistar rat pups were divided into control and experimental groups. $NaAsO_2$ alone or along with AOXs (ALA and Curcumin) was administered by intraperitoneal (i.p.) route from postnatal day (PND) 1 to 21 to experimental groups. Pups receiving distilled water/ethanol/DMSO served as the controls. On PND 22, the animals were perfusion fixed. Immunohistochemical localization of Calbindin D28k (CB) was carried out on parasaggital cerebellar sections (25 µm) using mouse monoclonal antibodies. The morphological features and morphometric parameters (linear density and perikaryal area (Pa)) of immunoreactive (IR) Purkinje cells were determined using NIS-Elements AR software attached to Nikon E 600 microscope fitted with Digital Camera Head DS-Fi1.
Preliminary observations revealed decrease in Purkinje cell density and cell area in *iAs* alone treated animals as compared to controls. However, these parameters were comparable amongst control and AOX supplemented groups.

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Poster

365. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Topic: A.02. Postnatal Neurogenesis

Support: Nserc discovery grant 2NS
CFI 2NS
CRC 2NS

Title: Age-mediated effects of short-term environmental enrichment on neuroplasticity

Authors: *K. CHANDLER[1], H. DOSSO[1], L. LAIRD[2], C. A. RUDYK[1], N. SALMASO[1]
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Abstract: Early in telencephalic development, neural stem cells are born in the ventricular zone and migrate through the cortex before differentiating into neurons and glia. Adult neurogenesis, however, is limited to specific niches in the brain: the dentate gyrus of the hippocampus (DG) and the subventricular zone (SVZ). The proliferation and differentiation potential of these neural stem cells is plastic and shows changes across states and in response to environmental manipulations. It has previously been shown that short-term environmental enrichment (Enr)

increases cognitive abilities on the Morris Water Maze and is sufficient to increase the proliferation of the GFAP+ stem cell pool in juvenile mice. However, in adults, longer-term Enr protocols are typically used to induce behavioural and functional recovery and few studies have examined the effects of short-term Enr in adults. We hypothesized that in conjunction with previous research, short-term Enr will be sufficient to induce an increase in cognitive abilities and in neural stem cell potential in juveniles, but that these changes will be attenuated in adults. To achieve this, we exposed juvenile (P35) and adult (P90) male C57 wildtype mice to two weeks Enr (including physical, social and cognitive enrichment), and examined cognitive behaviour as well as the potential of SVZ and DG NSCs in vitro using neurosphere assays. We also examined changes in doublecortin, SOX2, GFAP and synaptic protein levels to assess age-induced changes in neuroplasticity in response to short-term Enr. As hypothesized, we found that the short-term Enr increased learning and memory in juvenile mice, but not in the adult mice. These changes were paralleled by an increase in proliferation of the stem cell pool in juveniles that was less pronounced in adults, together suggesting an age-related decrease in NSC potential and plasticity in response to short-term environmental manipulations.

Disclosures: K. Chandler: None. **H. Dosso:** None. **L. Laird:** None. **C.A. Rudyk:** None. **N. Salmaso:** None.

Poster

365. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 365.13/A13

Topic: A.02. Postnatal Neurogenesis

Support: USAMRMC PC150494

Title: The effects of androgen deprivation therapy on the adult hippocampal neurogenesis and cognition in mice

Authors: *T. ALKAM, K. A. ATKINSON, 91766, J. JO, J. CHAN, E. SMITH, R. N. PECHNICK
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Abstract: Among the various therapeutic approaches, androgen deprivation therapy (ADT) is a well-established treatment for prostate cancer. Its goal is to lower levels of testosterone, the main factor driving the progression of prostate cancer. Although this treatment strategy can slow disease progression, patients receiving ADT show significant declines in executive functioning, spatial reasoning, spatial abilities and working memory. At the present time, however, little is known regarding the fundamental mechanisms underlying ADT-induced impairment of cognition. We hypothesize that the cognitive impairment observed in prostate cancer patients

following ADT is due to treatment-induced reduction in hippocampal neurogenesis. In this study, in order to assess neuronal survival, the number of BrdU-positive cells and percentage of BrdU-positive cells that co-express NeuN, a marker of mature neurons was determined in the dentate gyrus of mice using immunofluorescent double-staining and confocal microscopy. Proliferation was assessed by counting the number of Ki-67-positive cells and percentage of Ki-67-positive cells that co-express nestin (a marker of proliferating neural progenitors) and doublecortin (DCX), a protein expressed in immature neurons (i.e., neuroblasts). We found that ADT, using either surgical or pharmacological castration (i.e., the androgen receptor antagonist flutamide or down-regulating the secretion of gonadotropins using leuprolide), affected both neuronal proliferation and survival. In addition, the effects of ADT on learning and memory were determined using open field activity, Y-maze, Barnes maze, and novel object recognition tests. The results of the present study might lead to the development of an animal model to help understand the pathophysiological processes underlying ADT in humans. (Supported by USAMRMC PC150494).

Disclosures: T. Alkam: None. **K.A. Atkinson:** None. **J. Jo:** None. **J. Chan:** None. **E. Smith:** None. **R.N. Pechnick:** None.

Poster

365. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 365.14/A14

Topic: A.02. Postnatal Neurogenesis

Support: NIH 1ZIAMH002784

Title: Do new neurons affect motivation in a barrier T-maze task?

Authors: *K. B. HUNTZICKER[1], R.-M. KARLSSON[2], H. A. CAMERON[3]
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Abstract: Adult neurogenesis in the hippocampus has long been implicated in neural mechanisms for complex learning. Through the use of a transgenic rat model expressing herpes simplex virus thymidine kinase (HSV-TK) under the glial fibrillary acidic protein (GFAP) promoter, our lab can study the behavioral effects of pharmacogenetic ablation of adult neurogenesis. Previous studies from our lab have found that GFAP-TK rats treated with valganciclovir to eliminate adult neurogenesis show decreased motivation for weak rewards when compared with wild-type controls in two operant tasks that require high effort. To determine whether this difference stems from an aversive response to the uncertainty inherent to the progressive ratio and effort-based reward tasks or solely from a lack of motivation, we used a

barrier T-maze task to assess motivation when both effort level and reward outcome are certain. This paradigm employs a two-armed T-maze to compel animals to choose between a high-reward arm known to contain several sucrose pellets and a low-reward arm with fewer pellets. The addition of physical barriers of varying heights modulates the amount of effort that rats must expend to receive a reward, essentially forcing a decision between high-effort/high-reward and low-effort/low-reward outcomes. Results showed that GFAP-TK rats and wild-type controls behaved similarly with a high reward ratio (5:1), with both groups choosing the high-reward arm in more than 80 percent of trials, even when it required climbing a very high barrier. Ongoing studies will determine whether this behavior persists in the presence of a lower reward ratio and more evenly matched choices. If not, this would suggest that decreased motivation in rats lacking adult neurogenesis is unrelated to uncertainty about whether rewards will be earned. However, if GFAP-TK rats and wild-type rats continue to perform similarly in the T-maze task, this finding would suggest that GFAP-TK rats exhibit a differential response to reward motivation specifically in the presence of uncertainty, suggesting a role for adult neurogenesis in the cost-benefit analysis required for the execution of complex reward-seeking behaviors.

Disclosures: K.B. Huntzicker: None. **R. Karlsson:** None. **H.A. Cameron:** None.

Poster

365. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 365.15/A15

Topic: A.02. Postnatal Neurogenesis

Support: NIH R01 HD087288
Magee Womens Research Institute Clinical Trainee Research Grant

Title: Actions of a novel glucocorticoid in the neonatal rat brain

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Abstract: Bronchopulmonary dysplasia (BPD) remains a significant complication of prematurity, particularly extremely preterm infants. Its management often includes the administration of systemic glucocorticoids (sGC), mainly dexamethasone. However, sGC therapy carries significant concerns for not only short-term systemic side effects, but also detrimental impact on long-term neurodevelopment and structural brain development. As such, there remains a need for a GC therapy that will have the desired anti-inflammatory effect on neonatal lungs, but which would not produce negative systemic side effects seen with

dexamethasone. Ciclesonide is a new generation inhaled sGC currently used in children for asthma management. It is a prodrug activated by lower airway carboxylesterases and demonstrates low systemic bioavailability and rapid metabolism outside of the lungs, which allows for the avoidance of systemic side effects, in addition to equivalent efficacy to current sGC therapies. We hypothesized that exposure to ciclesonide will have limited detrimental effects on white matter in neonatal rat brain relative to dexamethasone due to its limited conversion in brain tissue to its active compound des-ciclesonide. Our study compared the effects of ciclesonide on myelin formation and astrogliosis via myelin basic protein (MBP) immunofluorescence and Western blot analysis, to those of dexamethasone, which has previously been shown to cause such abnormalities. We also assessed the pharmacokinetics of the conversion of ciclesonide to des-ciclesonide in various tissues in rat neonates via LC/MS/MS analysis. These studies provide an important step in examining the efficacy and impact on structural brain development, as well as brain and systemic pharmacokinetics, of ciclesonide in order to potentially establish its position as a superior alternative to dexamethasone. Development of an alternative and superior sGC therapy to dexamethasone in the management of BPD would present a significant clinical advancement in the treatment of extremely preterm infants.

Disclosures: E.M. Bargerstock: None. **J.D. Jaumotte:** None. **S.G. Wendell:** None. **D.B. DeFranco:** None.

Poster

365. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 365.16/A16

Topic: A.02. Postnatal Neurogenesis

Title: Neonatal phlebotomy-induced anemia, PIA, in developing mice increases expression of immune response genes in the hippocampus and is associated with behavioral and social deficits

Authors: *T. MATVEEVA[1], G. SINGH[2], T. GISSLEN[3], P. V. TRAN[4], J. C. GEWIRTZ[5], M. GEORGIEFF[3]
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Abstract: Background: Anemia is common in premature infants and is exacerbated by repeated phlebotomy for lab analyses. The behavioral consequences of infant phlebotomy remain understudied. vidence in humans suggests that the degree of anemia commonly tolerated in the NICU can negatively affect neurodevelopment, potentially through dysregulation of inflammatory pathways.

Objective: Determine the short-term effects of PIA on the hippocampal transcriptome and investigate long-term cognitive and social deficits in neonatal PIA mice.
Design/Methods: PIA was induced in mice by phlebotomizing 5.3 µL blood/g body weight twice daily through the facial vein beginning at postnatal day (P) 3 to target hematocrits (Hct) of 25% or18%, respectively. Thereafter, blood was drawn once daily (3.5µL/g) to maintain those hematocrit levels until P14. Non-phlebotomized pups served as controls. RNA isolated from P14 PIA and control hippocampus was sequenced and processed using EdgeR package to identify differentially expressed genes ((DGE, [FC] $\geq$ 1.5, P $\leq$ 0.05). DGEs were analyzed using Ingenuity Pathway Analysis (IPA) to uncover altered signaling pathways and functional gene networks. The formerly anemic and control mice were also assessed behaviorally using the NOR test and the Three-Chambered Sociability/Social Novelty Task immediately after recovery at P17-P21 and then again at $\geq$P120.
Results: 217 DEGs were identified from 18,380 sequenced genes. IPA analysis revealed multiple altered gene networks indicating an increased inflammatory response via NFATC2 and decreased estrogen receptor (ESR1) signaling. 18% Hct mice showed novelty aversion, while 25% Hct mice were indifferent to object novelty. Conversely controls spent more time exploring a novel object than a familiar one. During the Three-Chambered Sociability/Social Novelty task, both 25% and 18% Hct mice showed social novelty aversion, while 25% Hct animals also revealed deficits in sociability.
Conclusion(s): Neonatal PIA in mice similar to that of human premature infants displayed deficits in novelty-related exploration and social behavior. These effects could be related to increased inflammation and decreased estrogen receptor signaling- processes providing safeguards against injurious events in the neonatal period.

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Poster

365. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

Location: SDCC Halls B-H

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Program #/Poster #: 365.17/A17

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant K02DA023555
NIH Grant NNX15AE09G
NIH Grant R37HD059288

Title: Mild traumatic brain injury in the mouse results in transient increase of neurogenesis but sustained survival of newborn neurons

Authors: *K. L. CLARK[1,2], S. YUN[1,2], H. E. METHENY[1], A. S. COHEN[1,2], A. J. EISCH[1,2]
[1]Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: The majority of the 2.8 million traumatic brain injuries (TBIs) that occur annually in the US are classified as mild. Mild TBIs (mTBIs) can induce long-lasting impairment in memory and attention. Contextual memory is disrupted after mTBI, and is critically dependent on adult dentate gyrus neurogenesis. There are conflicting results about how TBI affects neurogenesis, with very little work examining the impact of a mild injury. Here we utilized a mouse model of mTBI and defined its influence on the dynamic process of adult hippocampal neurogenesis. Male C57BL/6J mice (6-8 weeks) received either sham surgery or mTBI via lateral fluid percussion injury (LFPI), and dentate gyrus neurogenesis was measured via stereology at early (3 days post-injury, dpi), intermediate (7 dpi), and late (31 dpi) time points. Mice received a single injection of BrdU (150mg/kg i.p.) 3 dpi to label proliferating cells. Brains from the early time point were collected 2 hours post-BrdU and assessed for indices of dentate gyrus neurogenesis: proliferation (BrdU+ and Ki67+ cells in subgranular zone [SGZ] of granule cell layer [GCL]) and immature neurons (doublecortin [DCX]+ cells in SGZ and GCL). Brains from the intermediate and late time points were collected 4 days and 4 weeks post-BrdU, respectively, and assessed for proliferation (Ki67+ cells), immature neurons (DCX+ cells), and survival and fate of newly-born neurons (BrdU+ cell number and phenotype). At the early time point, LFPI mice had ~50% more Ki67+ SGZ cells relative to sham mice, but the same number of DCX+ SGZ/GCL cells as sham mice. In contrast, at the intermediate time point, LFPI mice had ~30% more DCX+ SGZ/GCL cells than sham mice. At the late time point, Ki67+ and DCX+ cell numbers were the same between LFPI and sham mice, but LFPI mice had ~70% more BrdU+ SGZ cells than sham mice. Our current hypothesis is that these data suggest a transient increase in proliferation of neural precursor cells shortly after LFPI, which may relate to the subsequent transient increase in immature neurons and to a bolus of adult-born granule cell neurons that persist 1 month post-injury. Ongoing work is testing this hypothesis with additional immunohistochemical analyses (early and late timepoints: dentate gyrus GCL volume; intermediate: Ki67 and BrdU cell numbers, late: phenotype of BrdU+ cells; all time points: indices of glial activation), and exploring how these cells influence hippocampal physiology and function on both the circuit and behavioral level.

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Poster

365. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 365.18/A18

Topic: A.02. Postnatal Neurogenesis

Title: Oxycodone in the female rat: Development of an oral self-administration protocol and effects on infant behavior and communication

Authors: *G. ZANNI, M. J. DESALLE, A. A. DOUGHER, J. CHANDAR, H. M. DEUTSCH, G. A. BARR, A. J. EISCH
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Abstract: The use and misuse of prescription and other opiates during pregnancy in the US has increased 5-fold in the past 10 years. Indeed, from 2000-2012, 22,000 newborn infants in the U.S. were diagnosed with Neonatal Abstinence Syndrome (NAS, also called Neonatal Opiate Withdrawal Syndrome, or NOWS), which is marked by severe irritability and neurobehavioral complications. Little is known about the long-term consequences of oxycodone exposure. Here we explore the effects of gestational and early postnatal exposure to oxycodone using our newly-developed preclinical model of oxycodone self-administration and NAS. Adult female Long Evans rats received oxycodone in drinking water before, during, and after pregnancy and readily drank oxycodone (0.06-0.12 mg/ml), even when unadulterated water was available. Intake averaged about 10/mg/kg/day. Maternal liquid intake and body weight were measured daily, and the following measurements/behaviors were recorded in offspring from postnatal day 2 (P2) to P14: plantar thermal test, negative geotaxis, pivoting, olfactory spatial navigation, righting, cliff avoidance, and maternal separation-induced ultrasonic vocalizations. Oxycodone was detected in the serum of mothers and pups and there were no differences in the number of pups/litter, litter weight, or pup weight over time relative to pups of mothers that received water only. Compared to control pups, oxycodone exposed pups had longer latency to remove a paw in the plantar thermal test on P2. Together with detectable oxycodone in the serum of P0 pups and mothers, these data show oxycodone received via placenta and lactation has a functional effect in P2 pups. The analgesia was transient, though, as it was not evident in P14 litters. Oxycodone-exposed and control pups had similar performance on many of the tests (P3-P13), including motor coordination, cliff avoidance, righting time, and olfactory spatial learning. However, P7 oxycodone-exposed pups had reduced pivoting relative to control pups. Female but not male oxycodone-exposed rat pups vocalized more before and after reunion with their mother (P8) suggesting potential differences in attachment and social communication,. These data suggest prenatal exposure to opioids has a transient analgesic effect, a mild locomotor effect, and a sex-dependent effect on “emotional”-like behavior. We are currently employing this novel translational rat model for a longitudinal analysis of the effects of oxycodone on brain and behavior in both mothers and offspring, including hippocampal neurogenesis.

Disclosures: M.J. DeSalle: None. **A.A. Dougher:** None. **J. Chandar:** None. **H.M. Deutsch:** None. **G.A. Barr:** None. **A.J. Eisch:** A. Employment/Salary (full or part-time):; University of Pennsylvania, Department of Neuroscience and Mahoney Institute of Neuroscience.

Poster

365. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 365.19/A19

Topic: A.02. Postnatal Neurogenesis

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National Aeronautics and Space Administration NNX15AE09G
an Independent Investigator Award from the National Alliance for Research on Schizophrenia and Depression/Brain and Behavior Foundation

Title: GABAergic entorhinal cortex control of hippocampal function in stress-related behavior: Cellular and circuitry mechanisms

Authors: *S. YUN[1], R. P. REYNOLDS[2], M. SUAREZ[2], A. D. GIBSON[2], M. J. DESALLE[2], A. J. EISCH[2,1]
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]The Children's Hosp. of Philadelphia Res. Inst., Philadelphia, PA

Abstract: Major Depressive Disorder is marked by dysregulated hippocampal circuitry in both humans and rodent models, and antidepressant treatments, like pharamceutical and electroconvulsive therapies, ameliorate these hippocampal changes. From such work, a promising framework for discovery of new antidepressants has emerged: find treatments that "recalibrate" depression-linked dysfunctional neural circuits and behavior. Indeed, other approaches to "stimulate" neural circuits–such as deep brain stimulation (DBS)–have been successful in reversing depression-related symptoms and neuropathology, particularly in the hippocampus. In the context of depression, it is notable DBS has only been targeted to non-hippocampal brain regions, such as the nucleus accumbens and subcallosal cingulate. While direct stimulation of the hippocampus has generally negative effects, we recently showed chronic stimulation of the glutamatergic input from the entorhinal cortex [Ent] to the hippocampal dentate gyrus (DG) is antidepressive and improves fear memory. In addition to glutamateric projections, the Ent also hosts GABAergic interneurons that, despite their name, project to the hippocampus and contribute to network activity and function (i.e. memory). However, it is unknown if Ent GABAergic hippocampal-projecting neurons regulate depressive-like behaviors. Here we used chemogenetics and DREADD technology to control the excitability of efferent

GABAergic neurons via AAV-DIO-hM3Dq infusion into the Ent of either Somatostatin (SST)- or Parvalbumin (PV)-cre transgenic mice and examine the impact on social avoidance induces by chronic social defeat stress. Stimulation of Ent–CA1/CA3 projecting GABAergic neurons (SST+ or PV+ cells) shows longer total immobile time in FST after chronic restraint stress, implying depressive-like behaviors. In contrast, stimulation of Ent–DG projecting GABAergic neurons (SST+ cells) shows shorten total immobile time in FST. Given our interesting data, we are currently examining DG dysfunction-related depressive-like processes (e.g. pattern separation, social and non-social approach behavior, neurogenesis) after stimulation of SST+ Ent–DG projecting GABAergic neurons after chronic stress. Also, we are examining neuroanatomical and neuron functional complexity of Ent GABAergic interneuron via monosynaptic tracing techniques and in vivo Ca2+ imaging. Our study will reveal Ent–hippocampus projecting GABAergic interneurons circuit has broad implications for understanding pathological condition of depression and manipulating for depression treatment.

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Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.01/A20

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant MH094896
Angelman Syndrome Foundation
Dup15q Alliance

Title: Impaired BDNF-TrkB signaling in human Angelman syndrome neurons

Authors: *E. S. LEVINE, J. J. FINK
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Abstract: Individuals with a deletion of chromosome 15q11-q13 suffer from Angelman syndrome (AS), a neurogenetic developmental disorder characterized by intellectual disability, motor ataxia, autistic features, absent speech, and seizures. The specific gene that is responsible for AS encodes the ubiquitin protein ligase E3A (UBE3A). The expression of this gene in neurons is imprinted (silenced) on the paternal allele, and expression in neurons is only from the maternal allele. Thus, deletion or mutation of the maternal UBE3A gene cause a complete loss of Ube3A protein in neurons. However, the specific Ube3A targets and downstream signaling mechanisms underlying AS pathophysiology are unknown. Using neurons differentiated from patient-specific induced pluripotent stem cell (iPSC) lines, we have recently identified a cellular

phenotype in AS neurons that includes impaired maturation of resting membrane potential and action potential firing, along with decreases in spontaneous synaptic activity, synaptic plasticity, and dendritic complexity. In the present work, we explored whether disruption of signaling by brain-derived neurotrophic factor (BDNF), acting through the trkB receptor, is responsible for aspects of this phenotype. BDNF is a member of the neurotrophin gene family that plays well-established roles in neuronal differentiation and development as well as modulation of synaptic structure and function. Interestingly, BDNF-trkB signaling was recently shown to be disrupted in AS mice. In iPSC-derived neurons from control subjects, we find that removal of exogenous BDNF, along with blocking endogenous BDNF signaling, impaired maturation of resting membrane potential, action potential firing, and spontaneous synaptic activity, mimicking the phenotype of AS neurons. Preventing BDNF signaling in AS neurons, on the other hand, did not cause any further impairment. We also monitored acute responses to BDNF via electrophysiology and calcium imaging. BDNF application resulted in increased synaptic activity and action potential firing in control neurons, but failed to do so in AS neurons. Taken together, these results suggest that AS neurons are unresponsive to BDNF, which may account for the immature phenotype of AS neurons. Ongoing studies are examining whether disruptions in BDNF signaling are responsible for deficits in NMDA receptor-dependent synaptic plasticity in AS neurons. This approach sets the stage for identifying novel therapeutic targets for treatment of AS and related disorders.

Disclosures: E.S. Levine: None. **J.J. Fink:** None.

Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.02/A21

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant MH094896
Dup15q Alliance

Title: Effect of altered glucose levels on the activity of human Dup 15q syndrome neurons

Authors: *M. ELAMIN, T. M. ROBINSON, J. J. FINK, E. S. LEVINE
Neurosci., Univ. of Connecticut Sch. of Med., Farmington, CT

Abstract: Chromosome 15q11-q13 duplication syndrome (Dup15q) is a neurodevelopmental disorder caused by duplication of the maternal copy of this region of the long arm of chromosome 15. Affected children typically present with autistic behavior, epileptic seizures, and a wide range of intellectual and motor disabilities. We are using neurons derived from induced pluripotent stem cell (iPSC) lines from Dup15q patients and unaffected controls to

identify cellular phenotypes related to the underlying pathophysiology. We have recently identified differences in excitability and synaptic transmission between Dup15q neurons and unaffected controls over the course of *in vitro* development. In addition, several studies have reported mitochondrial dysfunction and metabolic irregularities in individuals with Dup15q that could contribute to the underlying pathophysiology. However, cells studied *in vitro* are typically grown in supra-physiological levels of glucose, which by itself could cause mitochondrial dysfunction, thereby obscuring physiological differences between control and Dup15q-derived neurons. Furthermore, Dup15q neurons could be more sensitive to elevated glucose levels if they have mitochondrial dysfunction, or alternatively, mitochondrial dysfunction may mask the effects of altered glucose levels. The present studies were designed to compare neuronal and synaptic activity of iPSC-derived neurons from Dup15q patients and unaffected controls under a range of glucose levels. We found that, in control cells, 48 hours of incubation with 3 mM glucose increased spontaneous synaptic activity and increased action potential amplitude compared to incubation with normal culture media (25 mM glucose). In contrast, neurons derived from Dup15q individuals had higher baseline synaptic activity compared to controls in normal culture media (25 mM), consistent with our previous results, but were unaffected by lowering glucose levels. In a parallel set of experiments, we are examining spontaneous action potential firing and neuronal synchrony using population calcium imaging. Preliminary results suggest that the availability of energy substrates can significantly modulate neuronal activity and support the existence of underlying metabolic abnormalities in Dup15q-derived neurons.

Disclosures: M. Elamin: None. **T.M. Robinson:** None. **J.J. Fink:** None. **E.S. Levine:** None.

Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

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Program #/Poster #: 366.03/A22

Topic: A.03. Stem Cells and Reprogramming

Support: Dean’s Competitive Fund for Promising Scholarship (Faculty of Arts and Sciences, Harvard University)

Title: Rapid production of stem cell-derived human neural progenitors for the study of Zika virus neuropathogenesis

Authors: *M. F. WELLS[1,2], M. R. SALICK[3], E. J. HILL[1], M. SIEKMANN[1], A. KAYKAS[4], K. EGGAN[1,2]
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Abstract: Neural progenitor cells (NPCs) play a vital role in early brain development by acting as an intermediate proliferative cell type in the pathway from pluripotent stem cells to fully functional neurons and glia. NPC dysfunction has been linked to several neurodevelopmental disorders, including schizophrenia, autism, and Zika (ZIKV) Congenital Syndrome. Our understanding of these brain diseases has been greatly improved by advancements in *in vitro* stem cell-derived NPC model systems, which usually take anywhere from 14-50 days to generate using conventional methods. Here, we describe human Stem cell-derived Ngn2-accelerated Progenitor cells (SNaPs), which are produced using a novel 48 hour induction protocol. Large quantities of highly pure SNaPs that express several canonical transcript and protein markers of human NPCs can be manufactured in a fraction of the time required for standard techniques. SNaPs are proliferative, multipotent, and able to self-aggregate into neurospheres under low attachment conditions. Importantly, SNaPs are susceptible to ZIKV infection and viral-mediated cell death, while also being able to support active replication of this virus. Furthermore, ZIKV infection elicits an antiviral response in SNaPs that is similar to that found in human patients, and can also inhibit neurosphere development and outgrowth. Given the efficiency of the SNaP system, we were also able to perform a first-of-its-kind whole genome CRISPR-Cas9 screen to identify genetic factors that confer resistance to ZIKV-induced cell death. Together, our findings support the use of SNaPs for the rapid 2-D and 3-D modeling of ZIKV neuropathogenesis in developing human neural cell types.

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Poster

366. Stem Cells and Disease Modeling II

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.04/A23

Topic: A.03. Stem Cells and Reprogramming

Support: CIRM Bridges Training Grant
LouLou Foundation (UPenn Orphan Disease Center)
Caley J. Brown Foundation, & Rettsyndrome.org

Title: CRISPR/Cas9-mediated homologous direct repair disease modeling and lentiviral gene therapy strategies for CDKL5 deficiency disorder

Authors: *T. NGUYEN[1,2,3], J. HALMAI[2,3], J. WALDO[1,2,3], D. CAMERON[2,3], S. CAMPO[2,3], J. CARTER[2,3], P. DENG[2,4,3], J. NOLTA[1], K. FINK[2,3], D. SEGAL[4]
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Abstract: CDKL5 deficiency disorder (CDD) is a rare X-linked epileptic encephalopathy caused by *de novo* mutations in the cyclin-dependent kinase-like 5 (*CDKL5*) gene located on the X-chromosome (Xp22). Pathogenic mutations in *CDKL5* lead to CDD and causes early onset of seizures, developmental delay, and intellectual disability. To date, there is still limited understanding of CDD and how *CDKL5* deficiency affects brain function. Recently, reactivation of the inactive X-chromosome containing the silenced, healthy CDKL5 in neurons has become an attractive therapeutic strategy to treat CDD. However, due to males having only one *CDKL5* gene, patients with a hemizygous *CDKL5* variants would not be candidates for this therapeutic approach. Our goal was to model a pathogenic *CDKL5* variant in male induced pluripotent stem cells (iPSC) and to generate a lentivirus encoding healthy *CDKL5* as a potential avenue to restore *CDKL5* levels. The CDKL5 mutation (Arg178Gln) was knocked into male iPSC using CRISPR Cas9-mediated HDR to generate a hemizygous *CDKL5* pluripotent cell model. This variant has been reported to be pathogenic and common in male and female CDD patients. CDD iPSCs were isolated through serial dilution to obtain purified cell population containing the CDD variant as assessed using Kompetitive Allele-Specific PCR (KASP). CDD iPSCs were then differentiated into neuronal-like cells for quantification of *CDKL5* expression level. CDD neurons were used as a model to assess the restoration of normal *CDKL5* levels *in vitro* following lentiviral transduction. Our CDD-models and lentivirus could potentially be contributed to future development of CDD therapeutic treatments for both males and females.

Disclosures: T. Nguyen: None. **J. Halmai:** None. **J. Waldo:** None. **D. Cameron:** None. **S. Campo:** None. **J. Carter:** None. **P. Deng:** None. **J. Nolta:** None. **K. Fink:** None. **D. Segal:** None.

Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.05/A24

Topic: A.03. Stem Cells and Reprogramming

Title: Understanding neurological disease by utilizing high-throughput assays and CRISPR KO iPSC-derived cell types as an approach to functional genomic studies

Authors: *J. A. DIZON, E. WILLEMS, C. REVANKAR, X. LIANG, D. PIPER
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Abstract: Induced pluripotent stem cells (iPSCs) can be differentiated into numerous cell types and provide useful tools to create translatable neuronal assay systems, effective for both *in vitro*

disease modeling and drug discovery efforts. The advent of CRISPR/Cas9 genome editing platforms has expanded methods to study genetic defects in disease by enabling the introduction of genetic changes in iPSCs, which can subsequently be differentiated to the affected cell type. Although CRISPR/Cas9 mediated genome editing in iPSCs is the optimal approach to study disease-specific genetic defects, genome editing during or after differentiation would support high-throughput functional genomics screens using targeting panels such as the LentiArray CRISPR libraries to understand the biology of neuronal development or to identify novel targets associated with a particular disease. To facilitate LentiArray CRISPR screening in hiPSC-derived cell types, we generated an iPSC line that stably expresses the Cas9 nuclease. We have established proof-of-concept that these LentiArray tools can efficiently edit the genome of Cas9 hiPSC-derived neuronal progenitors, which suggests that this approach will support high-throughput functional genomic screens in disease-relevant neuronal models. Furthermore, we have developed a series of high content imaging and excitability assays to specifically study disease-relevant cellular phenotypes in progenitors or neuronal cells. After delivery of tools, Cas9 iPSC-derived progenitors and neurons can then be tested in a high throughput fashion for changes in cell health, neurite outgrowth, calcium influx and variations in channel activity, and/or electrophysiological changes. By combining the LentiArray CRISPR system with hiPSC-derived neuronal progenitors or neurons and these high-throughput assays, we have built a functional genomics platform that enables the discovery of novel genetic targets and/or drug leads that could ultimately result in therapies for neurobiological diseases.

Disclosures: J.A. Dizon: A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **E. Willems:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **C. Revankar:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **X. Liang:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **D. Piper:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific.

Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.06/A25

Topic: A.03. Stem Cells and Reprogramming

Title: Neurons derived from human induced pluripotent stem cells as a viable alternative to rodents' embryonic primary neurons for safety and drug discovery studies

Authors: *P. KITCHENER[1], D. HESS[2], F. SIMON[1]
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Abstract: In vitro models for drug discovery or safety screening need to be relevant for particular pathological or physiological contexts and have high levels of reproducibility in order

to repeatedly obtain valuable results. The easiest starting materials are cancer cell lines, which are widely used in neuroscience literature for simple screening and are handled with standard procedures. A major drawback, however, is that these cells lack many of the key neuronal features, such as spontaneous neurite outgrowth and the ability to form functional neuronal networks that respond to neuronal pharmacology. While more delicate to obtain and use, primary neurons classically obtained from rodents are a tool of choice to study most of neuronal biology in vitro. They are good tools for measuring neurotoxicity and pathophysiological modeling, however, obtaining these cells is labor intensive and time consuming, requiring the sacrifice of a number of embryos. Additionally a specific subpopulation of interest may be poorly represented as exemplified by primary dopaminergic neurons. Three iPSC derived neuronal types from two different providers were evaluated in different assays where primary rodent neurons where used. CNS.4U® cells (Ncardia) represent a highly physiological in vitro co-culture model of diverse Human neurons and astrocytes. Peri.4U® cells (Ncardia) are peripheral neurons. iCell DOPA® (Cellular Dynamics International) are Human dopaminergic neurons. These postmitotic ready to use neurons suppress the need for long and delicate expansion and differentiation steps and provide a short path to initiating neuronal cultures. Each of these cells responds to particular needs in terms of neuronal types, that may not otherwise be obtained as Human neurons for reasonable throughput experiments. We show that optimized experimental conditions to perform live content neurite outgrowth assays, endpoint immunofluorescent labeling and high throughput intracellular calcium measurements, allow for the measuring of neurotoxicity and neuronal network functionality in different physiological and pathological contexts such as in epilepsy (CNS.4U), Parkinson's disease (iCell DOPA) and peripheral neuropathy (Peri.4U). Comparisons with similar data obtained in rodent primary neurons can demonstrate the relevance of the tested Human iPSC-derived neuronal cells with added workability and species relevance for Human medicine. Therefore, while not only reducing the effort involved in using primary neurons obtained from rodents, iPSC-derived neurons, used in adapted experimental paradigms have good validity as models for neurotoxicity and drug discovery studies.

Disclosures: D. Hess: None. **F. Simon:** None.

Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.07/A26

Topic: A.03. Stem Cells and Reprogramming

Title: Transplantation and functional integration of human stem cell derived 3D forebrain spheroids into the rodent cortex

Authors: *O. REVAH, T. A. KAHN, N. SAKAI, J. ANDERSEN, J. R. HUGUENARD, S. P. PASCA
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Abstract: Three-dimensional human brain region-specific organoids or spheroids derived from pluripotent stem cells hold great potential for studying development and modeling brain disease. However, these in vitro cultures do not receive physiological input from other brain regions, do not generally include immune cells and are not vascularized. Here, we have developed a method of transplanting and integrating forebrain spheroids into the cerebral cortex of rats. We show that forebrain spheroids integrate anatomically and functionally, become vascularized and send long-distance projections. We use state-of- the-art live imaging, electrophysiological and calcium imaging techniques to systematically investigate the morphology and function of the human neural graft. This platform can provide novel insights into maturation and circuit integration of neurons and can be used to model human neuropsychiatric disease.

Disclosures: O. Revah: None. **T.A. Kahn:** None. **N. Sakai:** None. **J. Andersen:** None. **J.R. Huguenard:** None. **S.P. Pasca:** None.

Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.08/A27

Topic: A.03. Stem Cells and Reprogramming

Support: NCI - R21CA199295

Title: Human cerebrospinal fluid induces an increase in proliferation of glioblastoma cells *in vitro* and *in vivo*

Authors: *P. S. MEADE[1,2], A. CARRANO[2], J. PHILLIPS[2], M. LARA-VELAZQUEZ[3,2], N. ZARCO[2], S. JEANNERET[4,2], H. GUERRERO-CAZARES[2]
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Abstract: Glioblastoma multiforme (GBM) is the most common and aggressive primary malignant brain tumor in adults [1]. Despite advances in treatment, GBM has an extremely poor prognosis [1, 2]. Studies indicate that tumors located close to the lateral ventricles (LV) have higher proliferative rates, invasive capacity, and potential of multifocal recurrence at distant areas of the brain [3-7]. The explanation of this phenomenon remains controversial. However,

recent theories propose that tumors are originated from a subpopulation of cells called brain tumor initiating cells (BTICs), which are similar to the neural progenitor cells (NPCs) found at the subventricular zone that lines the LV [8-11]. BTICs have self-renewing and multipotent characteristics, critical for tumor growth and maintenance [8-11]. These malignant capabilities could be increased when BTICs are in close relationship with the LV components: the cerebrospinal fluid (CSF) and the neurogenic niche located at the SVZ [3, 12]. Therefore, we studied the migration and proliferation effects of human BTICs after CSF stimulation. To this end, we divided the study into two subsets. First, 15,000 GBM cells were grown *in vitro* and stimulated with cancer CSF, non-cancer CSF, or control conditions for 48h diluted in 1:200 with base media. We evaluated cell viability using Alamar blue assay, and performed western blot and immunocytochemistry against Ki67 and Nestin. To determine cell migration, cells were plated on a glass bottom culture dish; groups were established as described above and evaluated by time-lapse video microscopy for 30 hours and further analyzed using Matlab. Second, we used an *in vivo* model in which both female and male nude mice were implanted with luciferase-labeled GBM cells in suspension with human cancer or non-cancer CSF in a hydrogel as vehicle (n=8 per group). Tumor growth was followed with bioluminescence imaging. 14 days post tumor implantation, mice were euthanized and brains were stained with H&E and immunohistochemistry against for Ki67. We found that *in vitro* stimulation with cancer CSF increases proliferation of GBM cells, as we obtained higher growth rates and greater concentrations of Ki67 and Nestin. Results correlated with the *in vivo* studies, as animals receiving cancer CSF presented with greater tumor size and Ki67 proliferation index. Regarding cell migration, we did not find differences in the *in vitro* migration assays. Taken together, this study provides direct evidence proving that cancer CSF could have a direct effect on tumor growth. Understanding the interaction of brain tumors and CSF may lead to new therapeutic strategies that will target the CSF components in GBM patients.

Disclosures: P.S. Meade: None. **A. Carrano:** None. **J. Phillips:** None. **M. Lara-Velazquez:** None. **N. Zarco:** None. **S. Jeanneret:** None. **H. Guerrero-Cazares:** None.

Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.09/A28

Topic: A.03. Stem Cells and Reprogramming

Support: Ministry of Health and Department of Educational Assistance, University and Research of the Autonomous Province of Bolzano

Title: Creating a fluorescent knock-in reporter for midbrain dopaminergic neurons using CRISPR/Cas9 with hiPSCs

Authors: *C. ÜBERBACHER[1], J. OBERGASTEIGER[1], S. VENEZIA[1], M. VOLTA[1], D. BECCANO-KELLY[2], M. ZOLI[4], A. A. HICKS[1], P. P. PRAMSTALLER[1], S. COWLEY[3], C. CORTI[1]
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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by a focal loss of dopaminergic (DA) neurons the substantia nigra pars compacta (SNpc). The establishment of patient derived pluripotent stem cells (iPSCs) (Takahashi et al. 2007) and their efficient conversion into DA neurons (Kriks et al. 2011) (Chambers et al. 2009) fueled the possibilities for disease modeling and its implication in development and screening of novel drugs up to implementation of cell transplantation therapies. Human iPSCs can be differentiated into DA neurons and support the study of neurological diseases in vitro. However, current neuronal differentiation protocols lack the ability to produce a homogeneous population of midbrain DA neurons. So far, this has not limited positive outcomes of functional recovery in preclinical studies (Daadi et al. 2012; Peng et al. 2014; Hallett et al. 2015) as transplantation often occurs at neural stem cell state (Qiu et al. 2017). However, it still represents an important limitation with regard to predictive and translational application for in vitro models of PD.
To overcome this limitation, we have used the CRISPR/Cas9 system to generate human iPSC lines engineered in the tyrosine hydroxylase (TH) gene, a marker of DA neurons. The iPSC lines carry an enhanced GFP reporter knocked-into frame with the TH gene and under the control of its own promoter to allow expression together with TH during in vitro differentiation. We confirmed the accurate integration of the fluorescent protein reporter in the cell lines with ddPCR and fluorescent in-situ hybridization. With ddPCR we confirmed the simultaneous expression of TH and eGFP mRNA. The co-localization of the two proteins was confirmed via co-immunostaining using primary antibodies specific for TH and eGFP respectively. In addition, upon differentiation into DA neurons of the genetically engineered TH-eGFP hiPSCs, the endogenous eGFP reporter expression was clearly recognizable using flow cytometric analysis as early as 25 days in vitro. These results give promising perspective into the possibility of isolating this subpopulation and thereby obtaining a pure DA cell population. In conclusion, we provide a novel cellular model to study PD phenotypes in vitro, allowing to focus functional studies and screening of novel drugs for the treatment of PD. Moreover, we implement a platform that can be applied to any other cell model of neurodegeneration.

Disclosures: C. Überbacher: None. **J. Obergasteiger:** None. **S. Venezia:** None. **M. Volta:** None. **D. Beccano-Kelly:** None. **M. Zoli:** None. **A.A. Hicks:** None. **P.P. Pramstaller:** None. **S. Cowley:** None. **C. Corti:** None.

Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.10/A29

Topic: A.03. Stem Cells and Reprogramming

Support: Italian Ministry of Health, Ricerca Corrente 2014-2017 to ALV
ERC Starting Grant 260888 to EMV,
Association Revert Onlus
Fondazione Cellule Staminali

Title: Establishment of stable iPS-derived human neural stem cells lines suitable for cell therapies and neurological diseases modeling

Authors: J. D. ROSATI[1], D. FERRARI[5], F. ALTIERI[1], S. TARDIVO[6], C. RICCIOLINI[7], C. FUSILLI[2], C. ZALFA[5], D. C. PROFICO[3], L. BERNARDINI[4], G. LAMORTE[4], T. MAZZA[2], M. CARELLA[4], M. GELATI[3,7], E. VALENTE[6], A. SIMEONE[8], *A. L. VESCOVI[5,1]
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Abstract: Establishing specific cell lineages from human induced Pluripotent Stem Cells (hiPSCs) is vital for cell therapy approaches in regenerative medicine, particularly for neurodegenerative disorders. While neural precursors have been induced from hiPSCs, the establishment of hiPSCs-derived human neural stem cells (hiNSCs), with characteristics that match fetal hNSCs and abide by cGMP standards, thus allowing clinical applications, has not been described. We generated hiNSCs by a virus-free technique, whose properties recapitulate those of the clinical grade hNSCs successfully used in an ALS phase I clinical trial. *Ex-vivo*, hiNSCs critically depend on exogenous mitogens for stable self-renewal and amplification and spontaneously differentiate into astrocytes, oligodendrocytes and neurons upon their removal. In the brain of immunodeficient mice, hiNSCs engraft and differentiate into neurons and glia, without tumor formation. These findings now warrant the establishment of clinical-grade, autologous and continuous hiNSC lines for clinical trials in neurological diseases.
We have expanded our previous results and successfully applied the same neuralization protocol to iPSCs derived from patients affected by genetic neurological diseases. These hiNSCs lines reproduce in vitro disease-related features such as altered neuronal differentiation and trophic

factors expression, thus representing a potential tool to study new therapies for uncurable devastating pathologies.

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Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.11/A30

Topic: A.03. Stem Cells and Reprogramming

Title: Generation of Parkinson's disease models through CRISPR/Cas9 editing in human induced pluripotent stem cells

Authors: *R. E. LACAMBACAL[1], E. WILLEMS[1], T. GOKIRMAK[1], C. REVANKAR[1], J. A. DIZON[1], X. LIANG[1], R. NEWMAN[2], D. KUNINGER[2], D. R. PIPER[3]
[1]Thermo Fisher Scientific, Carlsbad, CA; [2]Thermo Fisher Scientific, Frederick, MD; [3]Biosci. Division, Cell and Synthetic Biol., Thermo Fisher Scientific, Carlsbad, CA

Abstract: Induced pluripotent stem cells (iPSCs) have been globally recognized as a multipurpose research tool for modeling diseases, developing and screening potential drugs, and implementing cell transplantation therapies to support regenerative medicine. The ability to differentiate human iPSCs into any cell type, including dopaminergic neurons, supports the study of neurological diseases in vitro. The emergence of genome editing tools, such as the CRISPR/Cas9 system, allows diseases to be modeled at the genetic level and studied at the cellular level. However, genetic mimicking of disease has been difficult due to challenges in developing neuronal assays that demonstrate cellular and genetic phenotypes for Parkinson's disease. Here, we describe the generation of clonal CRISPR-edited iPSCs to model Parkinson's disease and demonstrate several cellular phenotypes in neuronal assays associated with the disease.. Through use of an iPSC line that stably expresses the Cas9 protein, we obtained highly efficient editing in targets relevant for Parkinson's disease and obtained single edited iPSC clones that were isolated through the combination of an optimized cell sorting method and expansion of as single cell clones with reagents that support improved single cell survival. We then generated dopaminergic neurons from our clonal CRISPR-edited iPSCs using our PSC Dopaminergic Neuron Differentiation Kit, which generate neurons that express tyrosine hydroxylase (TH) indicative of dopaminergic neurons. Neurite outgrowth assays with these dopaminergic neurons indicated phenotypes typical of Parkinson's disease. Dopaminergic neurons derived from the disease models generated were more sensitive to neurotoxic

compounds as shown in neurite length and apoptosis assays, which again exhibited a disease-like phenotype. In addition, we observed that target selective genes associated with Parkinson's disease are exclusively affected in iPSC lines with mutations introduced in the respective target. In summary, we describe our genome editing platform to reliably produce clonal gene-edited iPSCs that can be used as cellular models to study neurodegenerative disease phenotypes in vitro. This genome editing workflow can be used to mimic these human diseases or revert disease mutations, and will support significant insights into neurodegenerative diseases at the genetic and cellular level.

Disclosures: R.E. Lacambacal: A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **E. Willems:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **T. Gokirmak:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **C. Revankar:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **J.A. Dizon:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **X. Liang:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **R. Newman:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **D. Kuninger:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **D.R. Piper:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific.

Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.12/A31

Topic: A.03. Stem Cells and Reprogramming

Support: NIH F31-NS103447
Project ALS A13-0416

Title: Intrinsic differences between cranial and spinal motor neurons as a route to ALS gene therapy

Authors: *D. E. IANNITELLI[1], D. AN[1], E. O. MAZZONI[1,2]
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Abstract: Amyotrophic lateral sclerosis (ALS) is marked by progressive degeneration of motor neurons resulting in near total paralysis. While in familial forms of ALS all cells in the body carry associated mutations, a subset of cranial motor neurons (CrMN) resist degeneration better than spinal motor neurons (SpMN), allowing patients to retain eye movements until late disease stages. Therefore, the gene regulatory program triggered during embryonic development in CrMNs allows them to later resist neurodegeneration better than SpMNs. The nature of the intrinsic resistance of this subpopulation of motor neurons to degeneration is not understood and

represents an under-exploited area of ALS research that will lead to a better understanding of the disease mechanism and the development of new therapeutic strategies. Direct comparison of sensitive and resistant MNs is hindered by challenges in extracting and culturing sufficient numbers of CrMNs from tissue. We overcome this hurdle by employing a stem cell-based platform wherein we program both types of motor neurons from embryonic stem cells. Expression of the NIL (Ngn2-Isl1-Lhx3) and NIP (Ngn2-Isl1-Phox2a) transcription factor cassettes induce (i) iSpMNs and iCrMNs respectively. To test their response to ALS toxicity, we established isogenic inducible lines to program iSpMNs and iCrMNs expressing hSOD1 with ALS-related mutations which induce protein misfolding. Thus, taking advantage of our ability to generate both iSpMNs and iCrMNs from mouse embryonic stem cells at high efficiency and model ALS-associated mutations *in vitro*, our goal is to understand the molecular nature of differential sensitivity to neurodegeneration ALS. Immunostaining shows that iCrMNs accumulate less p62-positive aggregates than iSpMNs, differentially modeling an early symptomatic feature of ALS. Inhibition of the proteasome gives the opposite result, suggesting that iCrMNs rely more on proteasome function than iSpMNs to prevent protein aggregation. To determine whether differences in how iCrMNs and iSpMNs prevent protein aggregation extend to how they cope with proteostasis stress, we treated iCrMNs and iSpMNs with CPA. We found that iCMrNs survive better than iSpMNs to chemically induced proteostasis stress. With an *in vitro* platform in hand to model ALS pathology in iCrMNs and iSpMNs, we want to investigate if Phox2a expression (the master regulator of CMN fate) will be sufficient to 1. induce the gene regulatory network which confers resistance to CrMNs and 2. protect SpMNs from ALS stress. These findings will be used to determine if conferring CrMN attributes to SpMNs is sufficient to protect SpMNs from ALS-related degeneration.

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Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.13/A32

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant NS085011

Title: Multiplication of the SNCA locus exacerbates neuronal nuclear aging in hiPSC-derived neuronal models of Parkinson’s and dementia with Lewy bodies

Authors: *O. CHIBA-FALEK, L. TAGLIAFIERRO, M. E. ZAMORA
Duke Med., Durham, NC

Abstract: Overexpression of alpha-synuclein gene (*SNCA*) has been implicated in the etiology of synucleinopathies, such as Parkinson's (PD) and dementia with Lewy bodies (DLB). However, the mechanisms that mediate the pathogenic role of *SNCA* overexpression in synucleinopathies remain to be fully elucidated. Human induced Pluripotent Stem Cell (hiPSC) models advanced the field, however, age is critical in the etiology of synucleinopathies and hiPSC-derived models represent rejuvenated neurons which are not fully suitable to model age-related diseases. We developed a new approach to induce aging in hiPSC-derived neurons to further the understanding of the genetic etiologies and molecular mechanisms that are commonly perturbed in synucleinopathies, and those that may underlie the heterogeneity amongst the different diseases in this group. Using hiPSC from a patient with *SNCA*-triplication and a control, we applied the new protocol to generate *isogenic* hiPSC-derived aged dopaminergic and cholinergic neurons, that model PD and DLB, respectively. We characterized age-related phenotypes of the nuclear architecture, specifically markers for heterochromatin organization and nuclear envelope structure. In addition, we evaluated features of the alpha-synuclein aggregation including, aggregates distribution, size, number and confirmation. The hiPSC-derived aged neurons recapitulated aging-features, demonstrating that our protocol is fully suitable for studying late-onset neurodegenerative diseases. The *SNCA*-triplication neurons exhibited advanced-aging signatures of nuclear architecture and chromatin organization already at the juvenile-stage, suggesting that *SNCA* overexpression exacerbates nuclear aging. The aged *SNCA*-triplication dopaminergic neurons showed increased number of alpha-synuclein aggregates per-cell compared to the *isogenic* juvenile neurons. However, the effect of age on the number of aggregates was not observed for the cholinergic neurons. The aggregates in the cholinergic neurons presented a diffuse conformation, while aggregates in the dopaminergic neurons appeared more defined with a punctuate structure. In conclusion, we described a link between *SNCA* overexpression and neuronal nuclear architecture features of aging. We suggest that wild-type *SNCA* functions in pathways related to neuronal nuclear organization, and that *SNCA* overexpression exacerbates nuclear aging and by that contribute to synucleinopathies. Moreover, the interplay between aging and *SNCA* overexpression have a differential phenotypic effect on different neuronal types, providing new insights into the heterogeneity in synucleinopathies.

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Poster

366. Stem Cells and Disease Modeling II

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Program #/Poster #: 366.14/A33

Topic: A.03. Stem Cells and Reprogramming

Support: Cure Alzheimer's Fund

NIH Grant AG023084
NIH Grant NS034467

Title: Modeling PICALM Alzheimer's disease variants in human iPSC-derived cells

Authors: *E. J. LAWSON[1], Z. DAI[1], X. XIE[1], S. A. BAZZI[1], A. P. SAGARE[1], Z. ZHAO[1], R. E. TANZI[2], B. V. ZLOKOVIC[1]
[1]Physiol. and Neurosci., USC, Los Angeles, CA; [2]Massachusetts Gen Hosp, Harvard Med. Sch., Charlestown, MA

Abstract: PICALM is abundantly expressed in neurons and brain capillaries in the human brain, playing an essential role in regulating neuronal function and providing a major pathway for amyloid-beta clearance *in vivo* across the blood-brain barrier. Impaired transvascular and/or paravascular clearance of Amyloid-beta across the BBB contributes to an accumulation of Aβ, accelerating neurovascular and neuronal dysfunction in the Alzheimer's Disease brain. Our research has shown that PICALM is required for Aβ endocytosis and trafficking across the BBB through endocytosis via the LRP1 receptor. Moreover, diminished PICALM levels in the brain endothelium are associated with increased neuronal vulnerability to Aβ species resulting in neuronal dysfunction and, concurrently, neurodegeneration. Several PICALM variants have been identified to influence neuronal toxicity *in vivo*. Using CRISPR/CAS9 genome editing technology, we explored the protective *PICALM* SNP *rs3851179*^{A} and the risk *rs3851179*^{G} variants. We successfully generated iPSC lines homologous for allelic variants of *PICALM* SNP *rs3851179* which were further differentiated into neurons, microglia, and endothelial cells. We confirmed by western blot analysis higher PICALM expression in iPSC-derived neurons and endothelial cells carrying protective *rs3851179*^{A} alleles than those carrying non-protective *rs3851179*^{G} alleles. Neurons carrying protective or non-protective alleles of PICALM can be functionally compared throughout differentiation in 3D and 2D *in vitro* models. Interestingly, neurons generated from iPSCs with the protective *rs3851179*^{A} were less vulnerable to Aβ toxicity. By generating *in vitro* models with neurons, microglia, and endothelial cells derived from human iPSCs with other allelic variants of PICALM, we can explore the fundamental characteristics of AD pathology as it relates to PICALM expression.

Disclosures: E.J. Lawson: None. **Z. Dai:** None. **X. Xie:** None. **S.A. Bazzi:** None. **A.P. Sagare:** None. **Z. Zhao:** None. **R.E. Tanzi:** None. **B.V. Zlokovic:** None.

Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.15/A34

Topic: A.03. Stem Cells and Reprogramming

Support: MRC MC_UP_1203/2

Title: Efficient derivation of distinct dopaminergic neuronal subpopulations from mouse and human pluripotent stem cells

Authors: P. GARCAO, E. MOLES-GARCIA, T. OOSTERVEEN, C. SOLEILHAVOUP, K. PATRICK, *L. PANMAN
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Abstract: Midbrain dopaminergic (mDN) neurons constitute a highly diverse neuronal population controlling important brain functions, such as motor action, cognition, motivation, reward and emotions. These neurons can be broadly subdivided into two major anatomically and functionally groups, which form the substantia nigra (SN) and ventral tegmental area (VTA). The SN neurons innervate the dorsal/lateral striatum and are critical for controlling motor functions, while neurons within the VTA innervating cortical and limbic areas of the forebrain are primarily associated with emotional behaviours. SN dopaminergic neurons selectively degenerate in Parkinson's disease, while the neighbouring VTA neurons remain relatively unaffected despite the commonalities in developmental origin and gene expression profile. Pluripotent stem (PS) cells offer important opportunities for disease modelling and cell replacement therapy. However, it is important that cell types of the desired identity are generated. Parkinson's disease (PD) is the second most common neurodegenerative disease, which is caused by the selective degeneration of SN dopaminergic neurons located in the ventral midbrain. Despite the commonalities in gene expression and developmental origin, dopaminergic neurons within the VTA remain relatively spared in PD. Access to enriched cultures of these distinct dopaminergic subpopulationsSN will offer important opportunities for disease modelling and cell replacement studiesmodelling Parkinson's disease. Although, dopaminergic neurons have been efficiently generated from embryonic stem (ES) cells, it is unclear how enriched cultures of either SN or VTA subpopulations can be obtained. We have established novel mouse and human embryonic stem (ES) cell differentiation protocol that results in the generation of dopaminergic cultures highly enriched for SN neurons. The SN enriched cultures display the predicted sensitivity to mitochondrial toxicity, which is not observed in cultures mainly consisting of VTA neurons. In addition, applying our differentiation protocol to a PD patient specific iPS cell line resulted in increased levels of alpha-synuclein selectively in SN enriched cultures. Altogether our platform provides us with a model system that can be used to model PD disease and to get novel insight into the selective vulnerability of SN neurons.

Disclosures: P. Garcao: None. **E. Moles-Garcia:** None. **T. Oosterveen:** None. **C. Soleilhavoup:** None. **K. Patrick:** None. **L. Panman:** None.

Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.16/B1

Topic: A.01. Neurogenesis and Gliogenesis

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

Title: Stem Cell derived human central nervous system lineages and models of neurodegenerative diseases

Authors: *M. CARNA, V. POZO DEVOTO, M. FEOLE, V. LACOVICH, G. STOKIN
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Abstract: To date, different protocols have been published to differentiate human stem cells into neuronal and glial lineages. Current protocols of human stem cell drive differentiation towards a specific CNS lineage at the expense of the others. However, efficiency of target cell generation varies widely, characterization of cells profile and functionality differs between studies, which leads to highly variable protocols outcomes. Recent evidence indicates that differentiation of CNS cell lineages requires intimate exchange of growth and transcription factors between differentiating cells. Cell to cell communication is essential for coordination of cellular events, better development and homeostasis. To test whether NSCs can be differentiated into comparable amount of fully differentiated CNS cell lineages, namely neurons, astrocytes and oligodendrocytes, a novel protocol was developed to allow for exchange of all necessary factors and optimal maturation of cells. The medium was designed to mimic brain-like serum-free environment to test cells interactions and functionality. We find that this newly designed system exhibits comparable gene and protein expression patterns characteristics for mature CNS cell lineages. The optimal stem cell differentiation into the target cell type was confirmed by demonstrating appropriate morphology lineage specific markers. The function of cells was characterized by their electrophysiological properties, which regulate cell specific activities. The new protocol significantly improved glutamate responsive calcium transients in astrocytes and enhanced glutamate uptake; adequately supported neuronal activity exhibited by increased density of synapsin-immunoreactive punctae in culture, but moreover robustly promoted myelination confirmed by significant MBP immunoreactive cell bodies ensheathing axons. PCR array gene analyses uncovered cell-type specific secreted factors regulating differentiation process. Additionally, RNAseq of single cell-types was performed in order to discover unique regulatory pathways participating on the specific cell features. The novel 2D *in-vitro* model allows for optimal maturation and physiological function of human stem cell derived neurons, astrocytes and oligodendrocytes. It is a powerful tool which enables to investigate the

neurobiological impact of glial cells on neuronal differentiation and development and to study human neurological diseases *in-vitro.*

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

Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.17/B2

Topic: A.01. Neurogenesis and Gliogenesis

Title: Characterization of neural cells derived from human dental pulp stem cells

Authors: *Y. ARIMURA[1], T. KIKUCHI[2], R. YAMANAKA[3], Y. SHINDO[4], K. HOTTA[4], M. MOCHIZUKI[5], T. NAKAHARA[5], K. OKA[6]
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Abstract: Human dental pulp stem cells (hDPSCs) that can be obtained from deciduous or permanent teeth are notable stem cells. The hDPSCs are capable of differentiation into osteogenic, adipogenic, and also neurogenic cells and have proliferation ability (Tamaki et al., 2013). However, characteristics of these derived cells are decided as only static ones by using immunochemistry not functional ones. We, therefore, investigate the functional characteristics of the neuron-like cells derived from hDPSCs by using several fluorescent imaging techniques. We obtained hDPSCs from the cell bank of The Nippon Dental University School of Life Dentistry. In the specific neurogenic culture condition, hDPSCs differentiated into neuron-like cells. We introduced fluo-4 (calcium ion indicator) to these cells at 0, 7, 14, 21, 28 days after differentiation, and observed calcium mobilization before and after the application of several chemicals (glutamate, ATP, and high-KCl). We observed 4 types of hDPSC-derived neuron-like cells characterized by calcium responses; induction of calcium transient after glutamate application, calcium transient after ATP application, calcium oscillation after ATP application, and calcium transient after high-KCl application. In addition, calcium response was also obtained by stimulation with veratridine, an activator of voltage-dependent Na+ channel. After calcium imaging experiment, we identified each cell types (neurons or astrocytes) by immunostaining using the antibodies against glial fiber acidic protein (GFAP) as glial maker and βIII-tubulin as neuron marker, and characterized each cells with specific 4 types of calcium responses. The cells with calcium mobilization by the application of KCl showed intense immunoreactivity to βIII-

tubulin compared with that of GFAP. Regarding the localization site, strong positive reaction of βIII-tubulin was obtained in the cell processes and GFAP in the cell bodies. This results suggested that βIII-tubulin is involved in processes elongation.

Disclosures: Y. Arimura: None. **T. Kikuchi:** None. **R. Yamanaka:** None. **Y. Shindo:** None. **K. Hotta:** None. **M. Mochizuki:** None. **T. Nakahara:** None. **K. Oka:** None.

Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.18/B3

Topic: A.01. Neurogenesis and Gliogenesis

Support: Jiangsu Province Natural Science Foundation Grant BK20130891
Jiangsu Province's Innovation program

Title: Generation of cholinergic neurons in hPSC-derived organoids

Authors: *K.-H. FANG, F. YUAN, X.-Y. TANG, Y. HU, M. XU, Y. LIU
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Abstract: Cholinergic neurons play essential roles in regulating learning and memory, which mainly originate from medial ganglionic eminence (MGE). Current available protocols to generate cholinergic neurons are relied on 2D culture system, which is unaccessible for long term culture. This study is aiming at exploring a high efficiency method for differentiating cholinergic neuron in human pluripotent stem cell-derived basal forebrain (BF) organoids. With the combination of XAV939 (Wnt pathway inhibitor) and Purmorphamine (Shh signaling activator), we generate human BF organoids with ventricular and sub-ventricular structures which express MGE marker NKX2.1. To further promote the differentiation of cholinergic neurons, we treat the BF organoids with NGF (nerve growth factor). After two weeks, cholinergic neurons are determined in the BF organoids. Then we transplanted BF organoids into SCID mice basal forebrain. After 3 months, numerous $ChAT^+$ human neurons were tested in the mice brain, and a great mount of human neurites were observed in the cortex. The results of this study demonstrated XAV939/purmorphamine/NGF medium could generate human cholinergic neurons in BF organoids, and grafted human neurons could survived and have long projections in mice brain.

Disclosures: K. Fang: None. **F. Yuan:** None. **X. Tang:** None. **Y. Hu:** None. **M. Xu:** None. **Y. Liu:** None.

Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

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Program #/Poster #: 366.19/B4

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant NS083009
NIH Grant HD059967

Title: A GEFS+ SCN1A mutation causes cell type-specific and temperature-dependent defects in neurons derived from isogenic hiPSC pairs

Authors: *Y. XIE[1], N. N. NG[1], O. S. SAFRINA[1], C. M. RAMOS[1], K. C. ESS[2], P. H. SCHWARTZ[3], D. K. O'DOWD[1]
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Abstract: Approximately 50 missense mutations in *SCN1A*, a gene that encodes the alpha subunit of the Nav1.1 voltage-gated sodium channel, are associated with genetic epilepsy with febrile seizures plus (GEFS+). Mechanisms of how specific *SCN1A* mutations alter neuronal activity and result in seizures are still obscure. To address this question we are assessing the functional properties of neurons differentiated from two pairs of isogenic human iPSC lines: 1) a patient line with K1270T *SCN1A* mutation (GEFS+), and an isogenic corrected line by removing the K1270T mutation using CRISPR/Cas9 editing; 2) a control (unaffected sibling of GEFS+ patient) and isogenic mutant line by introducing the K1270T mutation using CRISPR/Cas9 editing. Isogenic iPSCs were patterned into functional neuronal cultures that consist of 65% excitatory neurons and 35% inhibitory neurons. Electrophysiological analysis of inhibitory neurons 21-24 days post plating showed that the *SCN1A* K1270T mutation, in both genetic backgrounds, causes a reduction in neuronal sodium current density and evoked firing rate at room temperature. Excitatory neurons also exhibited reduced sodium current density but the evoked firing rate was not altered, giving rise to hyperexcitability of the neural network. To investigate the underlying temperature-dependent changes, we are now evaluating the neuronal activity in isogenic hiPSC lines at elevated temperatures, in both excitatory and inhibitory neurons. Preliminary data suggest that the K1270T *SCN1A* mutation results in temperature-dependent depolarized shift of action potential rheobase selectively in inhibitory neurons. This isogenic hiPSC model will be very beneficial to deciphering the links between *SCN1A* mutations and the impact on febrile seizures and epileptic disorders, facilitating the development of personalized anti-seizure medications and improved therapies.

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Poster

366. Stem Cells and Disease Modeling II

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.20/B5

Topic: A.03. Stem Cells and Reprogramming

Support: CEPID-FAPESP
FAPESP Grant 2017/19877-0
FAPESP Grant 2016/09618-5
INCT
CNPq
FAPESQ

Title: Loss-of-function mutation in inositol monophosphatase 1 (IMPA1) causes severe intellectual disability and schizophrenia-like/bipolar-like behaviors: A human model to study inositol pathway

Authors: *T. FIGUEIREDO[1,2], G. KOBAYASHI[1], D. MOREIRA[1], D. OLIVEIRA[1], E. GOULART[1], A. MENDES[2], F. KOK[1], S. SANTOS[3], R. Y. CHO[4], C. MARCHETTO[2], F. H. GAGE[2], M. ZATZ[1]
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Abstract: Inositol monophosphatase 1 is the critical enzyme for the recovery of the inositol cycle, and it is key for both the de novo synthesis of inositol and the recycling of inositol polyphosphates. The phospoinositol metabolism pathway is involved in normal physiological conditions, such as insulin and PI3K/Akt signaling, endocytosis, vesicle trafficking, exocytosis, cell migration, proliferation, apoptosis, neurotransmitter release, hormone secretion and in maintaining the state of homeostasis for second messengers. Thus, dysfunctions of the inositol cycle have been implicated in a variety of human diseases, including developmental defects, cancer, diabetes and neurological diseases. IMPA1 is thought to have an important role in the mechanism of bipolar disorder (BD), because in therapeutic concentrations, lithium, the main pharmacological treatment for BD, it is an uncompetitive inhibitor of this enzyme. Despite its many physiological functions, no human disease had been attributed to a malfunction of this protein. We identified a new homozygous 5 bp duplication (c.489_483dupGGGCT) in *IMPA1*, leading to a frameshift (p.Ser165Trpfs*10) mutation, in a large consanguineous family with severe intellectual disability and disruptive behaviors from a geographically isolated location in Northeastern Brazil (Figueiredo et al., 2016). Recently, we investigated resting EEG data from

30 participants (17 non-carriers (NC), 9 heterozygotes (HT) and 4 homozygotes (HM). We found disturbances in frontal theta and more global alpha band disturbances in individuals with homozygous *IMPA1* mutation. Interestingly, homozygote IMPA1$^{-/-}$ mice die *in utero*. To gain further insights on IMPA1 function in human cells, we developed induced pluripotent stem cell lines (iPSCs) from three HM, one HT, and two NC individuals from the same family. Using anti-IMPA1 polyclonal antibody we observed expression of IMPA1 in iPSCs from family control individuals and no expression in homozygotes, because of a premature stop codon generated by the frameshift mutation in both alleles. We are currently expanding these studies to patient-derived neuronal cell lines in order to clearly elucidate the mechanisms by which impairment of IMPA1 can alter signaling pathways leading to the development of mental dysfunctions and to correlate with *in vivo* measures of brain activity and cognitive capacity aiming to better understand the pathway from genetic variants to abnormal behavior.

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Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.21/B6

Topic: A.03. Stem Cells and Reprogramming

Support: NIH MH101634
NIH MH113924
Cystinosis Research Foundation
Schmitt Foundation

Title: new methods for studying network activity in human induced-pluripotent stem cell (ipsc) derived neural cultures for modeling diseases

Authors: *K. PADMANABHAN[1], C. MARCHETTO[2], R. SANTOS[4], F. H. GAGE[3]
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Abstract: Neuron cultures derived from induced-Pluripotent Stem Cells (iPSC) are rapidly being used to model circuits *in vitro*. In these cultures, electrophysiological recordings of populations of neurons has been used to understand how structure influences network dynamics, and the ways in which diseases processes disrupt these networks and consequently alter activity. While methods that use mean firing rate or degrees of synchrony can help to identify differences in networks, they are often insensitive to the complexity of single neuron dynamics and the higher

order interactions between neurons that underlie many principles of computation *in vivo*. One of the major challenges for studying the activity of populations of neurons, both *in vitro* and *in vivo* is that the number of possible network states scales exponentially with the number of neurons recorded. In this regard, the benefit *In vitro* recording methods offer in terms of allowing for the monitoring of large numbers of neurons at high temporal precision, carries with it the consequence of requiring new statistical and computational methods for describing patterns of activity. To address this challenge, we recorded large populations of iPSC-derived neurons *in vitro,* and then developed approaches to classify and studying network activity. First, we describe strategies for characterizing the spike count statistics of populations of cells, including estimating the entropy of the circuit (a measure of structure). Following this, we use these methods to describe the network topology that generates specific patterns of dynamics. We show that our method can identify differences in circuit structure, for instance the effect of network size and connectivity in generating different kinds of dynamics. Importantly, our methods could serve as a platform for interrogating how different neurological and psychiatric disorders alter network activity.

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Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.22/B7

Topic: A.03. Stem Cells and Reprogramming

Title: Altered neural calcium signaling in human iPSC-derived cortical organoids with cytomegalovirus infection

Authors: *A. D. EBERT[1], A. JOHNSON[2], S. L. SISON[1], S. S. TERHUNE[3]
[1]Cell Biology, Neurobiology, and Anat., [2]Dept. of Obstetrics and Gynecology, [3]Dept. of Microbiology and Immunology, and Biomed. Engin., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Human cytomegalovirus (HCMV) is a beta herpesvirus that upon infection during pregnancy can cause severe congenital birth defects including microcephaly, vision loss, and hearing loss. Currently, no approved treatment options exist for managing in utero infections. Our previous work showed that the antiviral compound maribavir (MBV) decreases HCMV infection in undifferentiated human induced pluripotent stem cell (iPSC) derived neural progenitor cells (NPCs) and allowed progression to early steps in differentiation. However, it remains to be determined whether MBV-treated NPCs can differentiate into functional glutamatergic neurons and astrocytes. Here we evaluated the impact of infection on calcium signaling using cultured NPCs as well as in three-dimensional iPSC-derived cortical organoids.

Functional changes were quantified using live-cell ratiometric imaging measuring intracellular calcium changes following stimulation of purinergic and voltage-gated channels with ATP and KCl, respectively. In the absence of infection, acutely plated NPC populations exhibited increasing percentages of ATP-responsive astrocytes and KCl-responsive neurons over time in culture consistent with maturation. Following infection using GFP-expressing HCMV, NPCs exhibited reduced baseline calcium levels and were unresponsive when evaluated 5 days post infection. In contrast, addition of MBV resulted in reduced numbers of GFP-positive cells, and the GFP-negative cells within the population exhibited normal ATP- and KCl-responsive signaling. To extend these studies, we used early and late stage multicellular iPSC-derived cortical organoids. We first confirmed proper differentiation and laminar organization within uninfected organoids. Upon HCMV infection, we observed GFP-expressing cells within the tissues regardless of the developmental stage. MBV treatment reduced the numbers of GFP-expressing cells, and the GFP-negative cells exhibited more normal ATP- and KCl-responsive signaling. Our studies demonstrate that HCMV infection significantly impairs neuronal and glial function. Moreover, our data indicate that MBV-mediated inhibition of HCMV infection allows NPCs within a complex population to develop into functional glutamatergic neurons and astrocytes.

Disclosures: A.D. Ebert: None. **A. Johnson:** None. **S.L. Sison:** None. **S.S. Terhune:** None.

Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.23/B8

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant HD067517
Swebilius Foundation

Title: An epilepsy-associated Slack mutation increases K_{Na} currents and enhances excitability of human iPSC-derived neurons

Authors: *I. H. QURAISHI[1], K. P. MANGAN[5], Y. ZHANG[2], M. MERCIER[3], S. STERN[6], M. C. MARCHETTO[6], F. H. GAGE[6], L. K. KACZMAREK[4]
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Abstract: Human mutations in the sodium-activated Slack potassium channel, also known as KCNT1, produce intractable early childhood epilepsy with very severe intellectual disability. In heterologous systems, mutant channels are functional but produce higher amplitude currents than

wild type channels. This has presented a quandary because Slack channels are highly expressed in many neuronal types including excitatory pyramidal cells in the cortex. Ordinarily, increases in potassium currents that are relatively voltage-independent such as Slack are expected to reduce rather than enhance excitability. There have, however, been no studies to determine whether these mutations produce increased Slack currents in neurons (as opposed to transfected cell lines or oocytes), nor any studies of the effects of such increases on neuronal excitability. Using human neurons derived from IPS cells and engineered to have an epilepsy-associated KCNT1 mutation, we now show that the introduction of a disease-causing mutation strikingly increases neuronal sodium-activated potassium currents despite similar expression levels. We find that this results in more rapid repolarization of action potentials and a larger afterhyperpolarization following each spike. As a result, neurons bearing the mutant channel fire many more action potentials in response to depolarizing stimuli. In networks of neurons in vitro, the enhanced Slack currents produce more intense bursts of action potentials and increase overall firing rates. Our findings provide new insights into the biological role of Slack channels in the regulation of neuronal excitability and provide very clear new therapeutic opportunities for the treatment of these devastating diseases.

Disclosures: I.H. Quraishi: None. **K.P. Mangan:** A. Employment/Salary (full or part-time):; Cellular Dynamics, International. **Y. Zhang:** None. **M. Mercier:** None. **S. Stern:** None. **M.C. Marchetto:** None. **F.H. Gage:** None. **L.K. Kaczmarek:** None.

Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.24/B9

Topic: A.03. Stem Cells and Reprogramming

Support: SKLN-201702

Title: Brian organoid-based approach to the pathogenesis of ketamine-induced neurodevelopmental disorders

Authors: *C. XU[1], L. JIANG[2]
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Abstract: The repeated or lengthy use of general anesthetic and sedation drugs in pregnant women during their third trimester potentially affects the development of children's brain and is mandated as “black box warnings” by the U.S. Food and Drug Administration. Although animal studies demonstrated clearly that prolonged exposure to ketamine leads to histological and

functional disorders on brain development, the extrapolation of preclinical research to human is still insufficient for providing bases for clinical treatment, which highlights the need for a better model of developing human brain. In recent years, human pluripotent stem cell (hPSC)-derived three-dimensional organoid culture system, termed brain organoid, has been developed to recapitulate features of human brain development and help to study mechanisms of human brain diseases. We applied hPSC-derived brain organoids to mimic human brain development and investigate the influence of ketamine on germinal zone organization, neurogenesis, gene expression and the distinct primate-specific outer radial glia cell layer by immunostaining, quantification PCR and RNA sequencing. Our preliminary results suggest dramatic effects of ketamine on brain organoid growth, neural apoptosis and survival in 24- hours, which is both dose- and time- dependent as well. Human brain organoids provide an efficient way to evaluate the influence of ketamine on human neural development. Extrapolating these hPSC-derived findings to guide clinical use of ketamine and even other general anesthetic drugs in pregnant patients would be more necessary, compared with studies based merely on animal models.

Disclosures: C. Xu: None. **L. Jiang:** None.

Poster

366. Stem Cells and Disease Modeling II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 366.25/B10

Topic: A.03. Stem Cells and Reprogramming

Title: Neuroinflammation could be associated with autism risk increase in infants born with congenital Zika syndrome

Authors: *P. BELTRÃO-BRAGA[1], C. M. Y. OHKI[2], F. B. R. RUSSO[1], P. E. C. LEITE[3], V. V. LINDEN[2]
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Abstract: Zika virus is associated with congenital malformations, including microcephaly, leading to a congenital syndrome (CZS), which affected hundreds of newborns in Brazil. Autism Spectrum Disorders (ASD) is a neurodevelopmental disorder characterized by communication and social impairment, also associated with restricted and repetitive patterns of behavior. Epidemiological and animal model studies have implicated both environmental factors and genetics in ASD etiology, which affects approximately 1% of worldwide population. The literature concerning viral maternal infections during gestation and ASD risk is still inconclusive. Induced pluripotent stem cells (iPSC) have been successfully generated modeling human neurological disorders, including ASD and CZS. Here we investigated the effects of Zika virus in astrocytes and neurons derived from iPSC and its possible correlation with autism. We found

that Zika virus-infected astrocytes trigger a cytokine profile compatible with neuroinflammation. It includes IL6, IL17 and TNFa, which increased levels are related to ASD. Besides, we investigated the effect of Zika virus infection in neurogenesis and synaptogenesis. We found that Zika causes an impairment in functional synapses in neurons, revealing that children infected during embryogenesis could produce defective neurons, reflecting in cognitive impairment. Actually, children born with CZS during Brazilian outbreak are around 2/3 years old and some of them have been already diagnosed as presenting autistic clinical phenotype. Our results revealed that CZS display a neuroinflammation cytokine profile comparable with the one found in ASD individuals, as well as a significant decrease in synaptic gene and protein expression levels. Altogether, such data could be associated with the increased risk for ASD in children born with CZS.

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Poster

366. Stem Cells and Disease Modeling II

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Program #/Poster #: 366.26/B11

Topic: A.03. Stem Cells and Reprogramming

Support: Canadian Institute of Health Research
Canada Research Chair award
Indonesian government
Central government of China

Title: Disruption of GRIN2B impairs differentiation in human neurons

Authors: *G. MAUSSION[1], S. C. BELL[2], M. JEFRI[2], H. PENG[2], J.-F. THEROUX[2], H. SILVEIRA[2], V. SOUBANNIER[1], H. WU[2], E. GALAT[3], S. G. TORRES-PLATAS[2], C. BOUDREAU-PINSONNEAULT[2], L. O'LEARY[2], V. GALAT[3], G. TURECKI[2], T. M. DURCAN[1], E. A. FON[1], N. MECHAWAR[2], C. ERNST[2]
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Abstract: Heterozygous loss-of-function mutations in *GRIN2B*, a subunit of the NMDA receptor, cause intellectual disability and language impairment. We developed clonal models of *GRIN2B* deletion and loss-of-function mutations in a region coding for the glutamate-binding domain in human cells, and generated neurons from a patient harbouring a missense mutation in the same domain. Transcriptome analysis revealed extensive increases in genes associated with cell proliferation and decreases in genes associated with neuron differentiation, a result

supported by extensive protein analyses. Using electrophysiology and calcium imaging, we demonstrate that NMDA receptors are present on neural progenitor cells, and that human mutations in *GRIN2B* can impair calcium influx and membrane depolarization even in a presumed undifferentiated cell state, highlighting an important role for non-synaptic NMDA receptors. It may be this function, in part, which underlies the neurological disease observed in patients with *GRIN2B* mutations.

Disclosures: G. Maussion: None. **S.C. Bell:** None. **M. Jefri:** None. **H. Peng:** None. **J. Theroux:** None. **H. Silveira:** None. **V. Soubannier:** None. **H. Wu:** None. **E. Galat:** None. **S.G. Torres-Platas:** None. **C. Boudreau-Pinsonneault:** None. **L. O'Leary:** None. **V. Galat:** None. **G. Turecki:** None. **T.M. Durcan:** None. **E.A. Fon:** None. **N. Mechawar:** None. **C. Ernst:** None.

Poster

367. Axon Growth and Guidance

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 367.01/B12

Topic: A.05. Axon and Dendrite Development

Title: Biogenesis of sonic hedgehog in the developing brain

Authors: *A. RIVELL[1], P. J. YAO[1], M. P. MATTSON[1], R. S. PETRALIA[2], Y.-X. WANG[2]
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Abstract: In addition to patterning embryonic neural tubes, the Sonic Hedgehog (Shh) signaling pathway continues to have functions in the post-embryonic brain including the development and plasticity of neurons. We have previously found that the Shh pathway receptor Patched and transducer Smoothened are situated in the dendritic spines and shafts of hippocampal neurons. We have also found that activation of the Shh signaling pathway stimulates the growth of axons and presynaptic terminals in hippocampal neurons. In this study, we investigate the production and biogenesis of Shh. We begin by examining Shh mRNA and protein levels in developing brains and hippocampal neurons. We then examine potential regulatory factors that affect global Shh levels. Using antibodies that react with either the N-terminus or C-terminus of Shh, we compare the levels as well as subcellular distributions of different Shh protein species. The findings of our study provide a necessary step towards a full understanding of Shh signaling in brain functions. "This research was supported by the Intramural Research Program of the NIH, National institute on Aging".

Disclosures: A. Rivell: None. **P.J. Yao:** None. **M.P. Mattson:** None. **R.S. Petralia:** None. **Y. Wang:** None.

Poster

367. Axon Growth and Guidance

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 367.02/B13

Topic: A.05. Axon and Dendrite Development

Title: Growth cone turning on soft-substrate environments requires paxillin-drebrin interaction

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Abstract: Soft tissue environments have profound effects on axon guidance and growth. However, the molecular mechanisms that underlie growth cone responsiveness to guidance factors on soft substrate are still unclear. Here we found that soft substrate (0.1 kPa) promotes BDNF-induced growth cone turning via modulating the paxillin-associated structural dynamics of hippocampal neurons. By using MALDI-TOF MS and immunoprecipitation, we identified an actin-microtubule crosslinking protein-drebrin as one of the paxillin interacting proteins in embryonic brain lysates. On soft substrate, paxillin associated with drebrin and co-localized at growth cone T-zone. When cells asymmetrically exposed to the guidance factor, i.e., BDNF, their growth cones exhibited significantly larger turning angles with a higher degree of drebrin-paxillin co-localization on 0.1 kPa soft substrates as compared with on glass. Down regulation of drebrin expression by siRNA approach resulted in a reduction in the distribution of paxillin on T-zone. Ectopic expression of a paxillin variant, which has lost drebrin interactions, led to reduced turning responses of growth cone to BDNF-coated stripes on soft substrates. Together, these findings suggest that growth cone response to BDNF on 0.1 soft substrate requires paxillin association with drebrin.

Disclosures: C. Chen: None. **R. Lin:** None. **T. Chang:** None. **P. Cheng:** None.

Poster

367. Axon Growth and Guidance

Location: SDCC Halls B-H

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Program #/Poster #: 367.03/B14

Topic: A.05. Axon and Dendrite Development

Support: DST-INSPIRE, Govt of India
DST SERB Grant, EMR/2016/002306

CSIR, Govt of India
RGCB Intra-mural fund

Title: Guiding retinal ganglion cell axons to brain visual centres: Is Pax6 the key molecule?

Authors: *L. SOUNDARARAJAN[1], V. MEERA[1], S. SURYA[1], R. ANN PAUL[1], S. PARVATHY[1], B. BASU[1], A. BHATTACHARJEE[2], N. M. ABRAHAM[2], J. JAMES[1]
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Abstract: Intra-retinal axonal guidance is a complex process involving a number of axonal guidance genes that direct the nascent axons of retinal ganglion cells (RGCs) to fasciculate together to reach the optic disc. It is known that Pax6 is a key molecule for RGC genesis but its expression persists in RGCs after they are formed. We were curious to understand why the expression of Pax6 persists in the RGCs during late retinogenesis. Hence, we hypothesize that it could possibly be involved in axonal guidance after RGC fate specification. To understand the role of Pax6 in axonal guidance, we perturbed Pax6 expression in retinal explant culture and also we conditionally knocked out (cKO) Pax6 in the mice retina during the period of axon formation. Down regulation of Pax6 with siRNA in E16 retinal explants showed a significant reduction in RGC axonal growth and fasciculation. We further substantiated our results by knocking out Pax6 in E15.5 mice retina. Here, we have observed a decrease in the number of RGCs, amacrine cells, horizontal cells and photoreceptors which were confirmed by Brn3, Ap2-alpha, calbindin, and recoverin staining, respectively. Analysis of $Pax6^{+/+}$ (control) and $Pax6^{-/-}$ (knocked out at E15.5) retinal flatmounts showed a significant alteration in the axonal guidance and fasciculation in $Pax6^{-/-}$ retina compared to the control. To further understand axonal guidance molecules that Pax6 could be regulating, we performed ChIP-seq with Pax6 antibody and identified a number of axonal guidance genes that are regulated by Pax6. Out of which, the prominent ones were *EphB1* and *Sema5B*. The interaction and regulation of EphB1 and Sema5B by Pax6 was further confirmed with luciferase and qRT-PCR analyses. These findings highlight a novel role for Pax6 in the intra-retinal axonal guidance by regulating key guidance molecules. Our results could have far reaching consequences at a later stage where, it could be mimicked to guide the axons of RGCs transplanted to replace degenerating RGCs in conditions such as glaucoma.

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Poster

367. Axon Growth and Guidance

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 367.04/B15

Topic: A.05. Axon and Dendrite Development

Support: JP Grant 16K08478

Title: PlexinA1 is crucial for the midline crossing of callosal axons during corpus callosum development in BALB/c mice

Authors: *M. HOSSAIN[1], T. ITO[2], T. TSUZUKI[1], F. IMAIZUMI[1], I. TAKAHASHI[1], T. NEGISHI[1], K. YUKAWA[1]
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Abstract: The corpus callosum (CC) is the biggest commissural tract that connects the right and left hemispheres of the brain. In the mouse cortical midline during CC development, the guidepost structures emerge and express axon guidance molecules including semaphorins 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, localized on the cingulate pioneers has a crucial role in the midline crossing of the pioneer axons through the interactions with semaphorins like Sema3C. However, it remains unproved which type of Plexin receptor actually functions as a component of Npn1-containing receptor complex during the midline crossing of cingulate pioneering neurons. To examine if PlexinA1 is crucially involved in CC development, CC phenotype was examined in PlexinA1-deficinet mice at postnatal day 0.5 (P0.5) under the BALB/c genetic background. All of the PlexinA1-deficinet mice at P0.5 showed partial or complete agenesis of CC (partial; 6 of 13, complete; 7 of 13 mice). To address the role of PlexinA1 in CC development, we examined the localization of PlexinA1, Npn1, Sema3A, Sema3C and calretinin (CR) by immunohistochemistry (IHC) with brain sections from wild-type (WT) and the mutant mice at embryonic day 15.5 to 17.5 (E15.5-17.5). The IHC revealed the expression of PlexinA1 on the dorsal side of callosal axons crossing the cortical midline at E17.5. The IHC confirmed the expression of Sema3A following the gradient from lateral to midline and the specific localization of both Sema3C and CR in the guideposts such as subcallosal sling. Coimmunoprecipitation assay demonstrated the direct association of PlexinA1 and Npn1 at the stage of CC development. To examine the role of PlexinA1 in the midline crossing of callosal axons, the extension of callosal axons across the midline was traced by both IHC of Npn1 and DiI anterograde axonal tracing. Both methods showed that the incidence in midline crossing of callosal axons at E17.5 was significantly lower in PlexinA1-deficient brain as compared with WT. The results indicate the crucial involvement of PlexinA1 in the midline crossing of callosal axons during CC development in BALB/c mice. To test if PlexinA1 could act as a functional receptor mediating Sema3A repulsive and Sema3C attractive activity, we are planning to conduct an axon guidance assay using explants derived from embryonic cingulate gyrus.

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Poster

367. Axon Growth and Guidance

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 367.05/B16

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS078030
NIH Grant NS095471

Title: Nerve growth factor induces mitochondria fission that is required for axon branching

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Abstract: Mitochondria have emerged as major determinants of the sites of axon branching. We report that NGF treatment decreases the length and increases the number of axonal mitochondria along chicken sensory axons in vitro by 15 min of acute treatment, indicative of fission. Consistently, live imaging of mitochondria following 5-10 minutes of NGF treatment revealed increased rates of mitochondria fission. By 15 min post-treatment NGF established a persistent new steady state of mitochondria length and number, an effect that was reversed following NGF withdraw. BDNF and NT3 similarly decreased the length and increased the number of axonal mitochondria. Pharmacological and peptide-mediated inhibition, and dominant negative expression of dynamin related protein 1 (Drp1), a required component of mitochondria fission, blocked NGF induced fission and axon branching. *In ovo* expression of dominant negative Drp1 decreased the number of branches along sensory axons extending in the spinal cord. Live imaging of eYFP-Drp1 revealed accumulation at sites of mitochondria fission and NGF increased the formation of Drp1 accumulations. NGF promoted phosphorylation of Drp1 at the activating site S616 trough Erk activation independently of the PI3K pathway. Inhibition of ERK signaling also blocked the effect of NGF on mitochondria fission, branching and Drp1 accumulation along mitochondria. PI3K activity is required for branching and its inhibition blocked fission but not phosphorylation of Drp1. Furthermore, live imaging revealed that axonal actin patches, which we have previously shown are driven by PI3K signaling, co-localize with sites of mitochondria fission. Inhibition of actin polymerization using Latrunculin-A blocked the effect of NGF on mitochondria fission and Drp1 accumulation. Pharmacological inhibition of the actin nucleating Arp2/3 complex also blocked the effect of NGF on mitochondria length and number. Finally, inhibition of fission blocked the effect of NGF on the intra-axonal synthesis of cortactin, a required and mitochondria-dependent component of branching, and the subsequent increase in axonal actin dynamics. Collectively, these observations unveil a novel biological action of neurotrophins; the regulation of mitochondria fission and setting of steady state

mitochondrial length and number in axons. Given the multitude of roles played by neurotrophins in the nervous system these observations are expected to have broad implications. This work was supported by the NIH NS078030/ NS095471.

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Poster

367. Axon Growth and Guidance

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Program #/Poster #: 367.06/B17

Topic: A.05. Axon and Dendrite Development

Support: European Commission FP6 NEST Programme (contract 028473)
University of Aberdeen Development Trust
Spanish Science Ministry MAT2011-24363, MAT2015-65192-R
La Marato de TV3 Foundation (ID number 110131)
Severo Ochoa Programme for Centres of Excellence in R&D (SEV-2015-0496)

Title: Controlling nerve growth using an electric field induced indirectly in transparent conductive substrates

Authors: *A. M. RAJNICEK[1], Z. ZHAO[1], J. MORAL-VICO[2], A. CRUZ[2], C. MCCAIG[1], N. CASAN-PASTOR[2]
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Abstract: Rationale:
Innovative neurostimulation therapies, including those for brain and spinal injury, require improved electrode materials, such as poly(3,4-ethylenedioxythiophene) (PEDOT) polymers or IrOx mixed ionic-electronic conductors. Little is known about how the electrochemical changes they undergo during stimulation influence nerve growth.
Method:
We performed time lapse microscopy of amphibian neurons growing on transparent films of electronic (metal) conductors and electronic-ionic conductors (conductive polymers and semiconducting oxides) prepared in house. Materials were not connected directly to the power supply but an electric dipole was created wirelessly within them by electrodes connected to the culture medium in which the materials were immersed.
Results:
Without electrical stimulation neurons grew well on films of gold, platinum, PEDOT-polystyrene sulfonate (PEDOT-PSS), IrOx and the mixed oxide (Ir-Ti)Ox but successful growth

was not related directly to surface texture or hydrophilicity. Electrical stimulation induced a dipole in all conductive materials but neurons responded differently to electronic conductors and mixed-valence mixed-ionic conductors. Electrical stimulation slowed, but steered neurite extension to the imposed cathode on gold but not on platinum. The rate and cathodally-directed neurite growth on PEDOT-PSS resembled that on glass, but on IrOx and (Ir-Ti)Ox neurites grew faster and in random directions.
Conclusions:
Our data suggest that electrochemical changes induced in these materials indirectly controlled neurite growth speed and direction selectively. Evidence that the electric dipole induced in conductive materials was sufficient to control nerve growth will impact CNS neuroregenerative electrotherapies that exploit wireless stimulation of implanted material arrays, with additional implications for situations (e.g. retina) where transparency is required.

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Poster

367. Axon Growth and Guidance

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 367.07/B18

Topic: A.05. Axon and Dendrite Development

Title: Birthdate and muscle target in chick oculomotor axons

Authors: *J. P. SCHWARTZ[1], B. M. BJORKE[2]
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Abstract: Early in neural development, oculomotor neurons send axons into peripheral tissue to innervate four of the six extraocular muscles: the dorsal, ventral, and medial recti, and the ventral oblique. These axons generate the oculomotor nerve (nIII). We previously found that nIII displays a complex innervation pattern where motor axons first form a plexus with extraocular muscle precursors prior to muscle formation. The nerve-muscle precursor interaction is critical in regulating axon growth and nerve branching. We are currently interested in further characterizing this interaction by examining how motor pools are represented within the plexus. The first goal of our study is to determine whether motor neuron birthdate reproducibility predicts muscle choice. This would suggest that motor pools are presorted prior to axon outgrowth, plexus formation and muscle development. Motor pools in the chick oculomotor nucleus are divided into distinct sub-nuclei in the adult. Previous research demonstrates that motor neuron birthdate drives the organization of these sub-nuclei, but it is unclear whether motor pools can be reproducibly labeled based on birthdate alone. To answer this question, we are labeling early-born motor neurons with GFP, and late-born neurons with RFP introduced by

in ovo electroporation and examining subsequent muscle innervation. By using birth-date to identify motor pools we hope to characterize how motor pools sort within the nerve-muscle plexus prior to muscle innervation.

Disclosures: J.P. Schwartz: None. **B.M. Bjorke:** None.

Poster

367. Axon Growth and Guidance

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Program #/Poster #: 367.08/B19

Topic: A.05. Axon and Dendrite Development

Support: NSERC

Title: Early trigeminal ganglion afferents enter the cerebellum before the purkinje cells are born and target the nuclear transitory zone

Authors: *H. MARZBAN[1], M. RAHIMI-BALAEI[2], R. HAWKES[3]
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Abstract: In the standard model for the development of climbing and mossy afferent pathways to the cerebellum the ingrowing axons target the embryonic Purkinje cell somata (around embryonic ages (E)13-E16 in mice). In this report we describe a novel earlier stage in afferent development. Immunostaining for a neurofilament-associated antigen (NAA) reveals the early axon distributions with remarkable clarity. By using a combination of DiI axon tract tracing, analysis of *neurogenin1* null mice, which do not develop trigeminal ganglia, and mouse embryos maintained *in vitro*, we show that the first axons to innervate the cerebellar primordium as early as E9 are direct projections from the trigeminal ganglia. Therefore, early trigeminal projections are *in situ* before the Purkinje cells are born. Double immunostaining for NAA and markers of the different domains in the cerebellar primordium reveal that afferents first target the nuclear transitory zone (E9-E10), and only later (E10-E11) are axons, either collateral projections from the trigeminal ganglia or a new afferent source (e.g., vestibular ganglia), seen in the Purkinje cell plate.

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Poster

367. Axon Growth and Guidance

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Program #/Poster #: 367.09/B20

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant R21DC015635

Title: Core PCP proteins direct peripheral axon guidance in the developing cochlea through a non-autonomous signaling mechanism

Authors: *M. R. DEANS[1,2], S. R. GHIMIRE[2]
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Abstract: Neural circuit assembly is critically dependent upon dynamic axon pathfinding events that enable individual growth cones to accurately navigate the developing nervous system and form synaptic contacts in the correct target areas with the correct synaptic partners. The commissural axons of the neural tube have emerged as a preeminent model for studying these events because their growth cones sequentially respond to axon guidance molecules and morphogen gradients as they project towards and cross the ventral midline, and subsequently turn to extend anteriorly. In this model Planar Cell Polarity (PCP) signaling in the growth cone allows commissural axons to turn 90 degrees and extend anteriorly in response to an increasing Wnt gradient. We have characterized a similar 90 degree turning event in the developing cochlea that is required for TypeII Spiral ganglion neurons (SGN2) to extend peripheral axons that innervate the three rows of outer hair cells. SGN2s are thought to contribute to important feedback circuitry that provides neuroprotection in extreme noise, perhaps by acting as nociceptors. SGN2 peripheral axon turning in the cochlea is also dependent upon PCP signaling and in mutant mice lacking the core PCP gene *Vangl2*, these peripheral axons frequently turn incorrectly and project towards the cochlear apex rather than the cochlear base. Similar errors occur in other PCP mutants including *CELSR1* KO mice suggesting a role for the non-canonical Wnt-signaling pathway in SGN2 turning. Using a *Vangl2* CKO line and a series of Cre drivers to further restrict *Vangl2* gene deletion to either neurons or the cochlear duct, we demonstrate that VANGL2 functions in a non-cell autonomous manner and is not required in the growth cone for turning. This is remarkably different from commissural axons where VANGL2 and PCP signaling have been documented in the growth cone. We further demonstrate that VANGL2 and CELSR1 are asymmetrically distributed at the intercellular boundaries between cochlear supporting cells. At this location, the polarized distribution of these proteins is oriented parallel to the short axis of the cochlea and perpendicular to the polarized distribution of core PCP proteins in hair cells which are oriented parallel to the long axis. As the SGN2 peripheral axons pass between cochlear supporting cells before turning, VANGL2 is strategically located at this

intercellular junction to act as an axon guidance cue. While these experiments are focused on developmental processes guiding cochlear innervation, core PCP proteins are broadly expressed throughout the developing nervous system and may direct other axon guidance events through similar mechanisms.

Disclosures: M.R. Deans: None. **S.R. Ghimire:** None.

Poster

367. Axon Growth and Guidance

Location: SDCC Halls B-H

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Topic: A.05. Axon and Dendrite Development

Support: the Korean government (MSIP) (2016R1A5A2945889)
a National Research Foundation of Korea (NRF)(NRF-2017R1A2B4001846)

Title: The E3 ligase SIAH regulates ubiquitination and degradation of Akt3

Authors: *B.-S. KIM[1,2], H. KO[1,2], J.-Y. AHN[1,2]
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Abstract: Besides its role in neuronal survival, Akt signaling is an important regulator of neural development including axonal growth and branching. However, the mechanism for coordination of Akt activity and the distinctive function of Akt isoforms in the brain development presents a challenge. Here we report the identification of SIAH1 as a novel E3 ubiquitin ligase that preferentially regulates ubiquitin-dependent degradation of the Akt3. SIAH1 most strongly interacts with Akt3 among Akt isoforms and facilitates ubiquitination and subsequent degradation of active Akt3. Notably, during differentiation of hippocampal neurons in culture, Akt3 is abundant in l axonal shaft but not branching point or tips where SIAH1 is prominently present. Depletion of SIAH1 enhanced Akt3 levels in the soma and axonal tips, promoting axon growth and multiple branching. These findings demonstrate that Siah serves as elective ubiquitin ligase that mediates ubiquitin-dependent degradation of Akt3 isoform and would regulate Akt3 turnover in the brain.

Disclosures: B. Kim: None. **H. Ko:** None. **J. Ahn:** None.

Poster

367. Axon Growth and Guidance

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Program #/Poster #: 367.11/B22

Topic: A.05. Axon and Dendrite Development

Support: 2018R1A2A1A05020292
2014M3A9B6034224

Title: Transcription factor c-Jun regulates perineuronal inflammatory activation in dorsal root ganglia following peripheral nerve injury

Authors: *Y. OH
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Abstract: Although regeneration capacity of CNS axons is very limited, peripheral nerve axons readily regenerate, allowing recovery of function after injury. As such, adult dorsal root ganglion (DRG) neurons are able to regenerate axons robustly after sciatic nerve injury (SNI) accompanied by upregulation of numerous regeneration-associated genes (RAGs). Using CCL2-deficient mice, we previously demonstrated that a chemokine molecule CCL2, expressed in DRG neurons following SNI, plays a critical role in the activation of perineuronal inflammatory reactions that lead to enhanced axon regeneration capacity. Intriguingly, c-Jun, one of the RAGs, was persistently activated in CCL2-deficient DRG neurons. This finding prompted us to hypothesize that c-Jun could act in an upstream pathway to induce perineuronal inflammatory reactions supporting the enhanced axon regeneration capacity. Luciferase assay showed that c-Jun overexpression markedly increased transcriptional activity of CCL2. Chromatin immunoprecipitation assay confirmed binding of c-Jun to the promoter region of CCL2 gene following SNI. To examine a functional role of c-Jun expressed in DRG neurons, we established a mouse line in which c-Jun is conditionally deleted only in neural lineage cells by crossing the c-Jun floxed line with the nestin-cre mice. Cultured DRGs neurons deficient of c-Jun could not enhance the extent of neurite outgrowth in response to SNI *in vivo* as robustly as wild type DRG neurons. Consistent with this finding, upregulation of RAGs such as ATF3 and GAP-43 following SNI was substantially attenuated in c-Jun deficient mice. Perineuronal activation of macrophages was significantly reduced in DRGs of c-Jun deficient mice compared to those of c-Jun floxed animals without cre-recombinase. These results suggest that c-Jun is necessary for the activation of perineuronal macrophages by regulating transcription of CCL2 following peripheral nerve injury.

Disclosures: Y. Oh: None.

Poster

367. Axon Growth and Guidance

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Program #/Poster #: 367.12/B23

Topic: A.05. Axon and Dendrite Development

Support: NIH-NICHD grant P01 HD083157

Title: Disruption of neural circuitry in the mouse model of pediatric dysphagia

Authors: *Z. MOTAHARI[1], A. S. POPRATILOFF[1,2,3], S. A. MOODY[1,2], A. S. LAMANTIA[1,2]
[1]Anat. and Cell Biol., George Washington Univ., Washington, DC; [2]Inst. for Neurosci., Washington, DC; [3]Nanofabrication and Imaging Ctr., Washington, DC

Abstract: Pediatric dysphagia, feeding and swallowing difficulty, is seen in 35% to 80% of children with neurodevelopmental disorders. Its consequences include malnutrition, acute choking, and food aspiration leading to nasal, middle ear, and lung infections. We defined the *LgDel* 22q11.2 Deletion Syndrome mouse as a dysphagia model. In *LgDel*, several cranial nerves essential for feeding and swallowing develop aberrantly. The trigeminal nerve (CnV), which provides sensory innervation to the face and oral cavity and motor innervation to muscles of mastication, is particularly compromised due to disrupted retinoic acid (RA)-mediated anterior-posterior hindbrain patterning. *LgDel* CnV sensory and motor neurons, as well as the neural crest that constitutes most of the cranial mesenchyme through which these axons grow, arise from RA "posteriorized" rhombomeres. This patterning change could render CnV neurons unable to extend
axons appropriately, due either to disrupted neuronal identity or altered extrinsic guidance. We labeled individual CnV axons via small tracer injections in living E11.5 embryos of several genotypes and prepared these embryos as whole mounts for confocal 3D imaging, which resolves compromised pathfinding of single axons that likely would be missed in histological sections. An initial assessment of fasciculation using dual fluorophore tracer injections did not show significant distinctions in *LgDel* versus WT axon segregation or mixing. However, *LgDel* CNV axons, despite reaching targets shared with WT, branch, loop, and sprout aberrantly at significantly higher frequencies than WT (p£0.01). The numbers of phenotypes/axon (p£0.01) and loops/axon (p£0.001) were significantly higher in *LgDel*. Consistent with our previous data, these *LgDel* CnV single axon phenotypes are rescued by diminishing RA signaling genetically. As predicted,
individual CnV axons*Tbx1*$^{+/-}$ embryos, a 22q11deleted gene responsible for Cn IX/X abnormality, exhibited a normal morphology as those observed in WT CnV. We are now analyzing other heterozygous and homozygous mutations in other single 22q11-deleted genes to assess their potential contributions to these CnV axon growth and guidance phenotypes.

Together, the axon trajectory phenotypes associated *LgDel* and other genotypes indicates 22q11 gene deletion disrupts axon guidance and alters construction of key sensory and motor circuits for feeding and swallowing in an RA-dependent manner, independent of *Tbx1*, perhaps contributing to perinatal dysphagia.

Disclosures: Z. Motahari: None. **A.S. Popratiloff:** None. **S.A. Moody:** None. **A.S. LaMantia:** None.

Poster

367. Axon Growth and Guidance

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 367.13/DP01/B24

Topic: A.05. Axon and Dendrite Development

Support: CIHR, MOP-258547
NIDA, R01DA037911

Title: Mapping neural function in DCC mutation carriers with and without mirror movements

Authors: *D. E. VOSBERG[1], V. BEAULÉ[4], A. TORRES-BERRÍO[5], D. COOKE[6], A. CHALUPA[1], N. JAWORSKA[7], S. M. L. COX[1], K. LARCHER[1], D. ALLARD[1], F. DURAND[1], R. LA PIANA[1], D. TAMPIERI[1], A. DAGHER[1], C. BENKELFAT[1], M. SROUR[1], R. JOOBER[1], F. LEPORE[8], G. ROULEAU[1], A. PASCUAL-LEONE[9], M. FOX[6], C. FLORES[2], H. THEORET[4], M. LEYTON[3]
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Abstract: *Objectives:* In laboratory animals, the axon guidance molecule receptor, DCC, directs the neural connectivity of corticospinal and commissural fibers, influencing the coordination of motor behaviors. Here, we investigated these potential effects in humans. Members of a large four-generational family were tested, two-thirds of whom carry a *DCC* mutation and exhibit mirror movements, involuntary contralateral responses that mirror voluntary unilateral actions. We hypothesized that the *DCC* mutation carriers with mirror movements would have the following features: an ipsilateral corticospinal tract, greater ipsilateral motor activations, reduced interhemispheric inhibition, and decreased *DCC* mRNA expression. *DCC* mutation carriers without mirror movements, it was predicted, would express fewer of these features.
Methods: The participants (n=52) were 13 *DCC* mutation carriers with mirror movements, 7

DCC mutation carriers without mirror movements, 13 relatives without the mutation or mirror movements, and 19 unrelated healthy volunteers. The multimodal approach comprised transcranial magnetic stimulation, task and resting state functional magnetic resonance imaging, diffusion tensor imaging, and quantitative real time polymerase chain reaction.
Results: DCC mutation carriers with mirror movements exhibited a functional ipsilateral corticospinal tract, diminished interhemispheric inhibition, greater ipsilateral motor responses, and reduced *DCC* mRNA expression. In comparison, both groups of *DCC* mutation carriers displayed lower resting state functional connectivity between the left and right primary motor cortex and reduced white matter integrity in the corpus callosum.
Conclusions: Diverse connectivity abnormalities were identified in mutation carriers with and without mirror movements, but corticospinal effects and decreased peripheral *DCC* mRNA were unique to those with the mirror movement phenotype.

Disclosures: D.E. Vosberg: None. **V. Beaulé:** None. **A. Torres-Berrío:** None. **D. Cooke:** None. **A. Chalupa:** None. **N. Jaworska:** None. **S.M.L. Cox:** None. **K. Larcher:** None. **D. Allard:** None. **F. Durand:** None. **R. La Piana:** None. **D. Tampieri:** None. **A. Dagher:** None. **C. Benkelfat:** None. **M. Srour:** None. **R. Joober:** None. **F. Lepore:** None. **G. Rouleau:** None. **A. Pascual-Leone:** None. **M. Fox:** None. **C. Flores:** None. **H. Theoret:** None. **M. Leyton:** None.

Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 368.01/B25

Topic: A.07. Developmental Disorders

Title: Antioxidants and autism: Teacher perceptions of behavior change

Authors: *A. SADEK[1], L. S. BERK[2], K. MAINESS[3], N. S. DAHER[2]
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Abstract: Children with Autism Spectrum Disorder (ASD) demonstrate a physiological imbalance between free radicals and antioxidants, also referred to as oxidative stress. Oxidative stress is linked to the pathogenesis of this neurocognitive disorder. Children with ASD tend to demonstrate maladaptive behaviors that hinder their participation in activities of daily living. The purpose of this pilot study was to examine the effects of consuming high antioxidant cacao on behavior of children with ASD as measured by teacher perceptions.
This was a 4-week pre-test post-test clinical trial (NCT 03195465). Participants with ASD consumed 4 squares of the dark chocolate twice per day which had a composition of 70% cacao

and 30% organic cane sugar and total antioxidant concentration of 8,320 mmol/100 grams. The two behavioral outcome measures, the *Aberrant Behavior Checklist- 2nd Edition* and the *Autism Spectrum Rating Scale*, were completed by the child's teacher at baseline and end of week four. Twelve participants (9 males, 3 females, mean age of 10.9 ±3.9 years) completed the study. Significant improvements were noted on the Autism Spectrum Rating Scales of Social/Communication (p=0.03, η^2=0.79), Unusual Behaviors (p=0.02, η^2=0.70), and Self-Regulation (p=0.04, η^2=0.59). No significant changes were noted on any of the Aberrant Behavior Checklist-2 subscales (p>.05). Results from this study support existing literature on the benefits of antioxidants in improving social communication, unusual behaviors, and self-regulation behaviors of children with ASD. Further randomized controlled trials are deemed necessary to expand on the validity of these findings.

Table 1 Median (min, max) Scores for ABC-2 and ASRS over time

Scale	Baseline	Four weeks	p value* (η^2)
ABC-2			
Irritability	5.0 (0,31)	6.0 (0,28)	0.27 (0.18)
Social Withdrawal	13.5 (1,33)	9.0 (0,32)	0.48 (0.13)
Stereotypic Behavior	2.5 (0,15)	3.0 (0,15)	0.61 (0.05)
Hyperactivity/Noncompliance	10.5 (1,36)	8.0 (0,43)	0.20 (0.13)
Inappropriate Speech	1.0 (0,9)	0.5 (0,9)	0.59 (0.04)
ASRS			
Social/Communication	63.0 (55,82)	58.5 (47,82)	0.03 (0.79)
Unusual Behaviors	66.5 (58,85)	60.5 (53,81)	0.02 (0. 70)
Self-Regulation	61.0 (51,80)	58.0 (43,83)	0.04 (0.59)
Total of all 3 Subscales T Score	69.5 (62,79)	59.0 (52,79)	0.007 (1.4)

Abbreviations: ABC-2, Aberrant Behavior Checklist 2nd Edition; ASRS, Autism Spectrum Rating Scale, η^2 = effect size
*Wilcoxon Signed Rank Test
Effect Size = $\frac{\text{mean of difference}}{\text{SD of the difference}}$

Disclosures: A. Sadek: None. **L.S. Berk:** None. **K. Mainess:** None. **N.S. Daher:** None.

Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 368.02/B26

Topic: A.07. Developmental Disorders

Support: Evelyn Gruss Lipper foundation
The Center for Absorption in Science, Ministry of Immigrant Absorption, State of Israel
the Rubenstein Family post-doctorate fellowship
the Lady Davis Foundation
Levi Eshkol fellowship of the Israel Ministry of Science

Title: Report of a parents survey of medical cannabis use in treatment autism

Authors: *G. M. LEWITUS, B. YELLIN, A. DAR, S. IMBERMAN, P. BERMAN, D. MEIRI
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Abstract: Autism spectrum disorder (ASD) is a group of neuro-developmental disabilities which are characterized by impairments in communication, social interaction, behavioral control, and emotional instability. In Israel, individual with ASD with sever aggressive behavior can get license for medical cannabis from specialized physicians. However, there is no treatment guidelines for the use of cannabis and caregiver use trial and error to find the right dosage and strains of cannabis that is most effective for them.
This naturalistic follow-up study follow 66 individuals with ASD who were legally prescribed medical cannabis in Israel. In-house caregiver-specific surveys were administered to track behavioral-specific treatment progress. The cannabinoid compositions of the cannabis used by the participants were analyzed.
We found that cannabis use reduced aggressive behavior and improved sleeping quality but had negligible effects on repetitive behaviors. Few participants reported exacerbations in abnormal behavior. Moreover, differences in cannabis treatment response depended on sex as cannabis treatments were reported to be more effective in male participants. In addition, the cannabis products that were used were not all equally effective in reducing abnormal behaviors. Purified cannabidiol (CBD) was the least effective in reducing aggressive behaviors compared to whole extracts from high-CBD Cannabis plants.
Our observations suggest that cannabis products produced from high-CBD Cannabis plants may be considered as a potential therapy for ASD individuals specifically to reduce aggressive behaviors.

Disclosures: G.M. Lewitus: None. **B. Yellin:** None. **A. Dar:** None. **S. Imberman:** None. **P. Berman:** None. **D. Meiri:** None.

Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 368.03/B27

Topic: A.07. Developmental Disorders

Support: CONICET, CONICYT/FONDECYT Regular (1170010)
FONDAP 15150012
INECO Foundation

Title: Social emotions in adults with autism spectrum disorder: A neuroanatomical study of envy and Schadenfreude

Authors: *S. FITTIPALDI[1], S. BÁEZ[2,3], L. SEDEÑO[2,4], A. M. GARCÍA[2,4,5], A. IBÁÑEZ[2,4,5,6,7]
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Abstract: Introduction. Autism Spectrum Disorder (ADS) is a neuropsychiatric condition characterized by severe deficits in emotional and social functioning, which is a critical component in their quality of life. Although the study of social cognition and its neural correlates in ASD has received considerable interest in recent years, few works have addressed a fundamental aspect of human interaction such as social emotions (i.e. those that are triggered by the presence or interaction with others). In this study, we aim to fill that gap by exploring how ASD patients experience envy and Schadenfreude (pleasure at others' misfortune), two social emotions that constitute counter-empathic responses and therefore can negatively impact interpersonal relationships. Methods. We administered a novel ecological paradigm to 15 adults with ASD and 15 healthy controls (HC) matched in age, gender, IQ and level of education. Subjects had to read short sentences describing different fortunate and unfortunate events that happened to different characters, and rate how much envy and pleasure they felt, respectively. We used SPSS to compare the performance between patients and HC in the task. Additionally, we obtained magnetic resonance imaging recordings from all subjects. Using MATLAB and SPM, we performed voxel-based morphometry to explore the neuroanatomical correlates of envy and Schadenfreude in ADS. Results. Behaviorally, ASD patients reported significantly lower levels of envy and Schadenfreude as compared to HC. VBM analysis revealed a positive

correlation between levels of Schadenfreude and gray matter volume of middle-frontal and parietal regions. Envy did not correlate with any structure. Discussion. This is the first study that explored the neuroanatomical correlates of envy and Schadenfreude in ASD. We found that patients with ASD had a difficulty in feeling those social emotions. Moreover, the experience of Schadenfreude was associated with fronto-parietal hubs, structures previously implicated in mentalizing processes and in ASD symptoms. The study of complex social emotions through ecological paradigms that resemble real-life situations is critical to better understand some of the core deficits of ASD. This findings may help the development of new assessment and intervention strategies to improve the patients' quality of life.

Disclosures: S. Báez: None. **L. Sedeño:** None. **A.M. García:** None. **A. Ibáñez:** None.

Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 368.04/B28

Topic: A.07. Developmental Disorders

Support: Conacyt Scholarship No. 576860 (RAV)

Title: Design and use of an ipad application to improvide reading and writing in children with autism

Authors: *R. AGUILAR, L. I. GARCIA, G. CORIA, R. TOLEDO, M. HERNANDEZ, J. MANZO
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Abstract: Autism is a neurobiological disorder that is mainly expressed during the first 3 postnatal years, and includes impairment in social communication and social interaction, as well as repetitive activity. Accordingly, it is important for patients diagnosed as autistic, to be exposed to different therapies or contexts that facilitate the development of social and communication skills. Herein, we show the effects of an iPad application we developed to help reading-writing skills of autistic kids. Accordingly, the application was used for 20-min twice a week during one year. The results indicated that there were positive effects in reading and writing in ten out of ten kids of different age observed in Xalapa, Veracruz, Mexico. All of them were capable of recognize their written name, and also wrote and read it. They also recognized, read and wrote the name of 90% of family members, and also words related to emotions, body parts, and activities (e.g. sleep, eat, sweep, etc.) These results allow us to conclude that this iPad application (LEA) can be used as a complement in therapy to enhance the development of reading-writing skills in autistic kids.

Conacyt Scholarship No. 576860 (RAV), Cuerpo Académico de Neurociencias (UV-CA-28) and Neuroquímica (UV-CA-304).

Disclosures: R. Aguilar: None. **L.I. Garcia:** None. **G. Coria:** None. **R. Toledo:** None. **M. Hernandez:** None. **J. Manzo:** None.

Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

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Program #/Poster #: 368.05/B29

Topic: A.07. Developmental Disorders

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AICTE Grant 11-25/RIFD/CAYT/POL-II/2013-14
University Grants Commission (20-29(12)/2012(BSR),
ICMR SRF (45/13/2014-Nan/BMS),

Title: Evaluation of naringenin & its surface modified nanocarriers as neurotherapeutic in autism spectrum disorders (asd)

Authors: *R. BHANDARI, A. KUHAD, 160014, J. PALIWAL, 160014
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Abstract: Aim of the study was to investigate pharmacotherapeutic potential of Naringenin and its brain targeted nanoformulations in Autism Spectrum Disorders (ASD). ASD like phenotype was induced by infusion of 1M Propanoic acid into anterior portion of lateral ventricle in rats. Naringenin (25, 50 and 100 mg/kg), uncoated and coated (glutathione & tween 80) naringenin loaded poly (lactic-co-glycolic acid) (PLGA) nanoparticles (25 mg/kg) and minocycline (50 mg/kg) were administered orally for 28 days. Neurobehavioural, biochemical, blood brain barrier permeability, TNF-α, MMP-9, HSP-70 and P-glycoprotein tests were performed. Naringenin and its nanoparticles significantly restored behavioural and biochemical deficits in ASD phenotype. Glutathione and tween 80 coated nanoparticles enhanced brain delivery of NGN by inhibition of P-glycoprotein. Naringenin (100 mg/kg) and its nanocarriers (25 mg/kg) demonstrated pharmacological efficacy comparable to minocycline (50 mg/kg). Naringenin and its coated nanocarriers have strong clinical potential as an adjunct neurotherapeutic moiety in attenuating neuropsychopathology associated with ASD. ***Keywords:*** Autism spectrum disorders (ASD), naringenin, neurobehavioural, naringenin loaded PLGA nanoparticles, glutathione (GSH), tween 80

Disclosures: R. Bhandari: None. **A. Kuhad:** None. **J. Paliwal:** None.

Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 368.06/B30

Topic: A.07. Developmental Disorders

Support: Cuerpos Académicos de Neurociencias (UV-CA-28) y Neuroquímica (UV-CA-304).

Title: Improvement of cardiorespiratory parameters following music stimulation in children with autism

Authors: L. NUÑEZ-ARCOS[1], I. FERNANDEZ-LECHUGA[1], P. CARRILLO[2], R. TOLEDO[2], *M. HERNANDEZ[3], J. MANZO[2]
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Abstract: Autism spectrum disorder is an alteration of neurodevelopment becoming visible in children about 2-3 years of age. Basic behavioral manifestations of the disorder are social isolation, language impairment, and motor problems. However, there are further manifestations related to autonomic dysfunctions modifying anxiety, stress, mood changes, and hyperactivity in response to sensory stimuli, that become manifested in cardiorespiratory alterations. On the other hand, it is known that music stimulation is an external variable with the potency to modify the cardiorespiratory function. A number of experiments have shown that Mozart's K448 Sonata is highly effective to trigger such benefit responses. Therefore, in this study we use this sonata for musical stimulation of 9 children with autism from 5 to 12 years of age. Auditory stimulation was delivered through headphones during 5 min, twice a week for a year, and with a volume at the lower threshold for tolerance of music of each child. Previous to music stimulation, an intelligent wristband was placed to the child (Bracelet 37º; 37 Degree Technology, Shanghai), that took cardiac and respiratory frequency and blood pressure during the stimulation period and send data via Bluetooth to an app in a cell phone. Data showed that cardiac frequency becomes reduced with music stimulation but return to altered values when the stimulus is not applied continuously (when the kid did not come to the test for holidays or other reason). High blood pressure was reduced to normal values even if the child was absent for some tests, but it was unchanged in children with normal blood pressure. The respiratory frequency did not show any modification. Thus, results show that music stimulation is effective in the long term to modify to normal values some cardiorespiratory parameters, what could be a response of physiological changes that deserve to be studied in further studies.

Disclosures: L. Nuñez-Arcos: None. **I. Fernandez-Lechuga:** None. **P. Carrillo:** None. **R. Toledo:** None. **M. Hernandez:** None. **J. Manzo:** None.

Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 368.07/B31

Topic: A.07. Developmental Disorders

Support: Cuerpo Académico de Neurociencias (UV-CA-28)

Title: Increased skin sensibility induced by different sensory stimuli in kids with autism

Authors: A. FERNANDEZ-LECHUGA[1], L. NUÑEZ-ARCOS[1], P. CARRILLO[2], L. I. GARCIA[2], G. A. CORIA-AVILA[2], *J. MANZO[2]
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Abstract: Autism spectrum disorder is an alteration of neurodevelopment becoming visible in children about 2-3 years of age. Basic behavioral manifestations of the disorder are social isolation, language impairment, and motor problems. However, there are further manifestations related to sensory perception that is now also considered as a diagnosis criterion. Tactile stimulation is almost unknown, notwithstanding it is known that an appropriate perception is critical for the activities in everyday life. Thus, with the aim to modify alterations in tactile sensitivity of autistic kids, in this work we applied as therapy two sensory stimuli to 6 kids from 5 to 12 years old. The used stimuli were bubble paper and a skin massager twice a week for a year. A carpet of 2x2 m of bubble paper was placed on the floor and tests consisted in each kid walking in socks for 2 min trying to burst the bubbles. A handheld massager (HoMedics) was used to stimulate the skin with a 2 level of intensity for 2 min on the arm and face. Following stimuli, the sensitivity threshold was obtained by using Von Frey Fibers on the same areas, as kids kept their eyes closed indicating perception by pointing with a finger the stimulated area. Results showed that as therapy was applied consecutively every kid was able to perceive smaller fibers. Thus, here we showed that an enriched sensory stimulation of autistic children modifies cutaneous sensitivity, suggesting an improved perception of the general environment.

Disclosures: A. Fernandez-Lechuga: None. **L. Nuñez-Arcos:** None. **P. Carrillo:** None. **L.I. Garcia:** None. **G.A. Coria-Avila:** None. **J. Manzo:** None.

Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

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Topic: A.07. Developmental Disorders

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The Weston Havens Foundation
Stanford's Child Health Research Institute
The Katherine D. McCormick Fund
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Title: Cerebrospinal fluid vasopressin, diagnostic classification, and symptom severity in children with autism

Authors: *O. OZTAN[1], J. P. GARNER[1], S. PARTAP[1], E. H. SHERR[2], A. Y. HARDAN[1], C. A. FARMER[3], A. THURM[3], S. E. SWEDO[3], K. J. PARKER[1]
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Abstract: Background
Autism is a brain disorder characterized by core social deficits and the presence of restricted, repetitive behaviors. Progress in understanding and treating autism has been hindered by the extraordinary difficulty in obtaining brain-relevant tissues [e.g., cerebrospinal fluid (CSF)] from children by which to study disease biology more directly. Here we bridge this critical gap in knowledge by testing whether CSF concentrations of the "social" neuropeptides arginine vasopressin (AVP) and oxytocin (OXT) differentiate cases and controls and are associated with symptom severity in the largest pediatric cohort studied for this purpose to date.
Methods and Findings
Children with autism were diagnosed based on clinical criteria which was confirmed with research diagnostic methods. All participants (N=72: 48 males, 24 females, aged 1.5 to 9 years, matched 1:1 on sex and age) underwent lumbar puncture; CSF neuropeptide concentrations were quantified using established enzyme immunoassays. CSF AVP concentration was significantly lower in the autism compared to control group ($F_{1,66}$=14.20; P=0.0004). CSF AVP concentration also significantly differentiated individual cases and controls (Likelihood Ratio Chi-Square=15.14; P<0.0001). Specifically, across the range of observed CSF AVP concentrations, the likelihood of autism increased over 1000-fold, corresponding to nearly a 500-fold increase in risk with each 10-fold decrease in CSF AVP concentration. Lower CSF AVP concentration also

predicted greater overall symptom severity ($F_{1,27}$=4.878; P=0.0359) on the Autism Diagnostic Observation Schedule Calibrated Severity Score (ADOS-CSS). Further analyses revealed that this was driven by social ($F_{1,27}$=7.708; P=0.0099), but not repetitive ($F_{1,27}$=0.0274; P=0.8698), symptoms on the ADOS Social Affect-CSS and Restricted and Repetitive Behaviors-CSS, respectively. Finally,
these findings were more pronounced in males than in females with autism, and were specific to AVP, as no evidence implicating the structurally related neuropeptide OXT was found.

Conclusions

These findings highlight the value of performing CSF-focused biomarker research in individuals with autism. As prior preclinical research has shown that AVP (rather than OXT) preferentially regulates social behavior in male mammals, these data also suggest that brain-related AVP signaling deficits may be particularly relevant to better understanding the male-biased risk for, and symptom presentation in, autism. Finally, although preliminary, these findings suggest that AVP may be a promising CSF indicator of, and potential therapeutic agent for, autism's social deficits.

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Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 368.09/C1

Topic: A.07. Developmental Disorders

Support: CONACyT´s scholarship

Title: Effect of different types of distractors over selective attention in children diagnosed with autism spectrum disorders

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Abstract: Autism Spectrum Disorder (ASD) is usually detected in the first years after birth and is maintained throughout life. Children diagnosed with ASD present social interaction difficulties, communication deficits, stereotyped and repetitive behaviors, difficulties processing and recognizing social stimuli, and sensory alterations. These abnormalities in processing sensory stimuli are present in 87% of the cases. Apparently, these deficits are not related to alterations in primary sensory processing, but rather to the systems involved in relevant stimuli selection and inhibition processes associated to interference control. It has been proposed that

selective attention is one of the main cognitive alterations in ASD. Two alternatives have been proposed about the type of attention alterations that patients with ASD may present: First, it has been proposed that children with ASD over focus their attention on specific stimuli or traits; on the other hand, other authors have proposed that they have a wide attentional beam that allows them to perceive stimuli far from the attention focus. The objective of the present study was to evaluate the effect of social and nonsocial distractors over detection of visual stimuli. We also assessed performance on an Attentional Network Task (ANT).
For this, we evaluated 15 children diagnosed with ASD (ages 6-12) and compared their performance with that of children with normal development. Groups were paired by IQ and school grade.
Participants performed two tasks: a task of detecting visual stimuli in which they were presented with distraction stimuli in the periphery of the focus of attention (social and non-social), and the Attentional Network Task (ANT). Results showed that children with ASD diagnosis have higher reaction times on conditions when social distractors appear, compared to when they are presented with nonsocial distractors, also, error percentage was significantly higher in ASD group compared to control group. In addition, we found significative differences in ANT performance; both groups had higher reaction time rates when incongruent distractors were presented, however, the ASD group showed significantly lower correct answers percentage than the control group. No group was benefited by spatial or temporal cues. We conclude that social stimuli do interfere with selective attention processes of children with ASD diagnosis. Results on the ANT could mean poor functioning on executive control attention network on ASD diagnosed children.

Disclosures: S. Meneses-Ortega: None.

Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 368.10/C2

Topic: A.07. Developmental Disorders

Title: The effect of difference of autism-spectrum quotient scores on role adaptation in interpersonal motor coordination

Authors: *K. MUKAI[1], A. MIURA[1], K. KUDO[2], S. TSUTSUI[3]
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Abstract: It has been suggested that the difference in social skills between two persons affects role adaptation (leading or following role) in rhythmic interpersonal motor coordination. We investigated this by using interpersonal motor coordination task that required participants to

perform a rhythmic movement featuring an interpersonal relative phase pattern 90° without verbal communication. They were required to decide two roles during the task and continued the task until they were able to create the relative phase of 90° stably. Social skill was assessed by the Autism-spectrum Quotient (AQ), and then we computed the ratio of AQ scores between the members of each pair. Pairs whose social skills differed greater (pairs with smaller AQ ratio) finished the task early, and the role was adapted early. On the other hand, pairs whose social skills were similar (pairs with greater AQ ratio) finished the task late, and the role was switched more often than in the early pairs. We examined the effect of the difference of the AQ ratio on the performance of the task (i.e., the number of trials until the pairs finished the task) by mediation analysis. The results showed that pairs with a smaller AQ ratio difference in social skills had a smaller number of role switching, and that the pairs with a smaller number of role switching were able to finish the interpersonal motor coordination task earlier. A bias-corrected bootstrap confidence interval that was calculated for the indirect effect using 10,000 bootstrap samples lay entirely above zero, which provides evidence of an indirect effect of the AQ ratio on the number of trials through the mediator (i.e., number of role switching). The direct effect of the AQ ratio on the number of trials was not significant. We confirmed that the difference in social skills between two persons affected the performance of interpersonal motor coordination task in terms of role adaption.

Disclosures: A. Miura: None. **K. Kudo:** None. **S. Tsutsui:** None.

Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 368.11/C3

Topic: A.07. Developmental Disorders

Support: University of Louisville 21st Century Initiative

Title: Autism diagnosis using rewards experiments task-based functional-mri

Authors: *M. ALI[1], O. DEKHIL[2], A. SHALABY[2], R. KEYNTON[2], M. GHAZAL[2], A. EL BAZ[2], G. N. BARNES[3]
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Abstract: Introduction: Autism Spectrum Disorder (ASD) is a neuro-developmental disorder. The main goal of using the task-based functional MRI in ASD is to localize task-evoked blood oxygen level-dependent (BOLD) effects in the brain and subsequently, to analyze those effects in order to find anatomical areas of activation related to that specific task. In this work, we adopt the monetary reward experiment for monitoring the activation areas in the brains of both ASD

and control subjects in response to a specific task. In this task, physicians asked the participants to press a button to classify dichotomous categories of images. These images were displayed to them during the experiment, and subsequently, a monetary feedback was provided. **Materials and Methods:** We used 20 subjects and 3T MRI was used to acquire this data. We have covered the whole cerebral volume through 180 functional images which lasted for 6 minutes. 5 seconds were the duration for each trial, where the subject was exposed to the images for approximately 2 seconds. The feedback, then, was displayed for 1.25 sec, and finally a rest period of approximately 1.75 sec occurs between trials. We applied preprocessing algorithms on each volumes. GLM was used to model The BOLD activation change during the feedback display, then we conducted (using SPM package) a one regressor describing the feedback event timing and a first group analysis. We have analyzed the brain's Brodmann areas (BAs) to reach a diagnosis decision on local areas, to move to the idea of personalized medicine. Every brain voxel is then mapped to certain BA. We have used Autoencoder-based classifier (AE) for space reduction and learning the most significant features. We extracted features from every BA and fed them to the AE. A local decision per area is made which indicates the significance of this BA. Finally, we fused all local decisions to have a final diagnosis. **Experimental Results:** We have locally analyzed every BA of all the 94 BAs and have fused them to obtain a global diagnosis decision. We achieved global accuracy of 90%. In addition to the global diagnosis, visualized local analysis is provided. We will show multipe different cases of ASD, where BAs of the cortices of subjects are color-coded according to the strength of association of each area with ASD. The two maps are different, which emphasizes the idea of personalized medicine that is the ultimate goal of our group. **Conclusion:** A novel CAD system for autism diagnosis by adopting functional task-based fMRI information is proposed. Our system provided local analysis for brain areas, which can allocate subjects on the autism spectrum and help to deliver personalized treatments to individuals with autism.

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Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 368.12/C4

Topic: A.07. Developmental Disorders

Support: JSPS KAKENHI [25861030]

Title: Cortical surface architecture endophenotype and correlates of clinical diagnosis of autism spectrum disorder

Authors: *Y. AOKI[1], B. YAMAGATA[2], T. ITAHASHI[1], J. FUJINO[1], H. OHTA[1], O. TAKASHIO[3], M. NAKAMURA[1], N. KATO[1], M. MIMURA[2], R.-I. HASHIMOTO[1,4]
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Abstract: Identifying endophenotype and neural correlates for a diagnosis of autism spectrum disorder (ASD) is particularly important because of its complex genetic influence. Focusing on the cortical volume and three surface-based parameters, cortical thickness (CT), fractal dimension (FD), and sulcal depth (SD), we aimed to identify a pattern of ASD endophenotype and neural correlates for the clinical diagnosis. Enrolling 30 people with ASD endophenotype (15 people with ASD and 15 of their unaffected siblings) and 30 people without (15 pairs of TD siblings), sparse logistic regression with a leave-one-pair-out cross-validation showed high accuracy for identification of the ASD endophenotype (73.3%), which is more evident in SD compared with other parameters. Focusing on SD, a bootstrapping analysis accounting for a difference in SD between typical siblings showed substantially large difference between individuals with ASD and their unaffected siblings in seven out of 34 regions-of-interest. These regions includeinclude left bank of superior temporal sulcus (<0.01 percentile), right pars triangularis, banks superior temporal sulcus, medial orbitofrontal cortex, precentral gyrus (<0.01 percentile). Mean SD values in three of these regions were correlated with ASD severity. Intriguingly, both endophenotype and clinical diagnosis emphasize the social brain but they did not completely match. These findings suggest that ASD endophenotype emerges as SD and the neural correlates for the clinical diagnosis can be dissociable from the endophenotype.

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Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 368.14/C6

Topic: A.07. Developmental Disorders

Support: R01 MH106518-01A1

Title: Adolescents and young adult with autism spectrum disorder show specific impairments in cognition

Authors: *R. M. GEDDERT[1], M. K. KRUG[1], V. P. REINHARDT[1], J. P. HOGEVEEN[1], E. FERRER[2], M. SOLOMON[1]

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Abstract: Adolescents with autism spectrum disorder (ASD) exhibit impairments in cognitive control (Solomon et al., 2008; Solomon et al., 2009; Solomon et al., 2017). To further investigate the development of these deficits from adolescence to young adulthood, we recruited 88 participants with typical development (TYP) and 88 age and gender matched individuals (age 12-22) with ASD, who were assessed using gold standard ASD diagnostic measures including the Autism Diagnostic Observation Schedule (ADOS-2). Participants completed the NIH Toolbox Cognition Battery (NTCB). The NTCB is a well validated measure of cognition, consisting of measures of cognitive flexibility (Dimensional Change Card Sorting; DCCS), inhibitory control (Flanker), episodic memory (Picture Sequence Memory; PSM), list-sorting working memory (LSWM), processing speed (Pattern Comparison Processing Speed; PCPS), and language skills (Vocabulary; PV; Oral Reading Test; ORT; Akshoomoff et al., 2014). More generally, Flanker, DCCS, PSM, LSWM and PCPS are considered measures of fluid intelligence, while PV and ORT are measures of crystallized intelligence. The ASD group performed significantly worse on the Flanker (F = 38.17, p < .001), DCCS (F =37.27, p < .001), PCPS (F = 30.91, p < .001), PSM (F = 8.04, p = .005) and ORT (F = 5.121, p = .025) tasks when covarying full scale IQ. The groups were not significantly different in LSWM or PV. Stepwise discriminant function analysis was used to examine the combination of NTCB variables that best predicted diagnosis. The overall chi-square test was significant (Wilks lambda = 0.636, Chi-Square = 69.139, df = 4, Canonical Correlation = 0.603, p < .001). The function extracted consisted of DCCS, PCPS, Flanker and PSM, and accounted for 37% of the variance in each group's cognitive performance on the NTCB. These results indicate that the cognitive profile of individuals with ASD is characterized by weaknesses in measures of fluid reasoning, specifically impairments in flexibility, processing speed, inhibition, and episodic memory while crystallized abilities are largely intact. Lastly, we explored how age relates to performance and group differences. Though Flanker, PV and ORT performance was positively correlated with increasing age, diagnosis did not significantly impact growth on any measure. Collection of longitudinal data in the future will allow for a more robust investigation of the developmental trajectories of cognition in ASD. Future analyses will also examine how these relate to clinical symptoms, functional outcomes and the connectivity of neural networks.

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Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

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Program #/Poster #: 368.15/C7

Topic: A.07. Developmental Disorders

Support: NSERC Postdoctoral Fellowship (PDF)

Title: Exploring the relationship between prosodic control and social skills in children with and without ASD

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Abstract: The ability to communicate thoughts, ideas, and emotions is integral to social development. We know this in part because of conditions such as autism spectrum disorder (ASD), where early social communicative deficits (e.g., joint attention) are predictive of later language deficits. As children develop, speech becomes the dominant form of social communication, as it allows complex ideas and emotions to be conveyed between individuals. Social aspects of speech production, such as prosody (e.g., vocal pitch), must be carefully regulated to accurately express information about the emotionality, excitability, and intent of the speaker. Thus, a child may acquire fluent, even excellent speech articulation, but their social communication may be compromised due to poor prosodic control. The objective of the current study is to gain a better understanding of how auditory information is used to regulate the prosodic aspects of speech in children and adults with and without ASD. Furthermore, this research aims to explore the relationship between the prosodic control of speech, or more specifically the control of vocal pitch, and social communication abilities. In this study participants with and without ASD produced vocalizations while being exposed to frequency altered auditory feedback. The size, timing, and variability of participants' responses to the frequency altered feedback were measured. The multidimensional social competence scale was also used to assess social communication abilities. Results indicate that both participants with and without ASD produced compensatory vocal responses to the auditory feedback manipulation. Specifically, vocal responses in the altered auditory feedback condition were significantly larger than vocal responses in the unaltered auditory feedback condition. Vocal response magnitudes were also found to differ across participants with and without ASD. These results represent a key step in the understanding of how atypicalities in the mechanisms supporting speech production may manifest in social-communication deficits, as well as broader social competence, and vice versa. Thus, these findings will establish important benchmarks for researchers studying auditory processing, speech development, and speech perception and production, while also uncovering the relations between these processes and social communication outcomes. This research has the potential to facilitate early detection of speech deficits and communication disorders that interfere with social adaptation across the lifespan.

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Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

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Title: Communicative misalignment in autism spectrum disorder

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Abstract: Autism spectrum disorder (ASD) is diagnosed on the basis of communicative impairments observed in everyday social interactions. While individuals with ASD show remarkable proficiency on lab tests of social cognition, face-to-face interaction proves problematic and has been associated with biases in processing biological and multimodal linguistic cues. Here, we provide empirical evidence characterizing a specific cognitive challenge raised by interpersonal communication in people with ASD, which persists even in environments stripped of those biases and in the presence of neurotypical social motivation and cognitive flexibility. During online, experimentally-controlled interactions, both adults with ASD (N=22) and neurotypical adults (N=30) spontaneously generated novel and intelligible non-verbal communicative behaviors toward their interaction partners. Furthermore, both groups showed a similar propensity for modifying their behavior when misunderstandings arose. However, communicative success was lower when individuals with ASD interacted with other individuals both on and off the spectrum. This impairment was proportional to the misalignment between the meanings of the communicative behaviors used by pair members across successive interactions. Individuals on the spectrum struggled to converge on single shared behaviors with their interaction partners, particularly when resolving the referential ambiguity of a behavior required interpersonal coordination. These findings illustrate the importance of considering human communication as a solution to a conceptual alignment challenge, and how ineffective the evolutionarily anomalous human communicative system is without this special interactional ingredient.

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Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 368.17/C9

Topic: A.07. Developmental Disorders

Title: Gesture processing and production in autism spectrum disorder

Authors: *E. FOURIE[1,2], E. R. PALSER[1,6,7], J. J. POKORNY[1], M. NEFF[3,4], S. M. RIVERA[1,2,5]
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Abstract: Individuals with autism spectrum disorder (ASD) show impairments in nonverbal communication, including diminished gesturing and difficulties with imitation, which may be explained by a deficit in processing biological motion (BM). This study examined whether this BM deficit is reflected in atypical neural activity when observing gestures and investigated the relationship between activation during gesture observation and quality of gesture production in a group of children and adolescents with ASD and typically developing (TD) controls, ages 9 to 15. During the fMRI task, participants (19 ASD, 17 TD) viewed animations (created by applying digitalized motion capture of actions performed by an actor to a 3D human model) of both functional pantomimes (e.g. driving) and communicative gestures (e.g. waving), which were computationally manipulated to vary in the amount of movement, from subtle to exaggerated. Gesture production was assessed using a charade-style paradigm, in which participants enacted a series of 15 familiar actions (e.g. brush teeth), which were double-coded by blind raters for quality of specific components (e.g. limb movement, hand posture). In whole brain analyses, both groups showed activation of the motion-sensitive area MT+. The TD group showed an additional cluster of activity in left premotor cortex. In both groups, communicative gestures elicited greater activity than functional ones, suggesting higher processing demand for actions with communicative intent. In a 3-way ANOVA (movement x type x group) of peak values extracted from left and right MT+ for each condition, there was a main effect of movement ($p < .01$), such that the most overt gestures elicited greater activity than midlevel, but not subtle gestures, indicating increased processing for both the most subtle and overt gestures. Left MT+ also showed a movement x type x group interaction ($p < .01$), where activation differed based on movement for communicative gestures in the TD group and for functional gestures in the ASD group. Compared with TD individuals, MT+ in ASD appears to be less sensitive to changes in

movement intensity of communicative gestures.In a subset of participants who completed both fMRI and behavioral sessions (8 ASD, 11 TD), we performed correlations between MT+ activity and gesture performance. Activity in the L MT+ was positively correlated with performance score in the TD group ($r = .65$, $p < .05$) but not in the ASD group ($r = .08$, $p = .86$). These results suggest that in typical development, increased neural processing of gestures is associated with better gesture production, and that dysfunction in the BM system in ASD may disrupt development of gesturing abilities.

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Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

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Topic: A.07. Developmental Disorders

Support: Internally funded by MIT Lincoln Laboratory

Title: Quantifying fine motor dependencies in autism spectrum disorder

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Abstract: Autism Spectrum Disorder (ASD) is a prevalent neurodevelopmental disorder that often results in the co-occurrence of impairment of fine sensorimotor systems, as for example in comorbid deficits in manual-motor skills and speech production (Mody et al., 2017). Yet there is little quantitative understanding of these complex dependencies. An essential hypothesis in our work is that estimation of coordination across sensorimotor impairments, through advanced correlation and modeling methods, can improve objectivity and accuracy of detecting and tracking the ASD state. Toward our objective of a quantitative understanding of fine-motor dependencies, we designed and pilot-tested a non-intrusive platform capturing audio, video, and hand movement, with protocols involving vocal and facial expression plus hand writing, drawing, and tracing. While administering the speech and face protocol through an iPad display via Apple Airplay by the clinician, high-quality audio and video are recorded with an acoustic microphone and a HD camera. For hand movement, displacement, velocity and pressure are measured through our custom-designed, web-based iPad application. The speech protocol consists of a phonetically-balanced short paragraph, a diadochokinetic sequence, sustained vowels, and response to questions. The face protocol involves mimicking facial expression with

and without emotion labels, while the hand dexterity component involves writing word descriptions of objects, drawing geometric shapes, and tracing templates. MIT and MGH IRB approvals were obtained and data collection of 10 controls and 10 ASD subjects is planned for mid-May. Subjects are 6-12 years of age, verbal, and able to read the simple paragraph in the speech protocol. Speech measures include phoneme- and articulatory-based features reflecting fine neuromotor timing and coordination, while facial measures reflect neuromotor coordination across muscle groups, effective in detecting various neurological conditions (Quatieri et al., 2017). Temporal aspects of hand motion and pressure, also reflecting timing and coordination, are extracted. Correlation analysis of these features across the three modalities, and their use in detecting ASD, will be presented at the conference.

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Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

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Program #/Poster #: 368.19/C11

Topic: A.07. Developmental Disorders

Support: The American Psychological Foundation Elizabeth Munsterberg Koppitz Child Psychology Graduate Student Fellowship

Title: Similarities in limbic system connectivity at resting state in autism and social anxiety disorder

Authors: *A. KIRKPATRICK[1], L. ANTEZANA[2], C. BROWN[2], N. LY[2], M. MAURIN[2], J. VOYACK[2], J. RICHEY[2], M. COFFMAN[2]
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Abstract: Background: The limbic system (LS) is associated with the regulation of fear (Anad et. al 2005). Brain connectivity within the LS at rest is hypothesized to correlate with anxiety (Mucci et. al, 2018) and hyperconnectivity with subcortical regions has characterized social anxiety disorder (SAD; Arnold et. al, 2014). However, no studies have examined connectivity transdiagnostically in individuals with comorbid Autism Spectrum Disorder (ASD+SAD), and it is not known whether individuals with ASD+SAD show similar patterns of connectivity in the LS compared to those without ASD. **Objectives:** To elucidate heterogeneous patterns of LS connectivity in SAD across adolescents with varying degrees of social impairment. Specifically, we used a graph theory approach, Group Iterative Multiple Model Estimation (GIMME) with community detection on fMRI resting state data to examine whether LS connectivity can

robustly differentiate SAD, ASD+SAD, and controls (CON). Secondary analyses examine the relationship between the LS and measures of social anxiety. **Hypotheses**: We predict that individuals with ASD+SAD will share similar neural profiles within the LS, but that these profiles will be separate from SAD alone and from CON participants. It is also hypothesized that scores on measures of social anxiety will be distinct among diagnostic classifications. **Methods:** 20 children with each ASD+SAD, SAD only, and CON adolescents participated in this study (N=60). Participants were matched on age (M=15.19) and FSIQ (M=108.38). Diagnoses were confirmed with semi-structured clinical interviews. fMRI data were collected on a 3T TIMTrio. Functional regions of the LS were identified via Neurosynth and included: bilateral amygdala, thalamus, anterior cingulate cortex, and bilateral orbitalfrontal cortex. GIMME was used with resting state data from each subject. Subsequently, a community detection algorithm was used to identify subgroups characterized by LS connectivity patterns (Gates et al., 2014). Social anxiety was measured via self-report with the Leibowitz Social Anxiety Scale-Child Adolescent (LSAS-CA). **Results:** Analyses in progress define subgroups based on LS functioning. Additional analyses will reveal relationships between social anxiety and LS connectivity. Preliminary results on the LSAS-CA suggest significant group differences in social anxiety (p >0.001), such that CON had less anxiety than ASD (p = 0.030) and SAD (p *>* 0.001). **Conclusions:** The results will highlight the similarities in network activity in adolescents with anxiety and comorbid ASD. Going forward these findings have an influence on treatment of SAD and SAD+ASD.

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Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

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Topic: A.07. Developmental Disorders

Support: UW Interdisciplinary Competition Award
Eunice Kennedy Shriver National Institute of Child Health and Human Development P30 HD003352 and U54 HD090256

Title: Detecting visual-motor challenges in autism using a robotically guided drawing task

Authors: *A. H. MASON[1], M. ZINN[2], G. SUBRAMANI[2], A. FISHER[3], S. JACQUOT[3], B. TRAVERS[4]
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Abstract: Background: Children with autism spectrum disorder (ASD) struggle with motor impairments, and these motor impairments have been found to predict difficulties with independent living skills and the severity of core social communication impairments. Motor skill therapy may offer an effective but under-utilized approach to improve skills of children with ASD. However, individuals with ASD have a unique motor learning style that necessitates consistency in presentation, which is difficult for a human therapist to enact with fidelity. To address this, we aim to develop a robotically-guided motor skill intervention paradigm for the treatment of children with ASD. As the first step, we set out to determine whether the robot could accurately detect performance differences between children with ASD and children with typical development (TD). **Objective**: Use a robotically guided drawing task to examine drawing and motor performance under varying visual-motor conditions in children with ASD compared to children with TD. **Methods**: 20 children with ASD and 20 children with TD (10-12 years old) completed the robotically guided drawing tasks, in which they used the robotic arm to draw shapes on a computer screen mounted below a glass table. Participants drew 4 different shapes (circle, square, star, and hexagon) under 3 visual-motor conditions (tracing the shape, copying the shape from a model presented on a horizontal screen, and copying the shape from a model presented on a vertical screen). Kinematic data from the hand, arm, and torso were collected, and participants completed standardized motor and autism assessments. **Results**: Hierarchical linear modeling (using the nested structure of the data) examined the root mean squared error of the drawn shape compared to the model as a function of the condition and diagnostic group. A significant interaction between condition and group emerged (p = .02). Groups performed almost identically in the tracing condition but differed in the horizontal and vertical copying conditions. **Conclusions**: The robotically-guided drawing task was able to detect motor performance differences in children with ASD compared to children with TD, particularly when the to-be-copied shape was out of the line of vision during drawing. This finding is consistent with previous findings of atypical visual motor integration in ASD and indicates that many children with ASD may benefit from an intervention aimed at enhancing visual motor integration during motor tasks. Future analyses will examine the kinematic data and predictors of individual differences that may inform personalization of the robotically guided motor intervention.

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Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

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Title: Distinct patterns of impaired contextual learning in autism spectrum disorders

Authors: *B. TRAVERS[1], A. SUNKARA[4], B. KIM[5], T.-Y. CHANG[2], H. JIANG[2], O. SURGENT[3], K. SABEL[3], S. JACQUOT[3], A. DINGES[3], A. ROSENBERG[2]
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Abstract: Recent theoretical work suggests that certain behaviors displayed by individuals with an autism spectrum disorder (ASD) reflect a diminished ability to use contextual information to interpret current sensory information. Here we tested this hypothesis experimentally by evaluating contextual learning in ASD and typically developing (TD) adolescent peers by manipulating context across multiple sessions. Participants viewed a computer screen divided into four quadrants, and were asked to search for a visual target (the letter "C") amongst letters "I" and "F", and to report the quadrant in which the target was located using a button box. A total of 9 sessions of ~300 trials/session were completed by each participant. Unbeknownst to the participants, contextual information about the target location was manipulated across sessions by co-varying the number of I's in a quadrant and the probability of the target being present in that quadrant. In the first three and last three sessions, the number of letter I's in a quadrant was informative (context present), whereas in the middle three sessions, there was no contextual relationship between the I's and target location (no context). Search time as a function of the proportion of informative cues in the target quadrant was used as a measure of contextual learning. We found that the performance of TD participants modulated strongly and quickly with the contextual changes. In contrast, the ASD participants showed impaired contextual learning and greater heterogeneity in their learning profiles. Cluster analysis of the search time data revealed two distinct groups of ASD learning profiles. The first group resembled the TD learning profile in the magnitude of contextual learning, but when the context was turned off, they showed a one session delay in disengaging from the context learned in the first three sessions. The search times of the second ASD group did not modulate with context, indicating that they did not learn the embedded context. The performance of this group could instead be explained by a greater subjective saliency of low-level visual features of the search field. These results provide experimental evidence for at least two distinct contextual learning profiles within the autism spectrum, and have practical implications for the development of individualized treatments and educational approaches to maximize behavioral therapy gains.

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Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 368.22/C14

Topic: A.07. Developmental Disorders

Support: NIH 1R01HD079432-01A1

Title: Classification of autism spectrum disorder improves with addition of motor and sensory components to the research domain criteria (RDoC) framework

Authors: *L. A. HARRISON, E. KILROY, C. BUTERA, C. ZEISLER, L. AZIZ-ZADEH
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Abstract: The National Institute of Health's Research Domain Criteria (RDoC) is a multi-level research framework for investigating mental disorders. While not diagnostic, it aims to describe where individuals fall on a spectrum of health in terms of biological and psychological dysfunction in five domains: (1) negative and (2) positive valence systems, (3) cognitive systems, (4) social processes, and (5) arousal and regulatory systems. Notably, the RDoC framework is currently lacking a motor functioning domain, despite growing evidence of the role motor functioning has been observed to play in various disorders, including autism spectrum disorder (ASD) and schizophrenia. To test the utility of adding a motor and/or sensory domains to the RDoC framework in order to characterize individuals with ASD, we attempted to use machine learning to classify individuals with ASD according to various psychological measures that (1) map onto current RDoC domains (positive and negative affect from the PANAS; anxiety severity from the CASI-Anx; ADHD symptomatology from the Conners; identification, communication, and externalizing of affect from the Alexithymia scale for children; empathy from the IRI; affect recognition and theory of mind from the NEPSY; and arousal from the PH-C, then (2) onto indices of both motor (coordination from the DCDQ; postural praxis from the SIPT) and sensory functioning (for different sensory categories from the SenSor); then (3) using both traditional RDoC and sensorimotor measures combined. In a group of 35 children (16 diagnosed with ASD), a support vector machine-based decoder using current RDoC domains classified children with ASD versus neurotypical controls with 85.7% classification accuracy. Motor measures alone were largely successful, with 74.3% classification accuracy. The addition of sensory processing to the motor measure improved classification accuracy to 85.7%, supporting the inclusion of sensory processing measures as well as motor measures. RDoC classification improved with sensory measures added (88.6% accuracy) and even more with motor measures added (91.4% accuracy). The combined use of all three - current RDoC, motor, and sensory measures - matched the accuracy of RDoC plus motor measures (91.4% accuracy). This suggests that all components work together to capture the heterogeneity of functioning in

our ASD sample. These results support the utility of adding both motor and sensory domains to the RDoC framework for characterizing ASD.

Disclosures: L.A. Harrison: None. **E. Kilroy:** None. **C. Butera:** None. **C. Zeisler:** None. **L. Aziz-Zadeh:** None.

Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 368.23/C15

Topic: A.07. Developmental Disorders

Support: NIH / NIMH R01 MH08532812

Title: Monkey see monkey do: Decreased spontaneous entrainment to rhythmical movement in children with autism

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Abstract: During social interactions, typically developing children (TDC) and adults spontaneously entrain their movement rhythm to others and such synchronization facilitates prosociality and bonding. To explore these links in autism spectrum disorders (ASD) –a neurodevelopmental disorder marked by social interaction and motor imitation deficits– we examined motor entrainment to external visual rhythms in the absence of any instruction to do so.
Hypothesis 1: Children with ASD will show less motor entrainment than TDC.
Hypothesis 2: Among children with ASD lesser motor entrainment will correlate with autism severity in Autism Diagnostic Observation Scale.
Preliminary data includes thirteen 8-12 year-olds (9 ASD, 4 TDC) matched on IQ. In the study, children moved their arms in a certain way to "collect food for the monkey in the video" while the monkey character moved in time to 3 prefixed speeds: 0.75Hz, 1Hz, 1.25Hz in three 90 sec rounds (counterbalanced). To ensure all children could move to these speeds a final round was played in which children purposely tried to synchronize with the monkey. We recorded children's movements using Microsoft Kinect Xbox interface, obtained xyz coordinates using iPi Motion Capture software and calculated periodograms on MATLAB. To analyze entrainment levels, we assessed the difference between the children's movement frequency and the stimulus frequency.
No group effect on peak frequencies was found in the elicited round; hence all children could

perform the speeds presented. In line with Hypothesis 1, frequency difference scores showed less entrainment to the stimuli in children with ASD than in TDC (Fig 1, $p < .0001$). Analyses of mean power at +/-10% of stimulus frequency further supported Hypothesis 1 revealing that TDC moved more consistently and distinctly at the stimulus frequency than children with ASD ($p < .0001$). As per Hypothesis 2 higher autism severity was correlated with lesser entrainment ($r = -.69$, $t(7) = -2.53$, $p = .04$). Overall, our findings support spontaneous motor entrainment to visual rhythms as a biomarker for diagnosis and treatment of ASD.

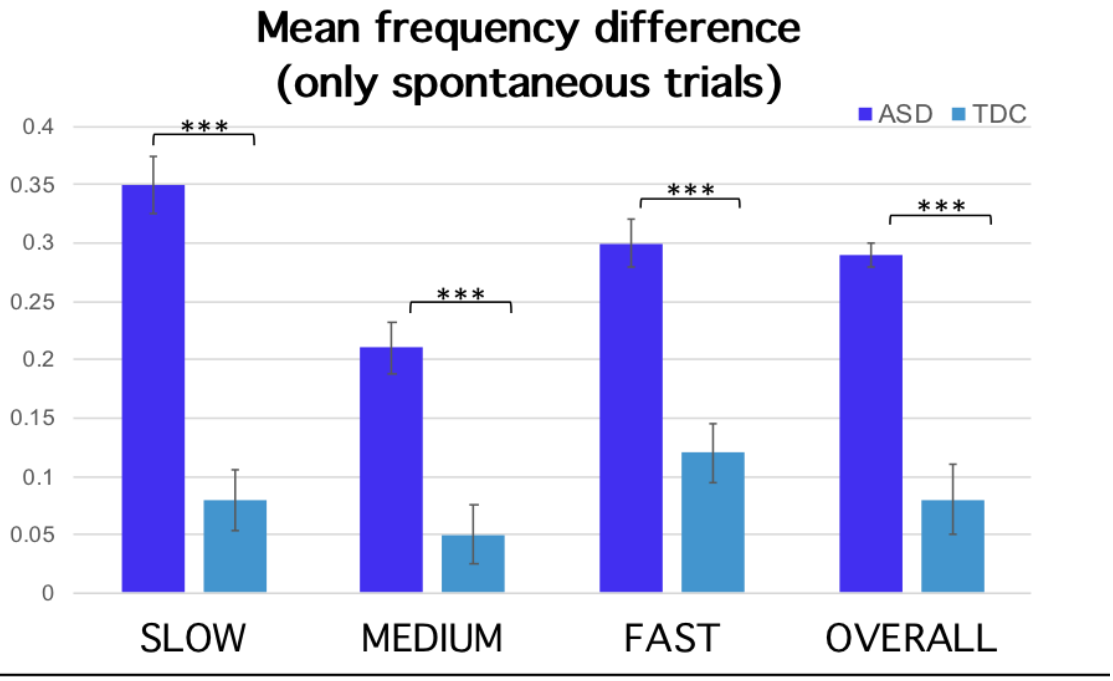

Fig 1. Mean frequency difference scores for ASD and TDC, with lower values indicating better entrainment with the stimulus.

Disclosures: B. Tuncgenc: None. **S.H. Mostofsky:** None.

Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

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Program #/Poster #: 368.24/C16

Topic: A.07. Developmental Disorders

Support: NIH Silvio O. Conte Grant 1P50MH100023

National Primate Research Center Base Grant #RR-00165, Animal Resource Program at NIH

Title: Acoustic properties of early life ultrasonic vocalizations are altered in the valproic acid rat model of autism

Authors: *M. L. MCNAIR[1], T. M. HENNESSEY[2], C. E. BARRETT[4], H. WALUM[3], D. RAINNIE[3]
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Abstract: Background: Early vocalizations and infant voice quality have been a research focus for early detection of autism spectrum disorder (ASD). Studies have examined social and behavioral impairments in animal models of ASD, though few have assessed parallel communication deficits or analyzed acoustic patterns of ultrasonic vocalizations (USVs) in rat models of ASD.
Objectives: This study investigated potential developmental differences in early life vocalizations between valproic acid (VPA)-exposed and control rat pups. We hypothesized that VPA-exposed pups would exhibit developmental delays in USV communication, emitting fewer USVs and expressing stunted acoustic development that mirrors the pre-speech delays seen in high risk, pre-ASD children.
Methods: Dams (VPA n=22, control n=20) received oral gavage of 500 mg/kg VPA or saline on embryonic days 11, 12, and 13. Five-minute recordings of VPA-exposed (n=54–74) and control (n=51– 75) pups in isolation were collected on postnatal days (P) 7, P11, and P14 using Sonotrack USV recording equipment. We examined USV number, duration, frequency, and structure at each postnatal day, emphasizing a comprehensive examination of acoustic property development within the VPA ASD model.
Results: VPA pups, compared to controls, called less on P7 (t_{150}=4.31, p<0.001) and P11 (t_{139}=2.88, p<0.005) but showed no difference in number of calls on P14 (t_{103}=0.56, p>0.05). VPA pups emitted more high frequency USVs. A three-way interaction was found between treatment, age, and frequency bin ($F_{8, 3230}$=6.0, p<0.001). Post hoc ANOVAs showed an interaction between treatment and frequency bin at P7 ($F_{8,1177}$=7.18, p<0.001), P11 ($F_{8,1112}$=13.08, p<0.001), and P14 ($F_{8,824}$=4.89, p<0.001). VPA pups showed a differential distribution of USV structure at P7 ($F_{1,35}$=864.81, p<0.05). Post hoc analysis revealed an interaction between treatment and call type in males for Short ($t_{10.41}$=2.35, p<0.05), Flat ($t_{16.47}$=2.78, p<0.05), Up ($t_{10.99}$=2.19, p<0.05), and Down structures ($t_{10.63}$=3.95, p<0.01), but only Short structures ($t_{8.41}$=2.93, p<0.05) in females. While prenatal VPA exposure did not alter USV duration, the pattern of duration changed from P7 to P11 to P14 for both VPA and controls.
Conclusions: Prenatal exposure to VPA alters early life vocalizations and may alter communication development in rat pups. These USV abnormalities may decrease functionality, differentially impact maternal behavior, and serve as a potential predictive factor of future ASD-like symptoms. Our study emphasizes a more complex analysis of USVs and supports using early life vocalizations as a potential form of early detection in both models and humans.

Disclosures: M.L. McNair: None. **T.M. Hennessey:** None. **C.E. Barrett:** None. **H. Walum:** None. **D. Rainnie:** None.

Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 368.25/C17

Topic: A.07. Developmental Disorders

Support: NIH Grant P50MH096891
Carver Chair

Title: Age- and sex-specificity of deficits in the Pcdh10 mouse model relevant to autism

Authors: *S. L. FERRI[1,2], H. DOW[3], E. S. BRODKIN[3], T. ABEL[1,2]
[1]Dept. of Mol. Physiol. and Biophysics, [2]Iowa Neurosci. Inst., Univ. of Iowa, Iowa City, IA; [3]Univ. of PA Sch. of Med., Philadelphia, PA

Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that affects nearly five times as many males as females. The protocadherin 10 gene (*PCDH10*) has been linked to ASD and is highly expressed in the amygdala and striatum. It encodes for an activity-dependent cell adhesion molecule involved in dendritic spine development. We previously reported that juvenile male mice (28-32 d) heterozygous for a deletion of *Pcdh10* ($Pcdh10^{+/-}$) exhibit deficits in a social approach task. Recently, we found that these deficits are ameliorated in adults (60-90 d), a phenomenon of social improvement also observed in individuals with autism. However, the cognitive deficits we have recently observed, in both contextual and cued fear conditioning, are present before and after puberty. These behavioral impairments, as well as amygdalar abnormalities we previously reported, including decreased expression of NMDAR and increased spine density, are observed in males only. Future studies will address the mechanisms involved in the age- and sex-specificity of the social and cognitive deficits.

Disclosures: S.L. Ferri: None. **H. Dow:** None. **E.S. Brodkin:** None. **T. Abel:** None.

Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 368.26/C18

Topic: A.07. Developmental Disorders

Support: NSERC
CIHR
Jonathan and Joshua Memorial Scholarship

Title: Sensorimotor gating and social behavior in adolescent ratsexposed to poly i:c maternal immune activation at mid-gestation

Authors: *F. HADDAD[1], L. LU[2], C. DE OLIVEIRA[2], S. SCHMID[2]
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Abstract: Maternal infection during pregnancy is associated with an increased risk of neurodevelopmental disorders such as autism spectrum disorder (ASD) and schizophrenia in the offspring. Interestingly, the association of multiple infections with similar disorders points to a common underlying mechanism for infection's effects on brain development. Results from rodent research have shown that a pathogen-free immune response during pregnancy is sufficient to cause symptoms linked to ASD and schizophrenia in the offspring, and that behavioral changes mainly manifest in adulthood. We used polyinosinic:polycytidylic acid (poly I:C) maternal immune activation (MIA) at Gestation Days (GD) 9.5 or 14.5 to more specifically describe the sensorimotor gating phenotype of the poly I:C model and investigate whether this phenotype is associated with aberrant social behavior in Sprague Dawley rats during adolescence and adulthood. Sensorimotor processes were measured through open field locomotor activity in addition to habituation and multimodal (auditory and visual) prepulse inhibition (PPI) of the acoustic startle response, while social novelty preference was measured in the three-chamber test. Results from MIA-exposed offspring show GD and sex-specific effects of MIA on startle reactivity, visual PPI and locomotor activity but no impairment in habituation of startle and social novelty preference. These results extend on past MIA studies, particularly in the specific characterization of PPI deficits across different stimuli.

Disclosures: F. Haddad: None. **L. Lu:** None. **C. De Oliveira:** None. **S. Schmid:** None.

Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 368.27/C19

Topic: A.07. Developmental Disorders

Title: The kynurenine pathway: A link between prenatal inflammation and disruption of offspring behavior in mice

Authors: *D. S. COELHO, A. TRAN, J. O'CONNOR
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Abstract: Infection and inflammation during pregnancy are risk factors for neurodevelopmental disorders such as autism. In rodent models, maternal immune activation (MIA) causes phenotypes in offspring that resemble many features of this human disorder, but the pathogenic mechanisms remain unclear. Inflammatory cytokines can upregulate the tryptophan metabolizing enzyme indoleamine 2,3-dioxygenase (IDO) which increases kynurenine metabolite levels, and experimentally increasing kynurenine levels of pregnant dams causes developmental consequences in the offspring. Therefore, we hypothesized that MIA-induced upregulation of IDO during gestation is a pathogenic mechanism by which fetal neurodevelopment is disrupted and autism-like phenotypes develop. Pregnant female IDO-/- mice or control C57BL/6J mice were administered 20 mg/kg polyinosinic:polycytidylic acid (PIC) or saline i.p. on gestational day 12.5. At post-natal day 7, isolation-induced ultrasonic vocalizations were recorded from pups after a brief separation from their dams. WT-MIA offspring exhibit decreased the number of vocalizations when compared to WT-Saline pups. IDO -/- animals did not exhibit a PIC-induced decrease in vocalization. Preliminary analysis of sound spectrograms shows that WT-PIC offspring display differences in the vocalization repertoire when compared to WT-saline pups characterized by fewer downward syllables and increased two-syllables. Repetitive/stereotypic behavior was also assessed, and IDO-/- offspring of PIC treated dams exhibited less repetitive grooming than WT pups of WT-PIC dams. Our data suggest that IDO plays a role in the development of communication deficits and repetitive behavior in the MIA model.

Disclosures: D.S. Coelho: None. **A. Tran:** None. **J. O'Connor:** None.

Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 368.28/C20

Topic: A.07. Developmental Disorders

Support: SFARI

Title: Deficits in learning are common across multiple mouse models of autism

Authors: *J. F. LYNCH, III[1], T. ABEL[1], T. NICKL-JOCKSCHAT[1], N. M. GRISSOM[2], S. E. MCKEE[3], H. SCHOCH[5], L. WALSH[4], T. M. REYES[6]
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California Irvine, Irvine, CA; [6]Psychiatry and Behavioral Neurosci., Univ. of Cincinnati, Cincinnati, OH

Abstract: Autism Spectrum Disorders (ASDs) are a set of neurodevelopmental disorders that cover a wide range of symptoms. The overall prevalence of ASDs continues to increase and is 4 times more common in males than females. Given the role of striatal and cortical deficits in ASDs and the large discrepancy in prevalence rates, the current experiments will assess different genetic mouse models in distinct learning and memory paradigms. The mouse models may include 16p11.2 microdeletion animals, which is the most common genetic link with ASD, CNTNAP2 knockout animals, and Shank3b knockout animals. All models will be trained in several distinct behavioral paradigms including operant conditioning, fear conditioning, social activity, and novel object recognition. Preliminary data suggests deficits in mutant mice in reward learning procedures and potentially reduced motivation as measured by a progressive ratio task. Overall, these preliminary findings suggest impaired goal-directed learning in males of 3 different ASD-linked genotype mouse models. Continuing work will demonstrate the depth and breadth of the deficits seen in mutants across behavioral procedures and tasks. Determining the mechanisms for these dysfunctions and understanding the sex-specific nature of these deficits are promising avenues for developing specific, targeted therapeutics for ASDs and other neurodevelopmental disorders.

Disclosures: J.F. Lynch: None. **T. Abel:** None. **T. Nickl-Jockschat:** None. **N.M. Grissom:** None. **S.E. McKee:** None. **H. Schoch:** None. **L. Walsh:** None. **T.M. Reyes:** None.

Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 368.29/C21

Topic: A.07. Developmental Disorders

Support: NIH Grant HD084209

Title: Fluoxetine alleviates effects of stress in pregnant mice but increases repetitive behaviors and reduces hippocampal synaptic plasticity in adult male and female offspring

Authors: *A. L. ARZUAGA[1], M. E. RAGOZZINO[2,3], J. LARSON[3,4]
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Abstract: Autism spectrum disorder (ASD) is characterized by social and communication impairments as well as restricted and repetitive behaviors (RRBs). RRBs can be exhibited as stereotyped motor behaviors or reduced behavioral flexibility. 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 in C57BL/6 (B6) mice examined whether restraint stress and/or treatment with selective serotonin reuptake inhibitor (SSRI) in pregnant females affects self-grooming behavior, behavioral flexibility, and/or hippocampal synaptic plasticity in male and female offspring. Pregnant female mice were subjected to chronic restraint stress from gestational days 4-18 (three daily 30-minute sessions) and/or administered fluoxetine (0.3mg/kg/day) on days 8-18. Offspring were tested as young adults (7 weeks of age) on self-grooming behavior and learning to inhibit a prepotent response in a spatial discrimination task. Offspring were also tested for alterations in long-term potentiation (LTP) induced by theta burst stimulation (TBS) of Schaffer-commissural synapses in hippocampal slices. The results indicate that restraint stress reduces preference for sucrose reward and suppresses weight gain in pregnant dams; these effects were reversed by fluoxetine. However, combined maternal stress and prenatal SSRI exposure increased self-grooming in both male and female offspring. These offspring also exhibited impaired behavioral flexibility in a spatial discrimination test. LTP in hippocampal field CA1 was reduced in offspring of dams subjected to restraint stress, SSRI exposure, or both treatments, relative to offspring of non-stressed, vehicle-treated dams. The findings suggest that restraint stress, which may model depression, combined with SSRI treatment during pregnancy increases RRBs and reduces hippocampal synaptic plasticity in adult offspring. Understanding the mechanisms by which maternal stress and SSRI exposure during pregnancy affect brain development in offspring is critical for better comprehension of ASD pathophysiology and may lead to the development of more effective treatments.

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

Poster

368. Behavioral Analyses of Autism in Humans and Rodent Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 368.30/C22

Topic: A.07. Developmental Disorders

Title: Effect of gluten on social behavior in rats

Authors: *P. MICHENER[1], S. N. MYERS[2], R. A. RICHARDSON[2], O. M. FAHR[2], I. M. NORTH[3], M. L. FEIGEN[2], T. R. SCHACHTMAN[2]
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Abstract: Previous research has shown that a gluten-free diet can have an effect on symptom reduction in children with autism spectrum disorder (ASD) (Knivsberg, Reichelt, Høien, & Nødland, 2003; Pennesi & Klein, 2012). Pennesi and Klein (2012) found that strict adherence to

a gluten-free diet for children with ASD led to an increase in social behaviors and a decrease in unusual, stereotyped behaviors compared to children who did not strictly adhere to the diet. In addition, the longer that the diet was implemented, the greater were the change in symptoms. Knivsberg et al. (2003) also found that after a 1-year gluten-free diet intervention, children with ASD on a gluten-free diet showed significant improvement in social behavior and unusual behaviors over a control group. These improvements included improved communication and social interaction and decreased isolation and stereotypic behaviors. The present experiment therefore was an exploratory experiment designed to examine social behaviors in male Sprague-Dawley rats that were on either a gluten-free (n = 2) or a high-gluten (n = 4) diet for 7 weeks. The rats were approximately 3 months old at the time of testing. The rats were placed into a plastic apparatus divided into two halves, with one half containing a clear plastic container with a nontarget rat underneath. Each target rat experienced a 3 min trial, and time spent in both the empty half and the half of the apparatus with the nontarget rat were recorded, as well as approach behaviors to the nontarget rat. The results showed that the high-gluten diet rats approached the nontarget rat significantly more times than the gluten-free rats. There were no differences between time spent in each side of the apparatus for the two groups; however, there was a slight tendency for gluten-free rats to spend more time on the nontarget side of the apparatus. These findings are inconsistent with the literature on children with ASD and gluten-free diets and the effects on social behavior. However, there were a total of 6 rats used in the present study, and the rats used were not a model of ASD. More data collection is anticipated.

Disclosures: P. Michener: None. **S.N. Myers:** None. **R.A. Richardson:** None. **O.M. Fahr:** None. **I.M. North:** None. **M.L. Feigen:** None. **T.R. Schachtman:** None.

Poster

369. Mechanisms of Developmental Disorders: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 369.01/C23

Topic: A.07. Developmental Disorders

Support: R01DA034097
UNM-HSC Research Allocation Committee (RAC)

Title: HuD regulates the biogenesis of synaptic plasticity and development-related circular RNAs

Authors: *M. DELL'ORCO, N. I. PERRONE-BIZZOZERO
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Abstract: The neuronal RNA-binding protein (RBP) HuD is involved in multiple steps of neuronal differentiation from neurogenesis to axonal and dendritic development and remodeling.

Post-transcriptional regulation of genes involved in neuronal development and plasticity is particularly relevant in neurons. RBPs not only bind to mRNAs but also interact with circular RNAs (circRNAs), a class of non-coding RNAs generated by pre-mRNA back-splicing. Due to their high expression in synaptic terminals, circRNAs are likely to be involved in neuroplasticity. Different RBPs control circRNAs production (e.g., MBL, QKI, ADAR1) or bind to circRNAs (e.g., FMRP, EIF4AIII, HuR). This study was aimed at exploring whether HuD regulation of circRNAs levels may be linked to synaptic plasticity and neuronal development. HuD controls target RNA's fate by binding to Adenylate-Uridylate Rich Elements (ARE). Using bioinformatics analyses, we searched for ARE sequences in all mouse circRNAs and found consensus HuD binding motifs in about 29%. By RNA immunoprecipitation (RIP) followed by circRNA arrays, we identified over 600 circRNAs bound by HuD. Pathways analysis showed an enrichment of HuD targeted circRNAs in genes regulating synapse density and central nervous system development (15.8%), neurites growth (14.3%), congenital brain malformations and dendrites morphogenesis (10.5%). To test whether HuD interactions with circRNAs could lead to changes in their levels, we analyzed differentially expressed circRNAs in HuD overexpressor (HuD_{OE}) and HuD KO (*Elavl4* $^{-/-}$) mice compared to wild type (WT) mice. Cross-correlations analyses of circRNAs altered in HuD_{OE} and *Elavl4* $^{-/-}$ revealed that HuD selectively regulates circRNAs deriving from neurogenesis and plasticity-related genes within the same biological pathways identified in the HuD RIP. These include: *Brwd1* and *Foxp1*, associated with neurodevelopment disorders and autism; *Ntrk3* neurotrophic receptor kinase; *Map1a* involved in neuronal development and regeneration; *Dock10* regulating dendritic spines morphogenesis; *Magi1* and *Lppr4*, members of the Plasticity Related Genes (PRGs) and membrane-associated guanylate kinases (MAGUKs) families respectively, controlling synaptic development and linked to psychiatric disorders. Interestingly, the linear counterparts of some of these circRNAs, such as *Brwd1*, *Foxp1* and *Ntrk3*, are listed in the ARE gene database (ARED) and likely targeted by HuD. Collectively these data suggest that HuD interactions with circRNAs regulates their biogenesis, and that the ensuing changes in HuD-targeted circRNAs and mRNAs may be related to altered neurogenesis and neuroplasticity.

Disclosures: M. Dell'Orco: None. **N.I. Perrone-Bizzozero:** None.

Poster

369. Mechanisms of Developmental Disorders: Animal Models

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Program #/Poster #: 369.02/C24

Topic: A.07. Developmental Disorders

Support: R01DA034097
UNM-HSC Research Allocation Committee (RAC)

Title: RNA binding proteins and circular RNAs are linked to nucleus accumbens gene expression changes during environmental enrichment following prolonged abstinence from cocaine self-administration

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Abstract: Aberrant neuroadaptative changes during the establishment of drug addiction are thought to involve alterations in post-transcriptional mechanisms controlling neuroplasticity in specific neural circuits. Multiple RNA binding proteins (RPBs), including neuronal HuD, have been previously associated with substance use disorders (SUD). Not only are HuD and many of its targets listed in the Knowledgebase of Addiction related genes (KARG) database, but HuD levels are also altered after cocaine exposure. Previous work by our group has shown that increased cocaine seeking after prolonged abstinence following cocaine-self administration is attenuated by environmental enrichment (EE); and that these changes in behavior are linked to changes in the expression of specific genes. RNA-seq analysis of gene expression in the nucleus accumbens (NAc) of EE animals compared to an isolated group, after 21 days of cocaine abstinence, demonstrated that differentially expressed genes are enriched in pathways involved in nervous system development; particularly synaptic transmission, neuronal differentiation, and axonogenesis. In this study, we aimed to explore whether HuD interaction with circular RNAs (circRNAs) could regulate gene expression during EE-induced neuroplasticity. CircRNAs are not only likely involved in synaptic plasticity and neurodevelopment, but also contain numerous consensus regulatory sequences for RBPs and microRNAs. Bioinformatics analyses from the same RNA-seq data identified several circRNAs that were linked to EE. Concurrently, analysis of circRNAs bound by HuD (see Dell'Orco et al, associated poster) showed that about 18% were derived from genes in KARG. Furthermore, using circRNA array data from HuD knock-out (KO) versus wild type mice, we were able to confirm that HuD also altered target circRNA expression. The set of genes that were targets of HuD or differentially expressed in HuD KOs were then compared with genes whose expression correlated with lever presses in the EE group. Specifically, we found that 87 of the circRNAs that were significantly downregulated in HuD KO mice and 49 of the bound circRNAs were associated with the EE gene set. These common genes belong to the same biological pathways that regulate synaptic plasticity and neuronal development. These analyses allowed us to generate a list of possible circRNAs targeted by HuD whose levels are altered during neuroadaptive processes induced by EE. Uncovering HuD regulation of circRNAs levels linked to the effect of EE on cocaine seeking can potentially reveal therapeutic targets to reduce drug craving and relapse.

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Poster

369. Mechanisms of Developmental Disorders: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 369.03/C25

Topic: A.07. Developmental Disorders

Title: Chronic prenatal interleukin-17 is sufficient to cause sex-specific ASD-phenotypes in a mouse model

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Abstract: Background: Studies in both humans and animal models have implicated interleukin-17 (IL-17) as a key pro-inflammatory cytokine in multiple psychiatric disorders and neurodevelopmental processes, respectively. In humans, both increased activity of Th17 lymphocytes and elevated blood levels of IL-17A have been linked to ASD and its severity. In animals, studies have implicated IL-17A signaling as being necessary for the offspring effects of viral infection during pregnancy, including behavioral phenotypes related to ASD. Offspring effects of chronic prenatal IL-17 exposure, however, have not been evaluated.
Method: C57BL/6 dams were chronically administered IL-17 (25ng/hr) via subcutaneous osmotic minipump (Alzet) throughout pregnancy. Some offspring tissues were collected at embryonic day 18 (E18), and some in adulthood. RNA-sequencing (Illumina HiSeq 4000) was performed on sexed, neocortical tissues across two lanes (approx. depth/lane: 15000). Gene-wise data analyses were conducted using Kallisto (used sequence based bias correction) and Sleuth. E18 and adult tissues were also analyzed for morphology via unbiased stereology and optical fractionator (StereoInvestigator). Behavior was assessed [three-chamber social task, open field, elevated plus maze, prepulse inhibition (Anymaze, SDI Lab)] in adult offspring.
Results: RNA-seq results demonstrated that there were significant, sex-specific changes to E18 neocortical gene expression with IL-17 exposure across four litters per group (320 genes were differentially expressed in males and none in females; n=4,4 per group, per sex). These altered transcripts were significantly enriched for ASD-related genes (37 matched ASD-related genes from the Simons Foundation Autism Research Initiative genes database). IL-17 male and female offspring had lower body weight at E18, as did males as adults. E18 males had smaller neocortical volume at E18 and as adults (total cell and neuronal density were unchanged). Additionally, IL-17 offspring exhibited male-specific social approach deficits and reduced anxiety-like behavior, though locomotor and sensorimotor gating behaviors were unaltered.
Conclusions: We report here that chronic IL-17 exposure during gestation was sufficient to result in ASD-like phenotypes in male offspring, including altered embryonic and adult neocortical size, embryonic neocortical gene expression signatures, and adult behavior. This echoes the male

bias and some altered cortical development and behavioral findings in the human ASD literature, suggesting that chronic prenatal IL-17 may serve as a robust ASD model and be a factor in altered neurodevelopment.

Disclosures: S.B. Gumusoglu: None. **A.S. Chilukuri:** None. **S. Haider:** None. **B.W. Hing:** None. **H.E. Stevens:** None.

Poster

369. Mechanisms of Developmental Disorders: Animal Models

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Topic: A.07. Developmental Disorders

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Title: Investigation of Foxp2 function in layer 6 corticothalamic neurons

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Abstract: Humans rely on language to convey information and form social bonds, but the neurobiology underlying this ability remains largely uncharacterized. One of few genes known to play a role in speech and language is *FOXP2*, which encodes a transcription factor expressed in the brain from development into adulthood. In the cortex, a brain region essential for language and cognition, layer 6 corticothalamic neurons comprise the major *Foxp2*-expressing cell type, yet few studies have investigated its function in these cells. Thus, we aimed to identify Foxp2-driven gene expression networks and neuronal functions in layer 6 corticothalamic neurons. Using single-cell RNA-seq of these neurons in mice lacking cortical *Foxp2*, we identified gene expression alterations related to neuromodulatory signaling. Furthermore, while mice lacking cortical *Foxp2* show normal cortical morphology and emit normal vocalizations throughout their lifespan, they display behaviors suggesting cognitive inflexibility. Altogether our data show that Foxp2 may promote gene expression programs in corticothalamic neurons that underlie cognitive functions beyond innate vocalization abilities. Understanding the cortical role of *Foxp2* will provide insights into language circuitry and the etiology of language-disrupting disorders of cognition.

Disclosures: M. Co: None. **A. Kulkarni:** None. **N. Usui:** None. **M. Harper:** None. **G. Konopka:** None.

Poster

369. Mechanisms of Developmental Disorders: Animal Models

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Program #/Poster #: 369.05/C27

Topic: A.07. Developmental Disorders

Support: ARC DE160100620

Title: USP9X missense variants associated with neurodevelopmental disorders disrupt signalling pathways critical to brain development

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Abstract: Variants in the X-linked gene USP9X have been associated with intellectual disability (ID) in both males and females. Nineteen mutations causing haploinsufficiency of USP9X in females with ID, congenital malformations and recognisable brain abnormalities have been reported. In males, only three missense mutations associated with ID had been reported, and another two associated with seizures, and as such the involvement of USP9X in male ID remained less certain. Here we report an additional 12 likely pathogenic USP9X missense variants in male ID cases, and an additional 25 variants of unknown significance for which in-silico prediction and allelic frequency data align well with pathogenicity. We describe an expanded phenotypic spectrum associated with USP9X missense mutations in males in which speech delay, hypotonia, seizures, autistic behaviour, aggressiveness and visual impairment were frequent. We also resolve a severe learning and memory deficit in our USP9X knockout mouse highlighting its utility to understand mechanisms of patient pathology. As a deubiquitylating enzyme, USP9X protects its substrates from proteasomal degradation. We previously reported altered levels of multiple USP9X substrates which regulate neurodevelopmental signalling pathways in embryonic brains of USP9X knockout mice. Furthermore, we show these pathways including mTOR, WNT, NOTCH and TGFβ are disrupted. We now identify several key substrates including SMURF1 and RAPTOR are also depleted in a panel of patient-derived fibroblast cells lines, with associated disruption to their respective signalling pathways TGFβ and mTOR. Collectively, our data demonstrate the involvement of USP9X in male ID and other

neurodevelopmental disorders, and identify mechanisms of pathogenesis centred on disrupted signalling pathways critical to normal brain development.

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Poster

369. Mechanisms of Developmental Disorders: Animal Models

Location: SDCC Halls B-H

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Title: Chd2 is necessary for neural circuit development and long-term memory

Authors: *Y. KIM[1], S. KHOSHKHOO[3], S. ABBASI[4], J. C. FRANKOWSKI[2], S. LEE[6], B. ZHU[5], Y. E. WU[7], R. F. HUNT[5]
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Abstract: Considerable evidence suggests loss of function mutations in the chromatin remodeler, *CHD2*, contribute to a broad spectrum of human neurodevelopmental disorders. However, it is unknown how *CHD2* mutations lead to impaired brain function. Here we report mice with heterozygous mutations in *Chd2* exhibit deficits in neuronal proliferation and a shift in neuronal excitability that included divergent changes in excitatory and inhibitory synaptic function. Further *in vivo* experiments show $Chd2^{+/-}$ mice displayed aberrant cortical rhythmogenesis and severe deficits in long-term memory, consistent with phenotypes observed in humans. We identified broad, age-dependent transcriptional changes in $Chd2^{+/-}$ mice, including alterations in neurogenesis, synaptic transmission and disease-related genes. We replicated our results in a second, independent cohort of mice with *Chd2* haploinsufficiency only in inhibitory interneurons and are now evaluating interneuron-based rescue experiments designed specifically for $Chd2^{+/-}$. Our results demonstrate a critical role for *Chd2* in neurodevelopment and provide initial insight into how *Chd2* haploinsufficiency leads to aberrant cortical network function and impaired memory.

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Poster

369. Mechanisms of Developmental Disorders: Animal Models

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Program #/Poster #: 369.07/C29

Topic: A.07. Developmental Disorders

Support: MH112763
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Title: Late stage embryonic disruption of Dnmt3a activity in pyramidal neurons alters glutamatergic transmission in frontal cortex

Authors: *A. PINTO-DUARTE[1], J. LI[2], C.-Y. LAI[1], C. LUO[4], J. LUCERO[1], T. J. SEJNOWSKI[1], E. A. MUKAMEL[2], S. B. POWELL[3], J. R. ECKER[4,5], M. M. BEHRENS[1]
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Abstract: DNA methylation is critical for regulating gene expression and establishing cell-type specificity. It occurs mainly in a cytosine-guanine (CG)-dinucleotide context, but brain tissue also contains a substantial amount of non-CG methylation (mCH). The accumulation of mCH depends on Dnmt3a, a *de novo* methyltransferase, whose levels peak at a time of intense synaptogenesis and neuronal maturation - the second postnatal week in mice. The dysregulation of Dnmt3a activity might, therefore, play an important role in the pathophysiology of neurodevelopmental disorders, such as autism or schizophrenia.
Previously, using a pyramidal-cell specific *Dnmt3a* knockout mouse, in which the conditional deletion occurs during the late embryonic stage (around embryonic day E15), we showed that the disruption of Dnmt3a activity caused the differential expression of nearly 1,000 genes in the medial prefrontal cortex (mPFC), a significant fraction of which were synapse or morphology related. Consistently, these mice presented sustained neurodevelopmental disease-related phenotypes, such as impaired working memory and sociability.
Our current results suggest two levels at which neural physiology might be compromised by the disruption of *Dnmt3a* expression. Firstly, we found that *Dnmt3a*-deficient pyramidal neurons in the mPFC were significantly hypoexcitable. Secondly, we observed a tendency for a greater variability in the amplitude of miniature excitatory postsynaptic currents onto those neurons, indicating possible impairments in the postsynaptic element.
In light of these findings, the detailed morphological and physiological analysis of the synapses,

which is currently underway, will further help understanding how Dnmt3a dysfunction might be involved in the molecular underpinnings of neurodevelopmental disorders.

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Poster

369. Mechanisms of Developmental Disorders: Animal Models

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Title: Dysfunction of RNA-binding protein Sfpq causes long-gene transcriptopathy in the brain

Authors: *A. TAKEUCHI, K. IIDA, M. HAGIWARA
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Abstract: From an evolutionary perspective, the pre-mRNA transcripts of vertebrates are comparatively expanded, and in mammals, genes preferentially expressed in the brain have significantly longer introns. Longer genes present a novel problem with respect to fulfilment of gene-length transcription and evidence suggests that dysregulation of long genes is a mechanism underlying neurodegenerative and psychiatric disorders, such as amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD), autism spectrum disorder (ASD), or Rett Syndrome. These observations have yielded the hypothesis that some neurodegenerative and psychiatric diseases are in fact “long-gene diseases” or “long genopathies.” Yet, it has remained unclear what mechanism specifically regulates long genes to ensure their long-distance transcription. Here, we report the discovery that RNA-binding protein Sfpq is a critical factor for maintaining transcriptional elongation of long genes (Takeuchi et al., *Cell Reports 2018*).
In mouse embryos, Sfpq was robustly expressed in the whole central nervous system including the spinal cord. In the cerebral cortex, Sfpq was specifically expressed in naïve cortical plate, where newly generated neurons are maturing, suggesting its crucial functions for the development of cerebrocortical neurons. Genome wide binding mapping using *in vivo*

crosslinking and immunoprecipitation (CLIP) indicated that Sfpq co-transcriptionally bound to the target pre-mRNAs. Loss of *Sfpq* dramatically reduced the expression of pre-mRNAs >100 kbp in length, which we have termed “long-gene transcriptopathy”. Mechanistically, we demonstrated that Sfpq is required for sustaining long-gene transcription by RNA polymerase II through mediating interaction of cyclin-dependent kinase 9 with the elongation complex. Phenotypically, *Sfpq* disruption caused neuronal apoptosis in developing mouse brains. Expression analysis of Sfpq-regulated genes revealed specific downregulation of developmentally essential neuronal genes longer than 100 kbp in *Sfpq*-disrupted brains; those genes are enriched in associations with neurodegenerative and psychiatric diseases. The identified molecular machinery yields directions for targeted investigations of association between long-gene transcriptopathy and neuronal diseases.

Disclosures: A. Takeuchi: None. **K. Iida:** None. **M. Hagiwara:** None.

Poster

369. Mechanisms of Developmental Disorders: Animal Models

Location: SDCC Halls B-H

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Topic: A.07. Developmental Disorders

Support: National Research Foundation of Korea(NRF) grants funded by the Korea government (NRF-2017R1D1A1B03029997
NRF-2018M3C7A1024148)

Title: Perturbation of overall activity of mTOR pathway in mouse model of focal cortical dysplasia

Authors: *J. KIM[1], S. BAEK[2]
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Abstract: Focal cortical dysplasia (FCD) is neurodevelopmental disorder associated with drug-resistant epilepsy. Histopathological spectrum of FCD is broad, including cortical dyslamination in the absence or presence of significant cellular defects, FCD Type I and Type II respectively. Somatic mutations in mTOR pathway genes such as *MTOR, TSC1* and *TSC2* have been found in FCD type II however, a clear genotype-phenotype correlation has not been established to explain FCD type I. In this study, we found that the severity of developmental defects is associated with the overall activity of the mTOR pathway. In developing mouse brain, ectopic overexpression of gain-of-function mutations in the components of upstream mTOR pathway caused cortical dyslamination and cellular defects seen in FCD type II, whereas expression of wildtypes caused cortical dyslamination only. We now aim to characterize transcriptional profiles to reveal the

molecular mechanisms underlying phenotypic differences caused by the perturbation of overall activity of mTOR pathway.

Disclosures: J. Kim: None. **S. Baek:** None.

Poster

369. Mechanisms of Developmental Disorders: Animal Models

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Title: FOXP1 regulates cell-type specific molecular pathways and function within striatal projection neurons

Authors: *A. ANDERSON, A. KULKARNI, S. CAVALIER, J. GIBSON, G. KONOPKA
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Abstract: *De novo*, loss-of-function mutations in the transcription factor *FOXP1* are among a handful of significant, recurrently-hit genes found in autism spectrum disorder (ASD). We previously reported that a *Foxp1* haploinsufficient mouse model (*Foxp1*$^{+/-}$) not only exhibited brain-region selective impairments within the striatum, but cell-type specific deficits particularly within dopamine 2 receptor (D2) expressing spiny projection neurons (SPNs). We therefore hypothesized that Foxp1 might govern striatal molecular pathways at risk in ASD, particularly within D2 SPNs. To test this, we deleted *Foxp1* from either D1, D2, or both neuronal populations in mice and used a combination of single-cell RNA-sequencing (scRNA-seq), physiological, and behavioral assays to better understand the cell-type specific mechanisms regulated by Foxp1 during striatal development. We found that Foxp1 is crucial for specifying a subset of D2 SPNs and D2 SPNs that remained are hyperexcitable with loss of Foxp1. Using scRNA-seq, we then identified the cell-type specific targets of Foxp1 and found that genes encoding KCN-family potassium channels were downregulated specifically in D2 SPNs. We also found evidence for cell-type specific alterations in striosome-matrix compartmentalization with loss of Foxp1 in D1 neurons. In addition, non-cell autonomous changes occurred with deletion of *Foxp1* in D2 SPNs specifically, including changes in local parvalbumin and calretinin positive interneuron populations. We connect these molecular findings to cell-type specific deficits in motor and limbic system-associated behaviors, including motor-learning, ultrasonic vocalizations, and fear conditioning. These results offer novels insights into the cell-type specific molecular pathways

regulated by Foxp1 during striatal development and provide the first evidence that Foxp1 specifies a subpopulation of D2 SPNs.

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Poster

369. Mechanisms of Developmental Disorders: Animal Models

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Topic: A.07. Developmental Disorders

Support: NIH R01NS045702,
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Title: Role of Sirt2 in oligodendrogenesis in white matter after neonatal hypoxia

Authors: *B. JABLONSKA, L.-J. CHEW, M. REIBNER, V. GALLO
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Abstract: Diffuse white matter injury (DWMI) is a major form of brain injury, which results in chronic neurological and behavioral disabilities in prematurely born infants, including a broad spectrum of cognitive and learning disabilities until young adulthood. Using a mouse model of neonatal hypoxia, we have previously demonstrated delayed oligodendrocyte (OL) maturation in white matter controlled by the $p27^{Kip1}$/Foxo1 pathway. Since Sirt2 is implicated in OL maturation, we determined whether neonatal hypoxia altered Sirt2 expression and function in white matter. We found that hypoxia reduced Sirt2 expression and the number of $Sirt2^+$ cells in white matter. The number of $Sirt2^+Olig2^+$ cells decreased after hypoxia, but $NG2^+Sirt2^+$ cells remained unchanged, indicating that hypoxia may affect Sirt2 primarily in mature OLs. Indeed, we observed a significant reduction of Sirt2 expression in mature $CC1^+$ and CNP^+ oligodendrocytes after hypoxia. Since Sirt2 siRNA treatment of cultured OL progenitor cells (OPCs) reduced percentages of $Olig2^+$, $GalC^+$, and $O4^+$ cells, it is likely that hypoxia-induced decrease in Sirt2 prevents OL differentiation. To uncover possible molecular mechanisms underlying OL dysmaturation mediated by Sirt2 changes, we investigated the $p27^{Kip1}$/FoxO pathway using immunoprecipitation. We found that the Sirt2/FoxO1 complex and deacetylated FoxO1 were reduced by hypoxia. Conversely, hypoxia enhanced formation of the Sirt2/FoxO3a complex, but reduced deacetylated FoxO3a levels. Since FoxO1 deacetylation regulates its activity, these changes induced by hypoxia suggest that reduced Sirt2-mediated deacetylation of FoxO1 reduces $p27^{Kip1}$ expression in injured white matter. These findings demonstrate that the functional involvement of Sirt2 may provide a link between enhanced OPC proliferation and delayed OL maturation in hypoxia-induced white matter damage.

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Poster

369. Mechanisms of Developmental Disorders: Animal Models

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Support: NIH R21MH105746
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Title: Neonatal death of conventional adenylyl cyclase 3 knockout pups

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Abstract: Primary cilia are microtubule-based organelles found on most mammalian cells, which function as signaling hubs for many physiological functions including sensation and development. Type III adenylyl cyclase (AC3) is highly expressed in olfactory cilia and neuronal primary cilia in the brain. While AC3 in olfactory cilia is known to mediate olfactory signal transduction, its function in primary cilia in the brain and other tissues remains unclear. An AC3 conventional knockout (KO) mouse line in C57Bl/6 background has been developed. We observed that most homozygous AC3 KO pups die within hours of birth (neonatal death). To identify the cause of neonatal death and to investigate if AC3 play a role in embryonic development E19 and P0 KO mice were compared to wildtype littermates and no differences in size or external morphological deformities were observed. P0 mice were sectioned and stained to look for possible internal malformations such as short rib thoracic dysplasia or other deformities but no gross defects were found and brain structures were almost identical between KOs and WTs. AC3 KO pups do not display cyanosis or pulmonary hypoplasia either, indicating normal pulmonary development. However, AC3 KO pups did not present milk spots in the belly and lacked movement coordination. We conclude that the neonatal death of AC3 KO pups is caused by lack of nourishment due to loss of smell, but not by AC3 ablation in other tissues.

Disclosures: M.R. Strobel: None. **R. LeBel:** None. **X. Chen:** None.

Poster

369. Mechanisms of Developmental Disorders: Animal Models

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Title: De novo mutations cause West syndrome and their pathophysiological effects

Authors: *K.-I. NAGATA[1], N. HAMADA[2], S. OGAYA[3], M. NAKASHIMA[4], T. NISHIJO[5], Y. SUGAWARA[6], I. IWAMOTO[2], H. ITO[2], Y. MAKI[3], K. SHIRAI[7], S. BABA[8], K. MARUYAMA[3], H. SAITSU[9], M. KATO[10], N. MATSUMOTO[4], T. MOMIYAMA[5]
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Abstract: Trio-based whole exome sequencing identified two *de novo* heterozygous missense mutations (c.1449T>C/p.(Leu500Pro) and c.1436A>T/p.(Asn479Ile)) in *PHACTR1*, encoding a scaffold molecule critical for the regulation of protein phosphatase 1 (PP1) and actin cytoskeleton, in unrelated Japanese individuals with West syndrome (infantile spasms with intellectual disability (ID)). We then examined the role of *Phactr1* in the development of mouse cerebral cortex and pathophysiological significance of these two mutations and another (c.1561C>T/p.(Arg521Cys), which had been reported in an undiagnosed ID patient. Immunoprecipitation analyses revealed that actin-binding activity of PHACTR1 was impaired by the p.Leu500Pro and p.Asn479Ile mutations while the p.Arg521Cys mutant exhibited reduced binding to PP1. Acute knockdown of *Phactr1* with *in utero* electroporation caused defects in cortical neuron migration and dendritic arbor formation during corticogenesis. Notably, these phenotypes were rescued by an RNAi-resistant Phactr1, but not by the mutants. Forced expression of the mutants *per se* also exhibited aberrant phenotypes in neuronal migration. These results suggest dominant negative effects of the mutant allele. In addition, electrophysiological analyses revealed abnormal synaptic properties in *Phactr1*-deficient excitatory cortical neurons. Taken together, *PHACTR1* abnormalities were found to cause West syndrome probably due to morphological and functional defects in cortical neurons during brain development.

Disclosures: K. Nagata: None. **N. Hamada:** None. **S. Ogaya:** None. **M. Nakashima:** None. **T. Nishijo:** None. **Y. Sugawara:** None. **I. Iwamoto:** None. **H. Ito:** None. **Y. Maki:** None. **K.**

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Poster

369. Mechanisms of Developmental Disorders: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 369.14/C36

Topic: A.07. Developmental Disorders

Support: MOST Grant 101-2320-B-010-077-MY2
MOST Grant 103-2911-I-010-504
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MOST Grant 106-2628-B-010-002-MY3

Title: A genetic screen by transposon-mediated somatic mutagenesis in the mouse brain identifies genes associated with malformations of cortical development

Authors: *I.-L. LU, J.-W. TSAI
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Abstract: Malformations of cortical development (MCDs) are heterogeneous neurodevelopmental disorders that often result in epilepsy and developmental delays in children. However, many genetic mutations involved in MCD pathogenesis remain unidentified. To identify new genes potentially involved in cortical development and the pathogenesis of MCDs, we took advantage of forward genetic screening by somatic mutagenesis during brain development. Here we developed a genetic screening paradigm by combining somatic mutagenesis with *in utero* electroporation in the developing mouse cortex. We identified 33 potential MCD genes, several genes have been previously implicated in neuronal development and disorders. Consistent with the screening results, functional disruption of these genes by RNA interference or using CRISPR/Cas9 causes alterations in the distribution of cortical neurons that resemble human cortical dysplasia. To verify potential clinical relevance of these candidate genes, we analyzed somatic mutations in brain tissue from patients with focal cortical dysplasia and found mutations enriched in these candidate genes. These results demonstrate that the approach is able to identify potential novel genes involved in cortical development and MCD pathogenesis.

Disclosures: I. Lu: None. **J. Tsai:** None.

Poster

369. Mechanisms of Developmental Disorders: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 369.15/C37

Topic: A.07. Developmental Disorders

Title: Mouse B3glct knockout a new model for hydrocephalus

Authors: *J. GOTO[1], P. SHULTZ[1], R. C. GRADY[2], C. SHULA[1], D. C. CAMERON[2], F. T. MANGANO[1], R. S. HALTIWANGER[3], B. C. HOLDENER[2]
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Abstract: Hydrocephalus is a life-threatening condition characterized by the excessive buildup of cerebrospinal fluid (CSF) in the cerebral ventricles. The development of excessive CSF can result from impaired CSF flow, immoderate production of CSF, or inabilities to reabsorb the CSF. Hydrocephalus affects one out of every 1,000 babies that are born and is the most common reason for children to have brain surgery. A better understanding of the CSF flow and factors that impair flow is needed to develop alternative treatment plans for more positive outcomes. CSF flow traditionally has been described as unidirectional from the lateral ventricles to the third ventricle, into the fourth ventricle, and finally along the subarachnoid space until the CSF is reabsorbed. Recent studies proposed additional CSF absorption sites, including nasal lymphatics and glymphatic pathways, but the molecular and cellular mechanisms involved in the development of CSF circulation system remained largely unknown.
Peters plus syndrome is caused by recessive loss-of-function mutations in *B3GLCT* (beta 3-glucosyltransferase) and is characterized by Peter's anomaly of the eye, craniofacial defects, shortening of the long bones and digits, and intellectual disabilities. *B3GLCT* mutations were also identified in late gestation fetuses with cleft-palate, eye abnormalities and structural brain disfigurements such as hypoplasia, Dandy-Walker malformation, and hydrocephalus. Here, we report the abnormal CSF flow pattern of the *B3glct* knockout mutant mice, a new mouse model with hydrocephalus phenotype. We examined the neural expression pattern of the *B3glct* gene in LacZ reporter allele inserted in the *B3glct* gene to identify the responsible cell types for the hydrocephalus phenotype. B3GLCT functions in *O*-glycosylation of 49 target proteins that have thrombospondin type 1 repeats (TSRs) with the consensus site C-X_{2-3}(S/T)C. Recent studies provide evidence that the B3GLCT-mediated modification accelerates folding of the native protein, suggesting that the neurological abnormalities in Peters plus syndrome result from defects in folding or function of one or more B3GLCT targets. Candidates include but are not limited to F-Spondin, Sco-spondin, SEMA5A, and SEMA5B.

Disclosures: J. Goto: None. **P. Shultz:** None. **R.C. Grady:** None. **C. Shula:** None. **D.C. Cameron:** None. **F.T. Mangano:** None. **R.S. Haltiwanger:** None. **B.C. Holdener:** None.

Poster

369. Mechanisms of Developmental Disorders: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 369.16/C38

Topic: A.07. Developmental Disorders

Support: DOD Grant 1181038304

Title: Dysregulation of mTORC2, but not mTORC1, underlies the neurophysiological and behavior abnormalities in Pten-deficient mouse model of ASD and epilepsy

Authors: *C.-J. CHEN[1], M. SGRITTA[1], J. MAYS[1], J. NOEBELS[2], M. COSTA-MATTIOLI[1]
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Abstract: The mechanistic target of rapamycin (mTOR) acts as a highly conserved signaling hub that integrates neuronal activity and a variety of synaptic inputs. Mutations in phosphatase and tensin homolog (*Pten*) leads to hyperactivation of both mTOR complex 1 and mTOR complex 2, and is associated with Autism Spectrum Disorder (ASD) and epilepsy. Chronic rapamycin treatment has been shown to inhibit both mTORC1 and mTORC2 and rescue neuronal and behavioral phenotypes in Pten-deficient mice. However, the individual contribution of mTOR complexes to the molecular, behavioral and neurophysiological abnormalities associated with Pten deficiency remains unknown. Here, we discovered that each mTOR complex differentially contributes to different aspects of ASD and seizures. Conditional *Pten* forebrain neuron knockout (*Pten* fb-KO) mice show enlarged brain size, ASD-like behaviors (including social and cognitive deficits as well as repetitive behaviors), increased neuronal excitability, seizure activity, and early mortality due to terminal seizure. Interestingly, genetic inhibition of mTORC1(*Pten;Raptor* fb-dKO) only restores normal brain size in *Pten* fb-KO. Genetic inhibition of mTORC2(*Pten;Rictor* fb-dKO) along, however, is able to rescue seizure and prolong survival of *Pten* fb-KO mice. Normal neuronal excitability, social behavior, and cognitive function are also restored in *Pten;Rictor* fb-dKO, but not in *Pten;Raptor* fb-dKO. We further showed that while the expression of glycolytic enzymes and glycolysis metabolites levels are increased in *Pten* fb-KO and *Pten;Raptor* fb-dKO brain, the restoration of in neuronal function in *Pten;Rictor*fb-dKO mice is accompanied by normalized glucose metabolism in the brain. Our findings not only provide new insight of understanding the molecular mechanism underlying brain mTORopatheis, but but alsohold promise for new specific mTORC2-based treatments for ASD, epilepsy, and related neurodevelopmental disorders in which mTOR function is dysregulated.

Disclosures: C. Chen: None. **M. sgritta:** None. **J. Mays:** None. **J. Noebels:** None. **M. Costa-Mattioli:** None.

Poster

369. Mechanisms of Developmental Disorders: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 369.17/D1

Topic: A.07. Developmental Disorders

Support: NIH Grant MH110911

Title: Optogenetic stimulation of the subthalamic nucleus reduces repetitive behavior in C58 mice

Authors: *A. M. MUEHLMANN[1], L. CURRY-POCHY[1], J. HART[1], M. SMELTZER[2], E. G. KRAUSE[3], M. H. LEWIS[1]
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Abstract: Restricted, repetitive behavior represents a range of responses including stereotyped movements, compulsions, and rituals that are diagnostic for autism spectrum disorders (ASD) and common in other neurodevelopmental and psychiatric disorders. Despite its clinical importance, effective medications for repetitive behavior are lacking. This is due to our rudimentary understanding of the relevant neural circuitry mediating such behavior. Identifying discrete neural pathways controlling repetitive behaviors is key to determining the precise neurobiological pathology and developing effective treatments. We use mouse models of repetitive behavior to accomplish these aims, including C58 mice that show very high rates of repetitive behavior in a laboratory environment. Our prior work using C58 mice revealed decreased neuronal activation and reduced dendritic spine density in the subthalamic nucleus. The overall goal of this project was to determine if reversing this hypoactivation through optogenetic stimulation could reduce repetitive behavior. Using this approach, we expressed channelrhodopsin (ChR2; pAAV-CaMKIIa-hChR2(H134R)-EYFP) or control virus (pAAV-CaMKIIa-EYFP) bilaterally in the subthalamic nucleus of C58 mice. The stimulation used was 10 ms pulses of 473 nm light at 40 Hz for five seconds on and ten seconds off. In ChR2-injected mice, repetitive behavior counts were equal to control mice when laser light was off (10 sec blocks). During photoactivation (5 sec blocks) repetitive behavior was significantly reduced relative to laser off in the ChR2 group and relative to the laser on in the control virus group. Other, non-repetitive, behaviors were not affected by photoactivation of the subthalamic nucleus. We are currently evaluating the widespread effect of the subthalamic nucleus stimulation on large-scale brain networks, as measured by BOLD signal changes, using optogenetic fMRI (ofMRI). These data will also be presented.

Disclosures: A.M. Muehlmann: None. **L. Curry-Pochy:** None. **J. Hart:** None. **M. Smeltzer:** None. **E.G. Krause:** None. **M.H. Lewis:** None.

Poster

369. Mechanisms of Developmental Disorders: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 369.18/D2

Topic: A.07. Developmental Disorders

Support: Telethon, GGP16083

Title: Altered inhibitory synaptic plasticity in the Neuroligin3-R451C mouse model of autism

Authors: *E. PETRINI, S. H. STANCHEVA, F. COLACI, L. MANNINO, A. BARBERIS
Inst. Italiano di Tecnologia, Genoa, Italy

Abstract: Autistic Spectrum Disorder (ASD) has been associated to genetic alterations of proteins that are crucial for the synaptic function such as Neuroligin 3 (NLGN3), a postsynaptic adhesion molecule, which binds to its presynaptic partner Neurexin at both excitatory and inhibitory synapses.
This study explores the R451C mutation on NLGN3 -found in autistic siblings- as a molecular determinant of ASD. We aim at assessing how this mutation affects the structure and function of inhibitory synapses and alters synaptic plasticity in a characterized transgenic mouse model of ASD, the $NLGN3^{R451C}$ knock-in (KI) mouse. We found that the $NLGN3^{R451C}$ protein was much less expressed at the neuronal surface and was significantly more mobile at GABAergic synapses as compared to WT conditions. Also surface synaptic GABAARs were more mobile in KI than in WT neurons. However, the immunoreactivity of the scaffold protein gephyrin and of GABAAR at synapses as well as the amplitude of inhibitory synaptic currents were comparable in WT and KI neurons, indicating that in basal conditions the higher lateral mobility of synaptic $NLGN3^{R451C}$ and GABAAR did not result in significant changes in the postsynaptic organization and function.
Next, we probed WT and KI neurons for the expression of postsynaptic inhibitory long term potentiation (iLTP) induced by a chemical protocol. As expected, upon iLTP induction, neurons from WT animals exhibited a significant increase in synaptic gephyrin along with the accumulation and the immobilization of GABAAR at synapses, leading to a persistent potentiation of the IPSCs amplitude as compared to control treatment. During iLTP we also observed enhanced stabilization of NLGN3 at GABAergic synapses in WT neurons. On the contrary, neurons from KI animals did not respond to the chemical stimulation, leaving the synaptic abundance of GABAAR and gephyrin as well as the amplitude of inhibitory synaptic currents comparable to controls. In line with this, in KI neurons the higher lateral diffusion of GABAAR and $NLGN3^{R451C}$ at inhibitory synapses observed in basal conditions persisted after

the chemical stimulation. Overall those results suggest that in NLGN3 KI neurons the altered stability of the molecular components of inhibitory synapses entail functional consequences only when major synaptic reorganizations are required, such as during synaptic plasticity. Those findings may shed light on how the $NLGN3^{R451C}$ mutation may unbalance the coordination of inhibitory and excitatory synaptic plasticity.

Disclosures: E. Petrini: None. **S.H. Stancheva:** None. **F. Colaci:** None. **L. Mannino:** None. **A. Barberis:** None.

Poster

369. Mechanisms of Developmental Disorders: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 369.19/D3

Topic: A.07. Developmental Disorders

Support: Simons Foundation
INSERM
FRM
ANR

Title: Defects in sensory information processing in the neocortex in a mouse model for fragile x syndrome — from ion channels to cell types and circuits

Authors: *A. A. FRICK[1], A. A. BHASKARAN[2], G. BONY[3], K. LE CORF[2], R. PROVILLE[2]
[1]INSERM U1215, Bordeaux cedex, France; [2]Neurocentre Magendie, INSERM U1215, Bordeaux, France; [3]Neurocentre Magendie, INSERM U1215, Bordeaux Cedex, France

Abstract: Sensory hypersensitivity is a common aspect of fragile x syndrome, and of autism spectrum disorder (ASD) in general. In spite of the prevalence of this phenotype there is a paucity of studies examining sensory information processing defects in animal models of ASD. We investigated sensory information processing in the neocortex of $Fmr1^{-/y}$ mice both at the network and cellular level using different tactile modalities, and a variety of methodologies. We present data illustrating that cellular and synaptic phenotypes depend on the particular cell-types within and across layers (layers 2/3 and 5). We also investigated the correction of aberrant cellular and synaptic properties by targeting a specific ion channel in these neocortical circuits — either using systemic delivery or direct neocortical application of the drug. In conclusion, our study contributes to an understanding of the role of cell diversity in neocortical function in both the normal and diseased brain. In addition, it lends further support for the importance of targeting neuronal excitability as a suitable approach for pharmacological correction of autism related neocortical defects.

Disclosures: A.A. Frick: None. **A.A. Bhaskaran:** None. **G. Bony:** None. **K. Le Corf:** None. **R. Proville:** None.

Poster

369. Mechanisms of Developmental Disorders: Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 369.20/D5

Topic: A.07. Developmental Disorders

Support: NRF-2017R1D1A1B03029997
NRF-2018M3C7A1024148

Title: Developmental mechanisms of sebaceous nevus syndrome caused by dysregulation of RAS/MAPK pathway

Authors: *Y. KIM[1], S. BAEK[2]
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Abstract: Sebaceous nevus syndrome is a neurocutaneous disorder that shows neurological symptoms such as epilepsy, mental retardation, cerebral defect and ocular abnormality with the skin lesion called nevus sebaceous. Recently, it has been reported that sebaceous nevus syndrome is caused by somatic mutation of KRAS and HRAS during development. Interestingly, other neurodevelopmental conditions that are associated with genetic mutations in the components of RAS/MAPK pathway also show characteristic neurological symptoms including mental retardation, epilepsy, seizures, and learning disability which may explained by perturbation of RAS/MAPK pathway. We found that the mis-activation of RAS/MAPK pathway resulted in neurological defects associated with sebaceous nevus syndrome. Activation of RAS/MAPK pathway by ectopic over-expression of KRAS p.G12D in developing mouse brain caused defective neuronal migration. Using human embryonic stem cell-derived neuronal progenitor cells, we aim to characterize the gene expression profile that may explain the underlying molecular mechanisms of neurological defects caused by the mis-regulation of RAS/MAPK pathway.

Disclosures: Y. Kim: None. **S. Baek:** None.

Poster

369. Mechanisms of Developmental Disorders: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 369.21/D4

Topic: A.07. Developmental Disorders

Support: NIH Grant R01MH106553
NINDS Grant R25NS095371

Title: Sex differences in microglia-neural signaling and learning following early-life immune activation in the juvenile rat

Authors: *B. OSBORNE, J. M. SCHWARZ
Dept. of Psychological and Brain Sci., Univ. of Delaware, Newark, DE

Abstract: Developmental disorders associated with learning deficits including Autism, ADHD, and developmental disorders have been linked to early-life immune activation, and notably, are male-biased. Microglia are the primary immune cells of the brain and are in constant communication with neurons, thus, activation of microglia can significantly influence the function of surrounding neurons. Recent evidence indicates that microglia-neuron signaling is also necessary for the proper formation of neural circuits that support learning during early brain development. We found that immune activation with lipopolysaccharide (LPS; 100ug/ml/kg) on postnatal day (P) 21 produces sex-dependent deficits in the *emergence* of hippocampal-dependent learning on P24 in rats. We examined gene expression in the hippocampus at 2-, 4-, 8-, and 24-hr following immune activation and found sex differences in the expression of inflammatory molecules including IL-1β in the hippocampus. Additionally, males, but not females, have a persistent decrease in brain derived neurotrophic factor expression starting 4hr post-LPS that persists until 24hr. Finally, we found that males, but not females, have a significant increase in the expression of C3, an immune molecule that tags synapses for phagocytic elimination, at 24hr. Currently, we are examining whether we can rescue learning deficits on P24 by treating males and females with the microglia inhibitor minocycline; however, we have found a sex difference in the effectiveness of minocycline such that minocycline effectively inhibits IL-1β production in males, but is not as effective in females. We also found that minocycline restores BDNF levels in males treated with LPS back to levels seen in control males. Finally, we examined whether LPS on P21 alters microglia phagocytosis of synapses in the hippocampus and whether measures of neuronal morphology and spine density are altered on P24. These results will be discussed. Our data suggest that changes in microglia-neural communication may be a mechanism underlying sex differences in the vulnerability to the emergence of developmental learning disorders following early-life immune activation and that LPS may cause lasting changes in microglia-neural communication in the hippocampus.

Disclosures: B. Osborne: None. **J.M. Schwarz:** None.

Poster

370. NMDA Receptors

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 370.01/D6

Topic: B.02. Ligand-Gated Ion Channels

Support: NINDS NS 088479
NIMH F30-MH115618

Title: Subtype-specific sensitivity of NMDA receptors to neuropsychiatric lupus autoantibodies

Authors: *K. CHAN[1], C. KOWAL[3], B. DIAMOND[3], L. P. WOLLMUTH[2]
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Abstract: Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disease where patients may develop perturbations in neurological and psychiatric function, leading to neuropsychiatric SLE (NPSLE). A subset of these patients produce anti-dsDNA antibodies (DNRAbs) that cross-react with the NMDA receptor. NMDA receptors are glutamate-gated ion channels that are essential for excitatory signaling in the nervous system. They are obligate heterotetramers, typically composed of two GluN1 subunits and two GluN2 subunits. DNRAbs mediate synaptic dysfunction, cognitive impairment, and excitotoxicity through NMDA receptor activity, largely in the hippocampus. The two major GluN2 subunits in the hippocampus are GluN2A and GluN2B and DNRAbs bind to both GluN2A and GluN2B-containing NMDA receptors. Nevertheless, the specific effect of DNRAbs on NMDA receptors and which NMDA receptor subunits are targeted remain elusive. A major function of synaptic NMDA receptors is to convert synaptically released glutamate into an electrical signal by opening of its associated ion channel, a property called ion channel gating. Here, we show that DNRAbs exert subunit-specific effects on NMDA receptor gating. At a moderate concentration (patient titers), DNRAbs increase peak amplitude of glutamate-induced currents only in GluN2A-containing receptors but not GluN2B-containing receptors. Using high-resolution single channel recordings, we show that DNRAbs increase the mean open probability (P_o) and mean open time (MOT) of GluN2A-containing receptors, but not in those containing GluN2B, suggesting that DNRAbs stabilize GluN2A-containing receptors in the open state. In the hippocampus, NMDA receptors are typically tri-heteromeric, containing both GluN2A and GluN2B subunits. We find that DNRAbs potentiate GluN2A/GluN2B tri-heteromeric receptors, suggesting that GluN2A confers dominance of antibody susceptibility to NMDA receptors. Our data supports *in vivo* findings of GluN2A-containing NMDA receptors being the primary target of excitotoxicity from DNRAbs.

Taken together, these findings suggest that targeted therapies for NPSLE patients to improve neurocognitive function should be directed towards GluN2A.

Disclosures: K. Chan: None. **C. Kowal:** None. **B. Diamond:** None. **L.P. Wollmuth:** None.

Poster

370. NMDA Receptors

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 370.02/D7

Topic: B.02. Ligand-Gated Ion Channels

Support: Biotechnology and Biological Sciences Research Council (BB/N015878/1)
Epilepsy Research UK (P1602)
SFARI
DBT, Govt of India

Title: Characterisation of intrinsic and synaptic properties of hippocampal CA1 pyramidal neurons in Grin2a$^{+/-}$ and Grin2a$^{-/-}$ rats

Authors: *F. YASMIN, S. A. BOOKER, R. LOUREIRO, K. F. M. MARWICK, G. E. HARDINGHAM, P. C. KIND, D. J. WYLLIE
Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: *N*-methyl-D-aspartate receptors (NMDARs) are ionotropic glutamate receptors that play pivotal physiological roles during development and maturation of the central nervous system (CNS). In the mammalian forebrain, the predominant NMDAR composition is a diheteromeric assembly of two GluN1 with either two GluN2A or two GluN2B subunits or a triheteromeric assembly of GluN1 and GluN2A and GluN2B subunits. The identity of GluN2 subunits in a NMDAR is a major determinant of its kinetic behavior with GluN1/2A and GluN1/2A/2B NMDARs having faster deactivation rates than those exhibited by GluN1/2B NMDARs. As such, loss or reduced expression of either GluN2A or GluN2B subunits will change the kinetic profile of NMDAR populations. Indeed, altered functional properties of NMDARs is considered to underlie the CNS dysfunction observed in individuals carrying mutations in either *GRIN2A* or *GRIN2B* (the genes encoding GluN2A and GluN2B, respectively). We have employed CRISPR/Cas9 gene editing methods to generate a genetically modified rat in which the *Grin2a* gene is deleted in order to investigate the influence of the GluN2A subunit in determining excitability and synaptic properties in CA1 hippocampal pyramidal neurons.
CA1 pyramidal neurons from P27-34 *Grin2a*$^{-/-}$ rats show an increase in the slope of the action potential frequency versus injected current (F/I plot) compared to *Grin2a*$^{+/+}$ controls but otherwise have unaltered intrinsic physiology. We also find that dendritic branch complexity,

assessed by 3-dimesional cell reconstruction and Sholl analysis, is reduced in *Grin2a*$^{-/-}$ rats. Loss of expression of GluN2A results in slowing of the NMDAR component of EPSCs as would be predicted if EPSCs were mediated by NMDARs with a greater GluN2B content (*Grin2a*$^{+/+}$: 104.33±5.74 ms, n=12; *Grin2a*$^{+/-}$: 140.58±13.19 ms, n=17; *Grin2a*$^{-/-}$: 186.59±10.2 ms, n=14). We also find that the frequency of mEPSCs is reduced in *Grin2a*$^{-/-}$ rats (*Grin2a*$^{+/+}$: 2.47±0.17 Hz, n=8; *Grin2a*$^{+/-}$: 1.59±0.33 Hz, n=10; *Grin2a*$^{-/-}$: 1.38±0.23 Hz, n=9). However, mEPSC amplitudes remain unaffected by the loss of GluN2A expression. Subthreshold EPSP summation and probability of action potential firing at different frequencies of Schaffer collateral stimulation (2, 5, 10, 20 and 50 Hz) appear unchanged by GluN2A reduction or deletion. Thus, while slower EPSCs are observed, EPSPs summate similarly in *Grin2a*$^{+/+}$, *Grin2a*$^{+/-}$ and *Grin2a*$^{-/-}$ CA1 pyramidal cells.

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Poster

370. NMDA Receptors

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 370.03/D8

Topic: B.02. Ligand-Gated Ion Channels

Support: GACR: P304/12/G069
GACR: 1702300S
TACR: TE01020028
880011

Title: Site of action of brain neurosteroid, pregnenolone sulfate, at the N-methyl-D-aspartate receptor

Authors: *L. .-. VYKLICKY JR[1], B. KRAUSOVA[2], B. KYSILOV[2], J. CERNY[2], V. VYKLICKY[2], M. LADISLAV[2], T. SMAJKALOVA[2], H. CHODOUNSKA[3], E. KUDOVA[3]
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Abstract: N-methyl-D-aspartate receptors (NMDARs) play a key role in excitatory synaptic transmission, and their dysfunction underlies some neurological and psychiatric disorders. Receptor hypofunction has been implicated in autism, schizophrenia, and various forms of intellectual disability, and compounds with a positive allosteric effect at NMDARs may have a beneficial effect in these diseases. The aim of this study was to characterize the site of action for pregnenolone sulfate (PES), an endogenous neurosteroid that has a positive allosteric effect at NMDARs. We have used patch-clamp technique to study PES effect at recombinant

GluN1/GluN2B receptors. Our results show that PES did not compensate for the diminution of NMDAR responses induced by cholesterol depletion, indicating that cholesterol and PES potentiation are mediated by distinct sites. Dose-response analysis of the positive allosteric effect of PE-S at NMDAR indicates that at biologically relevant concentrations the steroid exists in micelle form and together with the effect of methyl-γ-cyclodextrin indicates that the steroid acts at the NMDAR transmembrane domain. The steroid positive allosteric effect was observed only for PES added from the extracellular, not from the intracellular site. To identify the PES site, we sequentially replaced amino acid residues at the outer segment of the transmembrane helices M1 and M4 of both the GluN1 and GluN2B subunits. Single alanine substitution mutations included: GluN1(Q559A to V572A; T809A to V825A) and GluN2B(S555A to I568A; D814A to A830T). Relative effect of PES (100 μM) varied considerably from that found for the wild type receptors (104 ± 4 %; *n* = 158) and mutated receptors (spanning from inhibition 33 ± 2 % (*n* = 7) for GluN1/GluN2B(M824A) to augmented potentiation 507 ± 112 % (*n* = 4) for GluN1/GluN2B(G815A)). The effect of PES at GluN1/GluN2B(D816A; Y823A; and M824A) receptors was significantly reduced compared to WT and at GluN1(G815A; M818A; and G822A)/GluN2B receptors was significantly increased compared to WT. Electrophysiological results together with computational methods indicate that PES binds to the cavity between the M1 and M4 membrane domains of the GluN2B subunit of the closed conformation of the channel. Following receptor activation M1/M4 helices rearrange, including the steroid binding site - this explains why the effect of PE-S is disuse-dependent. Together our study has identified a novel site at the NMDAR by which endogenous neurosteroid PE-S augments the activity of the receptor.

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Poster

370. NMDA Receptors

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 370.04/D9

Topic: B.02. Ligand-Gated Ion Channels

Support: R01AA019455-01A1

Title: GluN2D-containing NMDARs modulate the excitatory activity of CRF neurons within the BNST and influence changes in anxiety and depressive-like behaviors

Authors: *G. J. SALIMANDO[1], T. A. WILLS[2], A. J. BAUCUM II[3], D. G. WINDER[1]
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Abstract: The bed nucleus of the stria terminalis (BNST) is a key regulator of affective stimuli. Previously, our lab has shown that disrupting glutamatergic signaling in the BNST via the ablation or inhibition of N-methyl-d-aspartate receptors (NMDARs) containing the GluN2B subunit alters regional synaptic plasticity and produces an antidepressant-like phenotype in mice. Using a proteomic screen of GluN2B-associated proteins, we identified GluN2D as another NMDAR subunit expressed in the BNST. GluN2D is a less well-studied NMDAR subunit with unique biophysical properties and a more restricted distribution in the CNS. Thus, we assessed the behaviors of GluN2D knockout mice in a variety of tasks designed to assess affective states. Interestingly, GluN2D KO mice exhibited a phenotypic profile across the elevated zero maze, open field and forced swim tests consistent with increased anxiety- and depressive-like behaviors, opposite to the phenotype of BNST-GluN2B knockouts. In addition to behavioral analyses, we have begun to assess the contribution of GluN2D to circuit activity in the BNST. We find that short-term plasticity (STP) is significantly reduced in GluN2D knockout animals compared to wildtype controls. Whole cell electrophysiological analysis of BNST neurons, however, showed no significant differences in the amplitude or frequency of spontaneous excitatory post synaptic currents (EPSC) or in evoked NMDAR EPSC kinetics between knockouts and wildtypes. We hypothesized that the changes we observed in both behavior and BNST plasticity could be due to altered signaling in select populations of BNST neurons that express high levels of GluN2D. Using RNAscope® fluorescent *in situ* hybridization, we found that GluN2D mRNA is highly co-localized with the neuropeptide corticotropin releasing factor (CRF, ~70-75% co-localization) in the BNST. To determine if GluN2D deletion on BNST-CRF neurons altered their excitatory activity, we crossed GluN2D knockout mice into a CRF-IRES-Cre/ROSA (Ai9) reporter line in order to interrogate these cells. CRF cells in GluN2D knockouts show increased spontaneous EPSC amplitude, and a subset of these cells also showed increased spontaneous EPSC frequency. Evoked NMDAR currents in GluN2D KO BNST-CRF cells also showed more rapid decay kinetics compared to GluN2D wildtype CRF cells, suggesting that GluN2D-containing NMDARs may play a role in regulating CRF signaling in the BNST. These changes in CRF cell activity may also underlie the behavioral phenotypes we and other have observed in the GluN2D KO mouse line.

Disclosures: G.J. Salimando: None. **T.A. Wills:** None. **A.J. Baucum II:** None. **D.G. Winder:** None.

Poster

370. NMDA Receptors

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Program #/Poster #: 370.05/D10

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH R01NS097802

Title: GluN3 containing triheteromeric NMDA receptor subunit interactions in the rat medial entorhinal area

Authors: *S. BEESLEY, S. S. KUMAR
Florida State Univ., Tallahassee, FL

Abstract: The subunit composition of native NMDA receptors (NMDARs) in the brain is not precisely known. Recent work from our lab has provided electrophysiological / pharmacological evidence for the expression of GluN3-containing triheteromeric (t-) NMDARs, comprising of one or more subunits from each of the three gene families (GluN1-GluN3), in excitatory layer 3 pyramidal neurons of the medial entorhinal area (MEA). The MEA is implicated in the pathophysiology of Temporal Lobe Epilepsy. Given the paucity of subunit specific pharmacological tools with which to query subunit composition of native receptors, we devised a cell biology approach to determining NMDAR subunit interactions within membrane-bound fractions of tissue excised from the MEA for immunoblotting and co-immunoprecipitation work. Using this approach we validated the expression of GluN3A containing t-NMDARs within the MEA, and the putative co-expression of GluN1/GluN2-containing diheteromeric (d-) NMDARs in these neurons. Based on our results, we propose co-expression of GluN1/GluN3A/GluN2B containing t-NMDARs along with $(GluN1/GluN2A)_2$–containing d-NMDARs in these neurons. Given the unique properties of GluN3-containing t-NMDARs, including enhanced Ca^{2+} permeability, these findings would facilitate the development of novel inhibitors which could curtail excitability of the MEA and prevent Ca^{2+} induced excitotoxicity and cell death of neurons that mediates temporal lobe epileptogenesis.

Disclosures: S. Beesley: None. **S.S. Kumar:** None.

Poster

370. NMDA Receptors

Location: SDCC Halls B-H

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Topic: B.02. Ligand-Gated Ion Channels

Support: NHMRC Grant GNT1099114
NHMRC Grant GNT1138452

Title: Sorting Nexin 27 controls the exocytosis of NMDA receptors

Authors: *V. ANGGONO[1,2], T. WANG[1,2], X. YU[1,2], M. VIEIRA[4], X. L. H. YONG[1,2], S. E. JANG[1,2], B. M. COLLINS[1,3], K. W. ROCHE[4]
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Abstract: Sorting nexin (SNX) is a family of cytoplasmic and membrane proteins commonly involved in the endosomal trafficking of surface receptors. SNX27 is the only sorting nexin to contain a postsynaptic density 95/discs large/zona occuldens (PDZ) domain, and is playing an important role in mediating PDZ-dependent endosomal sorting and recycling of cargo molecules to the plasma membrane. Mutations of *SNX27* gene is linked to intellectual disability, epilepsy and growth retardation. Mice lacking SNX27 display impairments in glutamatergic neurotransmission and long-term potentiation, as well as deficits in learning and memory. Previous studies have attributed synaptic dysfunction in SNX27 knockout mice to impairment in the trafficking of AMPA-type glutamate receptors. However, our recent finding found no evidence for direct interaction between SNX27 with AMPA receptor subunits. Instead, we found that SNX27 PDZ domain directly interacts with subunits of NMDA receptors and that this interaction is regulated by the phosphorylation of NMDA receptor near the carboxy-terminal PDZ ligands. Here, we report that SNX27 regulates the forward trafficking of GluN2A subunit of NMDA receptors in cultured hippocampal neurons. Overexpression of SNX27 upregulates surface expression GluN2A under basal conditions. In contrast, loss of SNX27 function abolishes activity-dependent insertion of GluN2A to the plasma membrane. Our results suggest that SNX27 plays a critical role in synaptic plasticity by enhancing the surface insertion of GluN2A-containing NMDA receptors during synaptic potentiation.

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Poster

370. NMDA Receptors

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Topic: B.02. Ligand-Gated Ion Channels

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Authors are members of the Mind-Brain College of the University of Lisbon

Title: Facilitation of synaptic, but not extrasynaptic, NMDA Currents CA1 pyramidal neurons by adenosine A_{2A} receptors in young adult rats

Authors: *A. M. SEBASTIAO, F. MOURO, D. M. ROMBO, R. B. DIAS, J. A. RIBEIRO
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Abstract: N-methyl-*D*-aspartate receptors (NMDAR) are calcium permeable ionotropic glutamate receptors that play pivotal roles in synaptogenesis, experience-dependent synaptic remodelling and for the induction of long-lasting changes in synaptic efficiency, as it is the case for several forms of synaptic plasticity. However, NMDARs also play a key role in neurodegeneration. The dual role of NMDAR is related to receptor location relative to the synapse, the synaptic NMDARs being mostly involved in synaptic plasticity while the extrasynaptic NMDARs are linked to neurotoxicity. Regulatory mechanisms that control NMDAR activity at specific membrane locations are therefore of particular importance and their knowledge relevant for strategies aiming to favour the balance towards neuronal survival and shape synaptic functioning. Adenosine is an endogenous neuromodulator and through membrane receptors of the A_{2A} subtype (A_{2A}Rs) can also influence both synaptic plasticity and neuronal death. We now evaluated the influence of adenosine A_{2A}Rs upon NMDAR discriminating between modulation of synaptic *versus* extrasynaptic receptors. Whole-cell patch-clamp recordings were obtained to evaluate NMDAR actions on CA1 pyramidal neurons of young adult (6-10 weeks) male Wistar rat hippocampus. Activation of adenosine A_{2A}Rs with CGS21680 (30nM) consistently facilitated chemical-evoked NMDAR-currents (NMDA-PSCs) and afferent-evoked NMDA-currents (NMDA-EPSCs), an action prevented by an A_{2A}R antagonist (SCH58261, 100nM) and a PKA inhibitor, H-89 (1µM). These actions were predominantly postsynaptically-mediated since there was no change in NMDA-EPSCs paired-pulse ratio (PPR). A_{2A}R actions were lost in the presence of an open-channel NMDAR blocker, MK-801 (10µM), but persisted in the presence of memantine, at a concentration (10µM) known to preferentially block extrasynaptic NMDARs. These results show that A_{2A}Rs exert a positive modulatory effect over synaptic, but not extrasynaptic NMDARs in CA1 neurons and therefore under non-pathological conditions may contribute to shift the dual role of NMDARs towards enhancement of synaptic plasticity.

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Poster

370. NMDA Receptors

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Program #/Poster #: 370.08/D13

Topic: B.02. Ligand-Gated Ion Channels

Support: NSF-IOS-BSF Grant 1655480
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Title: Zinc transporter ZnT1 regulates zinc inhibition of NMDARs

Authors: *R. F. KRALL[1], A. MOUTAL[2], R. KHANNA[2], M. HERSHFINKEL[3], E. AIZENMAN[1], T. TZOUNOPOULOS[1]
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Abstract: Synaptically released zinc modulates glutamatergic neurotransmission in a large number of brain regions, including neocortex, hippocampus and the dorsal cochlear nucleus (DCN), an auditory brainstem nucleus. Synaptic zinc is loaded into presynaptic vesicles by the transporter ZnT3 and released with activity. In the DCN, synaptically released zinc inhibits postsynaptic NMDARs and AMPARs. ZnT3 knockout mice, which lack synaptic zinc, show zinc-mediated inhibition of NMDARs at high frequency of stimulation, indicating that other transporters regulating extracellular zinc levels may contribute to the zinc-mediated inhibition of NMDARs. Consistent with this hypothesis, ZnT1, a plasma membrane-localized transporter that extrudes zinc from the cytoplasm, binds to the C-terminal domain of the highly zinc sensitive GluN2A subunit of the NMDAR. We hypothesized that through this association, ZnT1 regulates NMDAR inhibition by increasing zinc levels in the extracellular milieu surrounding NMDARs. We found that ZnT1 RNA expression *in vitro* increased developmentally, paralleling the expression profile of GluN2A. To test whether the ZnT1/NMDAR coupling regulates receptor function, we developed a peptide that competitively interferes with ZnT1-GluN2A association. The peptide sequence was identified by using a far-Western screen to determine the sequence of the C-terminal domain of GluN2A with the highest affinity for ZnT1. The peptide effectively displaced ZnT1 from Glun2A in our cultured neurons in a proximity ligation assay. In addition, this peptide, but not its scramble control, completely abolished endogenous zinc inhibition of NMDARs in rat cortical neurons *in vitro*. This effect was specific for NMDARs, as the peptide treatment reduced endogenous zinc inhibition of NMDAR-mediated synaptic currents in DCN slices, without influencing zinc-mediated inhibition of AMPAR-mediated responses. Finally, the peptide did not affect the IC50 of exogenously applied zinc of NMDA-induced whole cell currents in neurons and in CHO cells expressing GluN1/GluN2A-containing receptors, suggesting that it does not affect either the binding or the direct inhibition of zinc inhibition of NMDARs. These results suggest that ZnT1 regulates local extracellular zinc levels and zinc inhibition of NMDARs.

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Poster

370. NMDA Receptors

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Topic: B.02. Ligand-Gated Ion Channels

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Title: Neuromophological characterization of CA1 pyramidal cells expressing chimeric NMDAR GluN2 subunits: Changes during hippocampal development

Authors: *M. J. KEITH[1], J. REEVES[2], D. CHEN[2], R. E. KEITH[2], T. DUMAS[2]
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Abstract: N-methyl-D-aspartate receptors (NMDARs) are key regulators of dendritic growth. NMDAR composition changes throughout development in that GluN2B subunits are replaced by GluN2A subunits at roughly three weeks of age. Dysfunction of this process is known to impact activity-dependent synaptic plasticity and cognitive development and is believed to be involved in autism spectrum disorders. Calcium conductance through NMDARs [regulated by amino(A)-terminus and transmembrane domains (TMDs)] and intracellular signaling (regulated by carboxy(C)-terminus) are known to impact functional and structural synaptic plasticity. It remains unknown how these separate functional properties independently affect dendritic morphology. To determine the separate influences of NMDAR subunit regions on dendritic development, we constructed GluN2 chimeras and generated two transgenic mouse lines. One mouse line expresses the A-terminus and TMDs of GluN2A fused to C-terminus of GluN2B (termed ABc) and, vice versa, the other mouse line expresses the A-terminus and TMDs of GluN2B fused to C-terminus of GluN2A (termed BAc). Transcription was regulated by the TET-off expression system with tetracycline transactivator protein (tTA) expression under control of the CaMKII minimal promoter. tTA expression was seen in many forebrain regions, but predominantly in hippocampal pyramidal cells in area CA1. We measured neuromorphological characteristics of hippocampal CA1 pyramidal neurons in two postnatal periods: P17-P19, and P22-P24, by utilizing Thy-1 GFP fluorescence methods, confocal microscopy, and Neurolucida tracing. Our findings revealed that ABc animals appear to show reduced spine density at P17-P19 than BAc and WT animals. This suggests that ABc animals may go through synaptic pruning earlier than wildtypes due to the presence of the amino(A)-terminus and TM regions. In contrast to the ABc animals, the BAc animals appear to express greater synaptogenesis at P17-P19, possibly due to a prolonged development period compared to ABc and WT animals. The BAc animals express shorter than average branch length at P17-P19. This may be explained by prolonged development as mentioned previously. Also, the ABc animals have a longer average branch length in the P22-P24 age group suggesting that the GluN2B carboxy tail could be affecting growth factors following hippocampal maturation. This study furthers understanding of how NMDAR regions and their separate properties facilitate developmental changes, yielding insight into neurodevelopmental disorders.

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Poster

370. NMDA Receptors

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Program #/Poster #: 370.10/D15

Topic: B.02. Ligand-Gated Ion Channels

Title: Discovery of a novel antagonist selective for GluN2A-containing NMDA receptors

Authors: S. SIMAVORIAN, T. LOVENBERG, *R. NEFF
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Abstract: The discovery of NMDA modulators has long been a priority for both basic scientific investigation as well as for potential therapeutic purposes in many neurological and psychiatric diseases. Despite extensive efforts identification of compounds selective for GluN2A has proven difficult. Recent advances in the field, however, have generated tools and information that have facilitated new avenues of investigation. Specifically, the identification of GluN2A selective compounds and publication of the key structural determinants of their interactions with the channel have enabled virtual interrogation of ligand binding sites. Here, we report the discovery of a GluN2A selective antagonist that was identified in a *in silico* screen of the GluN2A glutamate binding site. The potency of this compound is right-shifted in the presence of increasing concentrations of glutamate. In addition, the molecule exhibits selectivity for antagonism of GluN2A over GluN2B, C, & D. Thus, we provide an additional tool for the targeted study of GluN2A function.

Disclosures: S. Simavorian: A. Employment/Salary (full or part-time):; Janssen R&D, LLC. **T. Lovenberg:** A. Employment/Salary (full or part-time):; Janseen R&D, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Johnson & Johnson. **R. Neff:** A. Employment/Salary (full or part-time):; Janssen R&D, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Johnson & Johnson.

Poster

370. NMDA Receptors

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant 1P01 GM118629-01A1
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Title: Ketamine trapping inside of NMDA channels affects spiking dynamics in Hodgkin-Huxley-like models

Authors: *M. M. KOWALSKI, M. M. MCCARTHY, N. KOPELL
Dept. of Mathematics and Statistics, Boston Univ., Boston, MA

Abstract: One of the most abundant ionotropic receptors for glutamate, the NMDA receptor, has received increased attention in recent years due to its fundamental role in synaptic plasticity, its dysfunction in the pathology of schizophrenia, and the prominent antidepressant, hallucinatory, and anesthetic effects induced by its direct channel blocker ketamine. At rest, closed NMDA channels trap physiologically available magnesium ions, which are expelled from the receptor during depolarization to make way for inward ion flow. Ketamine is believed to block NMDA receptors in a similar way to magnesium, but with higher affinity and higher net trapping, such that its negative effects on transmembrane currents persist for several seconds after application of glutamate to voltage-clamped cells. Previous studies report that high-dose ketamine anesthesia produces bursts of gamma oscillations in the EEG that appear regularly every 5-10 seconds and decay in frequency in a stereotypical fashion before disappearing completely for a couple of seconds. One possible explanation for the duration and frequency decay of these gamma epochs could be that high neural activity at the beginning of each burst facilitates ketamine entry into open NMDA channels, leading to progressive trapping and, in turn, periodic slowing and shutdown of the gamma-generating network. Given the complicated binding and trapping kinetics of ketamine, numerical network simulations are uniquely positioned to investigate this potential link, but all contemporary conductance-based models vastly reduce the complexity of NMDA kinetics by assuming instantaneous channel block. We thus incorporate a multiple-state, experimentally supported kinetic scheme of NMDA magnesium block into a Hodgkin-Huxley model of a single cell. We assume that ketamine acts via the same kinetic scheme as magnesium, but adjust for its higher affinity and trapping by increasing the rate constant of block. We find that, at the single-cell level, this significantly reduces excitability and produces a progressive decay in firing rate that can lead to complete shutdown of spike generation if the rate constant of block is set at a sufficiently high value. In the kinetic scheme we employ, this shutdown is partially driven by locking of the receptor into a distinct, desensitized state that requires a complete pause in input to recover the firing properties of the cell. We discuss the impact of extended trapping and blocking of NMDA receptors in the context of ketamine action on cortical network dynamics.

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Poster

370. NMDA Receptors

Location: SDCC Halls B-H

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Program #/Poster #: 370.12/D17

Topic: B.02. Ligand-Gated Ion Channels

Title: Control astrocyte proliferation through manipulating NMDA receptor signaling in mouse brain

Authors: *Y. GENG[1], C. FU[1], Q. WANG[1], J. KAMINKER[2], M. H. SHENG[2], Y. CHEN[1]
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Abstract: Astrocytic abnormality, such as alterations in its population, occurs in many brain diseases, but the underlying mechanisms are poorly understood. Particularly there is lack of tools to manipulate astrocyte proliferation to address the significance of alterations in its population. We report that astrocyte proliferation is bi-directionally regulated by neuronal activity via NMDA receptor (NMDAR) signaling in neurons. Using whole genome mRNA profiling, we found that a set of cell cycle-related genes were altered by prolonged treatment of hippocampal cultures with NMDAR antagonist AP5. These cell cycle-related genes were expressed in astrocytes rather than neurons. Consistent with this, NMDAR inhibition suppressed astrocyte proliferation, whereas NMDAR potentiation with a novel pharmacological tool promoted astrocyte proliferation *in vitro* and *in vivo*. In summary, we identified a mechanism regulating astrocyte proliferation and provided methods to manipulate it. These will be useful tools to study the functional significance of astrocyte.

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Poster

370. NMDA Receptors

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Topic: B.02. Ligand-Gated Ion Channels

Support: FCT Fellowship SFRH/BI/106010/2015

Title: Synaptic network dysfunction in neurological disorders: Understanding the impact of rare genetic variants of NMDAR subunits in autism-spectrum disorders and epilepsy

Authors: *M. M. VIEIRA[1], S. LIU[2], K. W. ROCHE[3]
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Abstract: Neurological disorders, including autism-spectrum disorders (ASDs) and epilepsy (EPI) are highly prevalent, complex syndromes, with genetic and environmental components. Intensive research efforts have been made to identify the causative genes. Notably, selective clusters of genes with copy number variations and rare variants have been identified. One such cluster is the synaptic protein network, which suggests that proteins related to synaptic function and activity may be dysfunctional in these pathologies. One of the recurrently affected genes in ASDs is *GRIN2B*, which encodes for GluN2B, a subunit of N-methyl-D-aspartate receptors (NMDARs). Indeed, many rare variants of GluN2B have been identified in ASD probands. The GluN2A subunit, on the other hand, is commonly identified in patients with EPI. This suggests that NMDAR dysfunction may be relevant for these neurological disorders.
We screened a variety of rare variants within the C-terminal domain (CTD) of GluN2A/B subunits and their effects on receptor function, spine density, and on the molecular complex associated with the receptors. Because the NMDAR CTDs mediate protein-protein interactions with synaptic scaffolds and signaling molecules, these variants might disrupt coupling NMDAR activity to downstream pathways.
We observed that GluN2B rare variants identified in ASD patients caused dramatic changes on NMDAR and AMPAR surface expression, spine density, and the interaction with post-synaptic proteins. To assess the implications of these defects observed in primary cultures, we generated an animal model of one of these rare variants, S1413L. Our results demonstrate that indeed this rare variant of GluN2B results in defects in the post-synaptic network. We observed that the PSD fraction of the mouse hippocampus had reduced levels of the NMDAR subunits as well as MAGUKs and CaMKII. In the cortical PSD fractions, we did not detect such differences relative to WT animals. Interestingly, our GluN2A rare variants, that were identified in EPI patients, also showed reduced spine density. This effect implies a common mechanism of dysfunction underlying both ASD syndromes and EPI.
Overall, our work supports a model in which dysfunction of the synaptic protein complex underlies defects seen in ASD and EPI. Elucidating these mechanisms will pave the way to the development of novel therapeutic strategies for these disorders.

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Poster

370. NMDA Receptors

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH NINDS Intramural Research Program

Title: Neurobeachin-mediated regulation of NMDA receptors and PSD-95 family members

Authors: *E. FINGLETON[1], K. W. ROCHE[2]
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Abstract: The PSD-95 family of scaffolding proteins regulate N-Methyl-D-Aspartate receptors (NMDARs), which play a key role in canonical forms of Long Term Potentiation (LTP). NMDARs are also key molecular players in neurological development and disease: over the course of development, NMDAR trafficking and expression patterns at the synapse versus extrasynaptic areas are dynamic and give rise to plasticity. PSD-95, among other scaffolding proteins, mediates NMDAR stability at the synapse. Neurobeachin, an autism candidate gene, is a known NMDAR interactor and regulator of spine dynamics. Neurobeachin has been shown to regulate the surface expression of several synaptic proteins, including NMDARs, and is thought to do so by facilitating local recycling. However, neurobeachin's role in shaping synaptic protein populations remains poorly understood. In the current study, we investigate the interaction between neurobeachin and PSD-95 family members. We virally transduce cortical neurons with shRNA to knock down neurobeachin expression and probe the role neurobeachin plays at the synapse throughout development. We find that neurobeachin differentially regulates synaptic expression of PSD-95 compared to other synaptic proteins. Furthermore, we find regulation of synaptic NMDARs by neurobeachin is developmentally-regulated and subunit-dependent.

Disclosures: E. Fingleton: None. **K.W. Roche:** None.

Poster

370. NMDA Receptors

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 370.15/D20

Topic: B.02. Ligand-Gated Ion Channels

Support: CIHR operating grant

Title: Non-ionotropic signaling through presynaptic NMDA receptors promotes neurotransmitter release in the developing retinotectal system

Authors: *M. VAN HORN, V. HIGENELL, E. S. RUTHAZER
Neurol. and Neurosurg., McGill Univ., Montreal, QC, Canada

Abstract: The NMDA receptor plays an important role in activity-dependent plasticity mechanisms underlying neural circuit formation. Recent experiments have reported NMDA receptor expression on presynaptic, as well as postsynaptic terminals. Presynaptic NMDA receptors (preNMDARs) have been found to modulate transmitter release and are essential for many forms of plasticity. Here we found evidence of preNMDARs on retinal ganglion cells (RGCs) in the *Xenopus laevis* tadpole visual system. Application of NMDA produced Ca^{2+} transients in RGC axon terminals separated from their somata, suggesting the presence of presynaptic NMDARs on RGC axons. Using whole cell electrophysiology, under postsynaptic NMDAR blockade, bath application of NMDAR antagonists, particularly targeting GluN2B-containing receptors, decreased miniature excitatory postsynaptic current (mEPSC) frequency. Moreover, the NMDAR antagonist APV resulted in a significant increase in paired -pulse ratios. These results suggest a presynaptic contribution of NMDARs to neurotransmission in the developing optic tectum. Interestingly, raising the animals in MK801, to block Ca^{2+} influx through the ion channel of the NMDARs, did not prevent the APV-induced decrease in mEPSC frequency, suggesting that presynaptic NMDARs in the optic tectum can signal non-ionotropically to regulate synaptic release. Taken together, these results suggest that NMDARs in RGCs modulate glutamate release via mechanisms both dependent and independent of Ca^{2+} influx through the ion channel.

Disclosures: M. Van Horn: None. **V. Higenell:** None. **E.S. Ruthazer:** None.

Poster

370. NMDA Receptors

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 370.16/D21

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant MH045817
Andrew Mellon Fellowship

Title: A hydrophobic path allows drug access to the NMDA receptor channel

Authors: *M. WILCOX[1], N. G. GLASGOW[2], S. MESBAHI-VASEY[3], A. NIGAM[1], M. B. PHILLIPS[1], A. L. TURCU[4], C. NARANGODA[3], M. G. KURNIKOVA[3], S. VAZQUEZ[4], J. W.

JOHNSON[1]
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Abstract: N-methyl-D-aspartate receptors (NMDARs) are ionotropic glutamate receptors that are critical for learning, memory and development. NMDARs are also involved in numerous diseases. In many pathological conditions NMDARs can become overactive and further disease progression. Molecules that inhibit NMDARs have been examined for their therapeutic potential in cases of pathological NMDAR overactivity. NMDAR inhibitors that act through an “open channel block” mechanism have shown significant therapeutic utility. Open channel blockers can enter the open NMDAR channel and bind there, preventing current flow. Memantine is an open channel blocker that is used to treat Alzheimer’s disease and shows promise in treating many other disorders. In addition to open channel block, memantine inhibits NMDARs through a second mechanism initially thought to involve memantine binding to NMDARs at a “superficial” site external to the pore. However, our recent data suggest that this second mechanism of inhibition involves accumulation of memantine in the membrane, followed by memantine transit from the membrane to the channel blocking site (membrane-to-channel inhibition, MCI). We further investigated MCI using whole-cell recordings from tsA201 cells transfected to express NMDAR subunits. Although very little uncharged memantine is present at physiological pH, experiments in which we varied pH suggest that the uncharged form of memantine is critical for MCI. The lack of MCI in experiments with a permanently charged trimethyl derivative of memantine also supports the importance of uncharged memantine in MCI. We used molecular modeling to predict a hydrophobic path through which memantine can travel during MCI, and examined the effects on MCI of mutating residues that line the putative hydrophobic path. We also discovered that numerous NMDAR channel blocking drugs in addition to memantine exhibit MCI. These data suggest a previously unexamined route of drug access to the NMDAR pore.

Disclosures: M. Wilcox: None. **N.G. Glasgow:** None. **S. Mesbahi-Vasey:** None. **A. Nigam:** None. **M.B. Phillips:** None. **A.L. Turcu:** None. **C. Narangoda:** None. **M.G. Kurnikova:** None. **S. Vazquez:** None. **J.W. Johnson:** None.

Poster

370. NMDA Receptors

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 370.17/D22

Topic: B.02. Ligand-Gated Ion Channels

Support: NSF 1456818
NIH NS104705

Title: Expression of GluN2C subunit in parvalbumin positive neurons and astrocytes: Relevance to schizophrenia

Authors: *P. J. GANDHI, G. SHELKAR, R. PAVULURI, J. LIU, S. DRAVID
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Abstract: Functional deficit in NMDA-receptors on parvalbumin (PV)-positive neurons is central to the pathophysiology of schizophrenia. Despite the proposed relationship of GluN2C-expressing PV-interneurons to the NMDA receptor hypofunction hypothesis in schizophrenia, the precise expression pattern of GluN2C subunit remains unknown. Using a novel EGFP reporter model, we found the expression of EGFP (GluN2C) with PV positive neurons in nucleus reticularis of the thalamus, globus pallidus externa and interna, ventral pallidum and substantia nigra. Interestingly, EGFP (GluN2C) showed co-localization with the astrocytic marker in the striatum, cortex, hippocampus and amygdala but not with PV positive neurons. GluN2C was found to be enriched in several thalamic relay nuclei including first order and higher order nuclei. We also studied the behavioral effect of GluN2C and GluN2D ablation on pure C57BL/6N mouse strain. GluN2D knockout mouse exhibited hypo-locomotion and anxiety-like behavior. Increase in startle response was found for GluN2C heterozygous, GluN2D heterozygous and GluN2D knockout (KO) while no significant difference in prepulse inhibition (PPI) was observed. We also found that CIQ, a selective positive allosteric modulator for GluN2C/GluN2D subunit, reversed the MK801 induced impairment in PPI in GluN2C wildtype and heterozygous mice but not in GluN2C KO mice. Furthermore, we observed that GluN2C KO mice but not GluN2D KO mice were less sensitive to phencyclidine-induced hyperlocomotion, a model of psychosis. Together, these results identify a unique expression pattern of GluN2C subunit in neuronal and non-neuronal cell population. It also demonstrates that GluN2C rather than GluN2D may underlie phencyclidine-induced hyperlocomotion and facilitation of GluN2C subunit may reverse the schizophrenic phenotypes.

Disclosures: P.J. Gandhi: None. **G. Shelkar:** None. **R. Pavuluri:** None. **J. Liu:** None. **S. Dravid:** None.

Poster

370. NMDA Receptors

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 370.18/D23

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant RO1MH045817

Title: Comparison of memantine and ketamine binding sites in the NMDA receptor channel

Authors: *M. B. PHILLIPS[1], C. NARANGODA[2], M. R. WILCOX[1], S. MESBAHI-VASEY[2], A. NIGAM[1], M. G. KURNIKOVA[2], J. W. JOHNSON[1]
[1]Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [2]Chem., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: N-methyl-D-aspartate receptors (NMDARs) are ionotropic glutamate receptors expressed at nearly all excitatory vertebrate synapses. NMDAR-mediated Ca^{2+} influx is essential for many critical neuronal functions and plays key roles in synaptogenesis, plasticity, dendritic integration, and cell survival. NMDAR dysfunction is implicated in nervous system pathologies such as neurodegenerative diseases, epilepsy, and cell death following stroke and ischemia. Thus, drugs targeting NMDARs are of great clinical interest. NMDAR channel blockers, antagonists that impede ion flux through NMDARs by physically occluding the channel, have shown significant therapeutic utility. Two of the most well-characterized and widely used NMDAR channel blockers are memantine (Mem) and ketamine (Ket). Despite sharing similar affinities, binding kinetics, and overlapping binding sites, Mem and Ket possess wildly divergent clinical profiles. Recent work from our lab has revealed substantial differences in how Mem and Ket act on NMDARs. Mem and Ket differentially augment NMDAR desensitization, with Mem enhancing Ca^{2+}-dependent desensitization of GluN2A-containing NMDARs. Here we test the hypothesis that the differential ability of Mem and Ket to augment NMDAR desensitization is due to differences in their interactions with the NMDAR channel. We used molecular simulations in which Mem and Ket were docked to our atomistic GluN1/N2A transmembrane domain (TMD) model to identify residues that differentially contribute to Mem and Ket binding. We then mutated channel residues predicted to specifically interact with Mem and examined effects on Mem and Ket potency. Whole-cell recordings from tsA201 cells transfected to express NMDARs revealed a mutation in the GluN2A TMD that altered Mem potency without affecting Ket potency, as predicted by our simulations. Strikingly, the mutation also powerfully enhanced NMDAR desensitization, suggesting that residues located in the Mem binding site are involved in the conformational changes associated with NMDAR desensitization. These experiments provide evidence that Mem and Ket interact with distinct NMDAR channel residues involved in NMDAR desensitization.

Disclosures: M.B. Phillips: None. **C. Narangoda:** None. **M.R. Wilcox:** None. **S. Mesbahi-Vasey:** None. **A. Nigam:** None. **M.G. Kurnikova:** None. **J.W. Johnson:** None.

Poster

370. NMDA Receptors

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 370.19/D24

Topic: B.02. Ligand-Gated Ion Channels

Support: NS065371

Title: Novel positive allosteric modulators for nmdars demonstrate enantiomer-specific actions

Authors: *S. F. TRAYNELIS[1], K. L. STRONG[1], M. P. EPPLIN[2], K. K. OGDEN[3], H. KUSUMOTO[1], S. BHATTACHARYA[1], G. SHAULSKY[1], R. E. PERSZYK[1], D. MENALDINO[2], K. B. HANSEN[4], T. J. WILDING[5], C. R. CAMP[1], M. J. MCDANIEL[1], J. ZHANG[1], P. LE[1], J. E. HUETTNER[5], D. C. LIOTTA[2]
[1]Dept Pharmacol, [2]Dept Chem., Emory Univ. Sch. of Med., Atlanta, GA; [3]Neurosci., Scripps Florida, Jupiter, FL; [4]Dept. of Biomed. & Pharmaceut. Sci., Univ. of Montana, Missoula, MT; [5]Cell Biol., Washington Univ. Med. Sch., Saint Louis, MO

Abstract: N-methyl-D-aspartate receptors (NMDARs) are ionotropic ligand-gated glutamate receptors that mediate a slow, Ca^{2+}-permeable component of synaptic transmission that is critical for brain development and function, different forms of learning and memory, and cognition. NMDARs are obligate heterotetramers made up of two GluN1 subunits and two GluN2 subunits from four different genes (GluN2A-D). Hypofunction of NMDAR activity is hypothesized to play a role in multiple neurological diseases including schizophrenia, which has driven interest in the discovery of drug-like small molecule positive allosteric modulators (PAMs) of NMDAR function. Here, we describe modifications to the tetrahydroisoquinoline scaffold of GluN2C/GluN2D-selective PAMs that expands their activity to include actions at GluN2A- and GluN2B-containing di- and triheteromeric receptors. This series (EU-1180-55) was active at all NMDAR subtypes in transfected HEK cells. However, separation of EU-1180-55 into (*S*) and (*R*) enantiomers, revealed enantiomer-specific actions on NMDARs that contained different GluN2 subunits. Specifically, (*S*)- EU-1180-55 showed pan-potentiation whereas (*R*)- EU-1180-55 was only active at GluN2C- and GluN2D-containing NMDARs. We exploited our triheteromeric expression system in *Xenopus laevis* oocytes, which utilizes masking of endoplasmic reticulum retention signals for control of subunit stoichiommetry, to evaluate the actions of compounds on different triheteromeric receptors. Evaluation of the actions of these two enantiomers on GluN1/2A/2B, GluN1/2A/2C, GluN1/2A/2D, GluN1/2B/2C, and GluN1/2B/2D triheteromeric receptors revealed unique pharmacological properties (n=5 oocytes from 2-3 frogs). Furthermore, we observed enantiomer-specific modulation of the NMDAR-component of evoked EPSCs at the CA3-CA1 synapse from mouse hippocampal slices. (*S*)-EU-1180-55 significantly prolonged the weighted decay time constant by 1.36-fold when compared to (*R*)- EU-1180-55 (1.18-fold) or vehicle (1.04-fold, one-way ANOVA, $p<0.001$, Tukey post hoc, N=8 cells). In summary, evaluation of the effects of (*S*)- and (*R*)- EU-1180-55 on triheteromeric NMDARs revealed a novel pharmacological profile that may be useful to probe the role of NMDARs comprised of different subunits in circuit function.

Disclosures: S.F. Traynelis: A. Employment/Salary (full or part-time):; Emory University. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeurOp, Inc.. F. Consulting Fees (e.g., advisory boards); Sage Therapeutics. **K.L. Strong:** A. Employment/Salary (full or part-time):; Emory University. **M.P. Epplin:** A. Employment/Salary (full or part-time):; Emory University. **K.K. Ogden:** A. Employment/Salary (full or part-time):; Scripps Flordia. **H. Kusumoto:** A. Employment/Salary (full or part-time):; Emory University. **S. Bhattacharya:** A.

Employment/Salary (full or part-time):; Emory University. **G. Shaulsky:** A. Employment/Salary (full or part-time):; Emory University. **R.E. Perszyk:** A. Employment/Salary (full or part-time):; Emory University. **D. Menaldino:** A. Employment/Salary (full or part-time):; Emory University. **K.B. Hansen:** A. Employment/Salary (full or part-time):; University of Montana. **T.J. Wilding:** A. Employment/Salary (full or part-time):; Washington University St. Louis. **C.R. Camp:** A. Employment/Salary (full or part-time):; Emory University. **M.J. McDaniel:** A. Employment/Salary (full or part-time):; Emory University. **J. Zhang:** A. Employment/Salary (full or part-time):; Emory University. **P. Le:** A. Employment/Salary (full or part-time):; Emory University. **J.E. Huettner:** A. Employment/Salary (full or part-time):; Washington University St. Louis. **D.C. Liotta:** A. Employment/Salary (full or part-time):; Emory University. F. Consulting Fees (e.g., advisory boards); NeurOp, Inc..

Poster

370. NMDA Receptors

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 370.20/D25

Topic: B.02. Ligand-Gated Ion Channels

Support: NS036654
NS092989

Title: Grin2a knockout promotes hyperexcitability in the mouse hippocampus: A model to understand epileptic activity in grin2a lof variants

Authors: *C. R. CAMP, S. F. TRAYNELIS
Emory Univ., Atlanta, GA

Abstract: N-methyl-D-aspartate receptors (NMDARs) are ionotropic ligand-gated glutamate receptors responsible for mediating a slow, Ca2+ permeable component of fast excitatory neurotransmission. These receptors are critical for neuronal development, learning and memory, and general cognition. Although there is high selective pressure against variation in regions of the *GRIN* genes essential for receptor function, numerous variants have been identified in patients with a range of neurological disorders. Among the four GluN2-subunits (GluN2A-D), variants within the GluN2A subunit are most commonly associated with epilepsy. Particularly interesting are the *GRIN2A* loss-of-function (LOF) mutations, which make up 67% (14/21) of currently identified *GRIN2A* variants and present an epileptic phenotype. This finding suggests that hypofunction of GluN2A results in compensatory mechanism(s) that promote hyperexcitability. To study these LOF mutants, we have used a *Grin2a*-knockout (KO) mouse model. Mouse hippocampal brain slices were studied using whole-cell patch clamp and extracellular field recordings. Slices were exposed to elevated levels of extracellular potassium that can generate spontaneous epileptiform activity. All *Grin2a*-KO brain slices (N = 3 animals/5

slices) showed spontaneous interictal field bursts originating in the CA3 region and propagating to the CA1 region of the hippocampus in 7.0 mM extracellular potassium. By contrast, no wild-type (WT) slices showed epileptiform activity (N = 3 animals/3 slices). Additionally, *Grin2A*-KO mice display an increased inhibitory tone, measured by an increased frequency of spontaneous inhibitory postsynaptic currents and a large gabazine-sensitive current, onto CA1 pyramidal cells when compared to WT. A better mechanistic understanding of how the loss of GluN2A activity impacts neuronal development and promotes these compensatory changes may allow novel therapeutics to be identified that can be used to treat patients with LOF *GRIN2A* mutations.

Disclosures: S.F. Traynelis: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeurOp, Inc.. F. Consulting Fees (e.g., advisory boards); Sage Therapeutics.

Poster

370. NMDA Receptors

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 370.21/D26

Topic: B.02. Ligand-Gated Ion Channels

Support: NS036654
Research grant from Janssen Pharmaceuticals Inc.

Title: Selective expression of triheteromeric NMDA receptors reveals unique properties

Authors: *S. BHATTACHARYA[1], F. YI[2], A. H. KHATRI[1], S. A. SWANGER[1], H. YUAN[1], K. B. HANSEN[2], S. F. TRAYNELIS[1]
[1]Deapartment of Pharmacol., Emory Univ., Atlanta, GA; [2]Dept. of Biomed. & Pharmaceut. Sci., Univ. of Montana, Missoula, MT

Abstract: *N*-methyl-*D*-aspartate receptors (NMDARs) are excitatory ligand-gated ion channels that play critical roles in CNS. Many studies on NMDARs have been performed on diheteromeric receptors containing 2 GluN1 and 2 identical GluN2 subunits. Four genes encode GluN2 subunits (GluN2A-D), and most neurons express 2 or more GluN2 subunits. Multiple lines of evidence suggest that some native NMDARs are triheteromeric assemblies of 2 GluN1 and 2 different GluN2 subunits. However, there are a few studies of the functional properties for triheteromeric NMDARs due difficulties in expressing triheteromeric NMDARs in heterologous systems without also co-expressing diheteromeric NMDARs. We developed a strategy that utilizes masking of endoplasmic reticulum retention signals to express triheteromeric NMDARs with two different GluN2 subunits, allowing evaluation of their properties. We have successfully utilized this strategy to express triheteromeric GluN1/2A/2B, GluN1/2A/2C, GluN1/2A/2D,

GluN1/2B/2C, and GluN1/2B/2D combinations by optimizing the amount of cRNA injected into oocytes and the post-injection incubation time. We also developed control experiments to estimate the percentage of the current response arising from diheteromeric NMDARs that might reach the cell surface despite the absence of masking of the ER retention signal. This was done by introducing two agonist binding site mutations to each GluN2 subunit that eliminate glutamate binding. These mutations, referred to as RK+TI, are inserted in each GluN2 subunit to act as controls for escaped diheteromeric receptors. We measured the "escape current" as a fraction of the total triheteromeric current for all combinations (*n=5*). Because we observed substantial escape current when GluN2A or GluN2B were expressed with GluN2C or GluN2D (nearly 10% in oocytes), we developed an approach to further reduce escape current, thereby increasing the fractional current response accounted for by triheteromeric receptors. To do this we introduced the N624K pore mutation into GluN2C or GluN2D subunits. When GluN1/2A/2D(NK) was expressed in the presence of 0.1-1.0 mM Mg^{2+}, there was virtually no detectable escape current. Similar results were found for GluN1/2A/2C(NK), GluN1/2B/2C(NK), and GluN1/2B/2D(NK). The reduced escape current allowed accurate pharmacological characterization of triheteromeric NMDARs, which typically showed altered EC_{50} values for agonists, endogenous ions, and allosteric modulators compared to diheteromeric receptors. This work advances our ability to study triheteromeric NMDARs with reduced contribution from diheteromeric receptors.

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Poster

370. NMDA Receptors

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 370.22/D27

Topic: B.02. Ligand-Gated Ion Channels

Support: Canadian Institutes of Health Research Foundation grant (FDN-154336)

Title: Alternative splicing of GluN1 tunes synaptic plasticity and learning in mice

Authors: *A. S. SENGAR[1], H. LI[1], W. ZHANG[1], C. LEUNG[1,2], N. M. SAW[1,2], V. WANG[1], Y. TU[1], Z. JIA[1,2], M. W. SALTER[1,2]
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Abstract: NMDA receptors (NMDARs) are important mediators of synaptic plasticity and memory. Native NMDARs consist of two GluN1 subunits with two GluN2A-D subunits, and possibly GluN3. The GRIN1 gene, which encodes GluN1 has 8 splice variants whereas the GRIN2s are unspliced. The functional roles for various GluN2 subunits in synaptic physiology have been well characterized but the biological functions of GRIN1 splice variants remain unknown in physiological contexts. Here, we examined the role of alternative splicing of GluN1 exon 5, which encodes the N1 cassette. We generated mice lacking exon 5 (GluN1a) or compulsorily expressing this exon (Glun1b). Here we show that GluN1a and GluN1b mice are viable, develop normally and have normal basal synaptic transmission at CA3-CA1 synapses of the hippocampus. The presence or absence of exon 5 did not change the input-output relationship of basal synaptic transmission. There were no significant differences between wild-type, GluN1a or GluN1b mice in their ratio of NMDAR- to AMPAR-mediated excitatory postsynaptic currents (EPSCs) nor in their paired-pulse ratio (PPR) of fEPSPs. Current-voltage relationship (I-V) of NMDARs does not change by exon 5 splicing, suggesting NMDAR channel function was not altered. However, we found that theta burst stimulation (TBS)-induced long-term potentiation (LTP) was significantly lower in hippocampal CA1 slices taken from GluN1b mice ($125.48 \pm 4.60\%$ n=10, $p<0.05$ vs WT or GluN1a) compared to slices taken from either wild-type ($150.39 \pm 4.61\%$, n=19) or GluN1a mice ($147.95 \pm 5.39\%$). In contrast, slices from GluN1a mice had significantly stronger LTP when induced by 20Hz stimulation versus GluN1b or wild-type slices ($148.03 \pm 2.86\%$ n=6 for GluN1a, $p<0.05$ vs WT or GluN1b; $130.15 \pm 1.64\%$ n=9 for WT; $130.02 \pm 2.26\%$ n=8 for GluN1b). Additionally, GluN1b mice had reduced learning and memory performance in the Morris-water maze test compared with wild-type mice while GluN1a mice performed better than wild-type mice. Together, these data show that splicing of exon 5 modulates LTP strength, and learning and memory behavior in mice. We have thus defined for the first time a biological function for alternative splicing of GluN1 in vivo.

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Poster

370. NMDA Receptors

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 370.23/D28

Topic: B.02. Ligand-Gated Ion Channels

Support: CIHR Research Foundation Grant (FDN-154336)

Title: GluN1 N1-cassette regulates glycine-primed internalization and NMDA channel activity in hippocampal CA1 pyramidal neurons

Authors: *V. RAJANI, H. LI, A. S. SENGAR, D. CHUNG, L. HAN, M. W. SALTER
Neurosciences & Mental Hlth., The Hosp. for Sick Children, Toronto, ON, Canada

Abstract: N-methyl-D-aspartate receptors (NMDARs) are a subtype of ionotropic glutamate receptors that play a central role in numerous physiological and pathological conditions of the central nervous system. NMDARs are assembled as tetramers composed of two GluN1 subunits and two of four possible GluN2 subunits. NMDARs are activated upon simultaneous binding of co-agonists glycine and glutamate to the GluN1 and GluN2 subunits, respectively. We reported previously that glycine (30μM-1mM) initiates a signaling event that primes the receptors for dynamin-dependent endocytosis upon addition of glutamate (*Nature* 2003). Here we show that glycine-primed internalization of recombinant NMDARs expressed in HEK293 cells is prevented by the presence of alternatively spliced exon 5 (N1 cassette) in GluN1. Western blot of co-immunoprecipitation revealed that glycine stimulation significantly increased the association of endocytosis adaptor protein AP-2 with recombinant NMDA receptors expressing exon 5-lacking (GluN1a) but not exon 5-containing (GluN1b) subunits. Secondly, high glycine did not induce the progressive, ~50% reduction in NMDAR-mediated current in HEK-293 cells expressing GluN1b subunits that we observed with cells expressing GluN1a subunits. To determine the role of alternative splicing of the N-terminal exon in neurons, we generated mice lacking exon 5 or compulsorily expressing this exon. In hippocampal slices from GluN1a mice, pretreatment with a high glycine concentration produced a subsequent and sustained decline in NMDAR-mediated synaptic currents (68.1±5.2 of baseline, n=13, $p<0.05$). However, in slices from GluN1b mice, NMDAR-mediated synaptic currents fully recovered to baseline levels (95.0 ± 6.5, n=7, p=0.22). Our results indicate that exon 5-lacking NMDA receptors are permissive to glycine primed internalization, while exon 5-containing NMDA receptors are not. This differential mechanism may regulate cell surface expression of NMDARs in neurons. Our findings suggest that GluN1a-NMDARs at synapses in the hippocampal pyramidal neurons may be primed for internalization by glycine, leading to the depression of NMDAR synaptic transmission.

Disclosures: V. Rajani: None. **H. Li:** None. **A.S. Sengar:** None. **D. Chung:** None. **L. Han:** None. **M.W. Salter:** None.

Poster

371. Non-NMDA Receptors

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 371.01/D29

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant 5R01NS061920
CNPq 204709/2014-8

Title: Phosphorylation of GluA1-S845 regulates clathrin-mediated endocytosis of AMPA receptors

Authors: *M. F. SATHLER[1,2], L. KHATRI[3], R. KUBRUSLY[4], S. KIM[2], E. ZIFF[3]
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Abstract: Introduction: During scaling-up, AMPARs accumulate at synapses, restoring synaptic strength. In most scaling-up protocols, GluA2-lacking AMPA receptors accumulate selectively at the synapse, despite the fact that the great majority of AMPARs are GluA1/2 heteromers. Although it has been shown that GluA1 S845 phosphorylation reduces endocytosis, the molecular basis for regulation of GluA1 endocytosis is not well understood. **Objective:** To study how GluA1 S845 phosphorylation regulates clathrin-mediated endocytosis during neuron activity. **Methods:** Cortical neurons were obtained from E18 Sprague-Dawley rat embryos and cultured as mixed cultures for immunofluorescence and co-immunoprecipitation, after 48h with TTX or chemical LTP induction. Membrane fraction of Sprague-Dawley rat whole brain was used for the GST pull-down assay. Unpaired two-tailed Student's t-tests were used in single comparisons. For multiple comparisons, we used one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test to determine statistical significance. P value < 0.05 was considered statistically significant. **Results:** Previous results from the group have shown that 48h TTX treatment increases GluA1 S845 phosphorylation (Kim and Ziff, 2014). 48h TTX treatment reduces GluA1 endocytosis (CTL=1; TTX=0.63±0.02) and increases surface expression (CTL=1; TTX=1.17±0.04). Blockage of dynamin with 25 µM Dynole increases binding of clathrin adaptor AP2 to GluA1 (CTL=1; DYN=1.46±0.13). 48h TTX treatment reduces B-Adaptin binding to GluA1 (CTL=1; TTX=0.43±0.11). Induction of chemical LTP increases phosphorylation of GluA1 S845 (CTL=1; cLTP=5.55±0.84) and decreases B-Adaptin binding to GluA1 (CTL=1; cLTP=0.24±0.08). GST pull-down assay show less binding of GluA1 to the S845D phosphomutant (GLUA1=1; S845D=0.31±0.06). **Conclusion:** TTX treatment for 48 hours, a well stablished scaling up protocol, diminishes GluA1 endocytosis rate and the binding of β-Adaptin, a subunit of the AP2 adaptor, to GluA1. GluA1 S845 phosphorylation decreases the binding of AP2 to the CTD of this AMPA receptor subunit, unveiling a mechanism of phosphorylation-regulated clathrin-mediated endocytosis of GluA1. **References**: Kim S, Ziff EB (2014) Calcineurin Mediates Synaptic Scaling Via Synaptic Trafficking of Ca2+-Permeable AMPA Receptors. PLoS Biol 12(7): e1001900. doi:10.1371/journal.pbio.1001900

Disclosures: M.F. Sathler: None. **L. Khatri:** None. **R. Kubrusly:** None. **S. Kim:** None. **E. Ziff:** None.

Poster

371. Non-NMDA Receptors

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 371.02/D30

Topic: B.02. Ligand-Gated Ion Channels

Support: DFG PL619/2-1

Title: Photoactive unnatural amino acids reveal functional modules of the AMPA receptor membrane domain

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Abstract: Ionotropic glutamate receptors (iGluRs) are ligand gated ion channels, which are fundamental for normal brain function and are proposed to be involved in numerous central nervous system (CNS) diseases. Consequently, considerable efforts have been dedicated to study the function of iGluRs. However, despite recent progress in resolving their structural architecture and dynamics, but current methods lack specificity in the membrane domain. Here, we present an optogenetic approach with the goal of elucidating receptor dynamics mediated by specific AMPA-type iGluRs. This method involves site-specific incorporation of unnatural amino acid (UAA) photo-crosslinkers by unnatural mutagenesis. Thus, we create photo-controllable AMPA receptors that can be regulated with high spatiotemporal precision, using light as the orthogonal input signal. We introduced the UAAs p-benzoyl-L-phenylalanine (BzF) and p-azido-L-phenylalanine (AzF) at individual sites throughout the transmembrane domain (TMD) of AMPA receptor subunits. Recordings of outside-out patches from HEK cells in combination with synchronised exposure to UV light via epi-illumination led to a range of effects on channel activity, from fast inhibition to potentiation. One of the most striking UV effects arose from the F579 site harbouring AzFin the base of the M2 re-entrant helix, which showed complex changes in receptor kinetics after crosslinking AzF upon UV illumination.Entry to desensitzation was reduced but the desensitized state also became more stable. These results suggest a distinct role of the M2 segment in channel gating, beyond its canonical role in ion selectivity

Disclosures: A. Poshtiban: None. **M. H. Poulsen:** None. **V. Ghisi:** None. **A. Plested:** None.

Poster

371. Non-NMDA Receptors

Location: SDCC Halls B-H

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Program #/Poster #: 371.03/DP02/D31

Topic: B.02. Ligand-Gated Ion Channels

Support: ANR "PAINT"
ERC "ADOS"
CNRS
Aquitaine Region

Title: Role of synaptic plasticity in AMPA receptor intracellular trafficking

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Abstract: Abundance of AMPA receptors (AMPARs) at the synapse is essential for the establishment and maintenance of synaptic function. Their synaptic localization is dependent on a highly dynamic exocytosis, endocytosis and plasma membrane mobility events. Our hypothesis is that synaptic localization of AMPARs is also regulated by their intracellular trafficking at basal state and during LTP. However, AMPARs post-ER trafficking toward the plasma membrane still remains poorly understood because of the lack of appropriate biological and imaging tools. Using a new biochemical tool combined with photonic live imaging, we controlled and followed the dynamic secretion of tagged GluA1 containing receptors in cultured rat hippocampal neurons and characterize AMPAR intracellular transport. These analyzes are performed in basal condition, during induction of LTP and 20-50 min after LTP. Finally we have studied the impact of GluA1 phosphorylation on its transport by expressing phospho-mimetic mutants known to be phosphorylated during synaptic plasticity.

Disclosures: F. Coussen: A. Employment/Salary (full or part-time):; CNRS France. 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.; ANR France / ERC Europe. **E. Hangen:** None. **F. Cordelières:** A. Employment/Salary (full or part-time):; CNRS France. **J. Petersen:** None. **D. Choquet:** A. Employment/Salary (full or part-time):; CNRS France.

Poster

371. Non-NMDA Receptors

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 371.04/D32

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant AG056603-01

Title: APP phosphorylation and internalization regulates synaptic removal of AMPARs

Authors: *S. N. GARCIA DUBAR[1], D. CASTELLANO[1], M. ABDEL-GHANI[1], A. SCHNEEWEIS[1], E. ANDRE[1], S. VICINI[2], D. T. PAK[3]
[1]Pharmacol. & Physiol., Georgetown Univ., Washington, DC; [2]Dept Parmacology & Physiol., Georgetown Univ. Med. Ctr., Washington, DC; [3]Pharmacol., Georgetown Univ. Med. Sch., Washington, DC

Abstract: Alzheimer's disease (AD), characterized by progressive cognitive impairment and neuronal cell death, is the most common neurodegenerative disorder. The initiating step in AD pathogenesis is considered to be the accumulation of amyloid beta (Aβ), a proteolytic cleavage product of amyloid precursor protein (APP) Thus, understanding the regulation of APP processing is critically important. Aβ production is known to be responsive to synaptic activity levels, and we have recently identified polo-like kinase (Plk) 2 as a novel molecular mechanism underlying synaptic activity-dependent production of Aβ. We identified two sites in the APP cytosolic tail (Thr668/Ser675) that were directly phosphorylated by Plk2 and regulated APP internalization as well as amyloidogenic processing. Here, we aimed to test the hypothesis that phosphorylation of APP by Plk2 at these residues governs the intertwined processes of APP internalization, production of Aβ, and synaptic removal of AMPARs. Hippocampal neurons from embryonic Sprague-Dawley rats were cultured and plated on poly-D-lysine coated glass coverslips. At DIV 19 neurons were transfected by Lipofectamine 2000 with constructs expressing either APP WT or APP-2A-HSP (Thr668/Ser675 sites mutated to nonphosphorylatable alanines). Forty-eight hours after transfection, the neurons were subjected to patch clamp electrophysiological analyses. Current clamp recordings from these neurons showed the occurrence of spontaneous paroxysmal depolarizing shift occurring at the frequency of ~1.9 Hz. AMPAR mEPSCs were recorded in the presence of 1 μm TTX and 25 μM bicuculline and morphological analysis of neuronal population subtypes was performed as described (Lee et al., Neuron 2013). In morphologically identified CA3 pyramidal neurons transfected with APP-2A-HSP, mEPSCs peak amplitude was significantly larger (-18.24±2.159 pA, mean±SEM) than in neurons transfected with APP-WT (-12.58±1.396 pA). No change was observed in mEPSC frequency or decay time. Our results support the hypothesis that APP phosphorylation and internalization regulates synaptic removal of AMPARs. Further

characterization of different APP phosphosite mutations under various synaptic plasticity paradigms will be presented. We propose that dysregulations in these mechanisms may contribute to synaptic deficits and disease progression in AD.

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Poster

371. Non-NMDA Receptors

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Support: NINDS intramural Research program (S.W., Y.L., and K.W.R.)
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Title: Subunit-specific regulation of synaptic AMPARs by $step_{61}$

Authors: *S. WON[1], S. INCONTRO[3], Y. LI[1], R. A. NICOLL[4], K. W. ROCHE[2]
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Abstract: AMPA receptors (AMPARs) are ionotropic glutamate receptors that mediate fast excitatory neurotransmission and are expressed throughout the central nervous system. AMPARs are composed of four subunits (GluA1-4), which combine to form tetramers, mostly heteromeric. In addition, AMPAR modulation is a critical mechanism that underlies many forms of synaptic plasticity. The precise regulation of AMPAR expression, trafficking, and localization is critical for proper neuronal function. Phosphorylation, a posttranslational modification, regulates surface and synaptic expression of AMPARs. The S/T phosphorylation of AMPARs has been well studied, whereas there are fewer studies on the role of tyrosine phosphorylation of AMPARs. It has been reported that tyrosine phosphorylation by Src family kinases regulates AMPAR trafficking by inhibiting endocytosis. Striatal-enriched protein tyrosine phosphatase (STEP) is a brain-specific protein tyrosine phosphatase. STEP has splice variants: $STEP_{61}$, $STEP_{46}$, $STEP_{38}$, and $STEP_{20}$. All variants are expressed in the striatum, whereas $STEP_{61}$ is only expressed in cortex and hippocampus. $STEP_{61}$ regulates a variety of synaptic proteins such as ERK 1/2, p38, Fyn, and Pyk2 kinases via dephosphorylation at regulatory tyrosine residues within their activation region, thereby inactivating them. $STEP_{61}$ also regulates NMDARs. In the case of GluN2B, $STEP_{61}$ dephosphorylates Y1472 in its endocytic motif and facilitates the internalization of GluN2B-containing NMDARs. To better understand the effect of $STEP_{61}$ on other receptors and synaptic proteins, we used mass spectrometry to identify the $STEP_{61}$ interactome using mouse cortex and hippocampus. We identified a number of known interactors

such as GluN2B, PSD-95, and Fyn, but also novel ones including the GluA2 subunit of AMPARs. Interestingly, the synaptic expression of the AMPAR subunit GluA2 and 3 are increased in STEP knockout mouse brain and knock-down of STEP increases AMPAR-mediated synaptic currents. Our findings demonstrate a differential effect of $STEP_{61}$ on NMDAR vs. AMPAR. In both cases, $STEP_{61}$ dephosphorylates receptors but there is an extrasynaptic effect on NMDARs. Therefore, we report a comprehensive list of $STEP_{61}$ binding partners, including AMPARs, and reveal a critical role for $STEP_{61}$ in regulating synaptic AMPARs.

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Poster

371. Non-NMDA Receptors

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Program #/Poster #: 371.06/D34

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH grant MH079407
NSFC grant 81471304
NSFC grant 31771189

Title: Regulation of AMPA receptor stability and trafficking by acetylation in Alzheimer's disease

Authors: *M. O'CONNOR[1], Y.-P. SHENTU[2], G. WANG[1], Y. ZHANG[1], R. LIU[2], H.-Y. MAN[1]
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Abstract: AMPA receptors (AMPARs) mediate fast excitatory synaptic transmission. Regulation of AMPAR trafficking and surface expression is crucial for synaptic plasticity and brain functions such as learning and memory. Disruption in AMPAR postsynaptic surface expression can result in dysfunctional synaptic activity and ultimately impairments in brain function. We have recently reported that AMPARs are subject to lysine acetylation on GluA1 C-terminals. This post translational modification competes with ubiquitination, leading to increased stability by antagonizing receptor internalization and degradation. Here we show that p300 is the acetyltransferase responsible for this modification. In cultured hippocampal neurons, overexpression of p300 or activation of endogenous p300 results in an increase in AMPAR acetylation levels. This increase in GluA1 acetylation also results in an increase in both total and surface expression of AMPARs. We find that application of Aβ to neurons causes a reduction in AMPAR acetylation. Post-mortem brain tissue from Alzheimer’s Disease (AD) patients show a significant reduction in GluA1 acetylation and an increase in GluA1 ubiquitination compared

with age matched healthy controls. Consistently, an increase in acetylation of GluA1 ameliorates the Aβ-induced removal of AMPARs from the cell surface. Overexpression of acetylation-mimetic GluA1 (GluA1-4KQ) reduces Aβ-induced changes in GluA1 internalization and turnover. Likewise, overexpression of p300 in the presence of Aβ reduced the GluA1 internalization and degradation. These findings indicate that p300 functions as the acetyltransferase responsible for the lysine acetylation on AMPARs. AMPAR acetylation likely plays a key role in the aberrant alterations in AMPAR distribution and dynamics in AD.

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Poster

371. Non-NMDA Receptors

Location: SDCC Halls B-H

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Program #/Poster #: 371.07/D35

Topic: B.02. Ligand-Gated Ion Channels

Title: Claudins: A novel group of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) auxiliary proteins and a stepping stone to understanding transmembrane AMPAR regulatory protein function

Authors: *S. OBST[1,2,3], S. HAERING[1], A. KUZULU[1,4], M. HOLLMANN[1]
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Abstract: Ionotropic glutamate receptors (iGluRs) are responsible for the majority of excitatory signal transmission throughout the central nervous system (CNS). Especially AMPA receptors (AMPARs) play an important role in fast signal transmission but are also crucial for the onset of learning mechanisms in the brain.
The group of transmembrane AMPAR regulatory proteins (TARPs) support AMPARs by interacting with them and modulating their functional properties. We recently identified the family of claudins, which show high structural homology to TARPs and were formerly only known to be involved in assembling tight junctions, as potential candidates for a new class of modulatory proteins of iGluRs and AMPARs in particular[1].
We now investigated additional claudins, co-expressed with the AMPAR GluA1(Q)flip from *in vitro*-transcribed cRNA injected into *Xenopus laevis* oocytes. Two-electrode voltage clamp was used to measure glutamate- and glutamate-trichlormethiazide-(TCM)-induced whole cell currents. Five to seven oocytes per condition and day were measured in at least three different oocyte batches, normalized to the mean current response of the receptor alone, and then pooled and statistically analyzed.

We identified another functionally interacting claudin that in oocytes shows a 10-fold higher modulation than the weakly modulating claudins 20 and 24 investigated before.[1] We also present screening data of the modulatory function of 24 mammalian claudins for homomeric GluA2(Q)flip and GluA2(R)flip receptors. Via domain exchange mutations and point mutations we investigated the mechanism of interaction and current modulation that might be shared between TARPs and modulatory claudins. A shared mechanism was expected due to their similar structure in, e.g., the transmembrane domain 4 (TMD 4) or the extracellular loop between TMD 3 and the extracellular β-sheet 5, which have been reported to be critical for TARP modulation.[2] Differences in structure, such as in the extracellular loop between β-sheets 1 & 2, shed light on the much more efficient modulatory properties of TARPs. These structural and functional similarities as well as the distinct differences between these two groups of proteins depict claudins as a stepping stone in the evolution of auxiliary protein modulatory function.
[1] [Haering, S., Dissertation, Ruhr University Bochum, 2015]
[2] [Riva et al., eLife 2017; 6:e28680, Control of AMPA receptor activity by the extracellular loops of auxiliary proteins]

Disclosures: S. Obst: None. **S. Haering:** None. **A. Kuzulu:** None. **M. Hollmann:** None.

Poster

371. Non-NMDA Receptors

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Topic: B.02. Ligand-Gated Ion Channels

Support: MRC grant MC_U105174197
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BB/N002113/1

Title: Subunit organization in heteromeric AMPA receptors - Rules and functional consequences

Authors: *I. H. GREGER, H. HO, O. CAIS, B. HERGUEDAS, J. F. WATSON, B. KOHEGYI
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Abstract: AMPA-type glutamate receptors (AMPARs) mediate the majority of fast excitatory neurotransmission in the brain. They belong to the ionotropic glutamate receptor (iGluR) family, consisting of four subfamily members that comprise tetrameric cation channels.
iGluRs predominantly assembly as heteromers – NMDA-type receptors (NMDARs) are strict (obligatory) heteromers, whereas AMPARs are preferential heteromers but they can also form homomers. The two subunit pairs in a tetramer are conformationally distinct in all three major iGluR subfamilies, with a pore proximal (A/C) chain pair and a pore-distal (B/D) pair. The B/D chains are believed to exert a greater force on the channel gate upon agonist-triggered channel

opening than the A/C pair. In NMDARs, GluN1 subunits occupy the A/C position and GluN2 subunits the B/D slots. This division of labour is expected to also pose constraints on the organization of AMPAR hetero-tetramers. However, current data on AMPAR subunit organization are conflicting. Here we present a series of functional and structural data investigating the architecture and organization of AMPAR heteromers and their consequence on receptor signalling in recombinant cells and at synapses of CA1 hippocampal neurons.

Disclosures: I.H. Greger: None. **H. Ho:** None. **O. Cais:** None. **B. Herguedas:** None. **J.F. Watson:** None. **B. Kohegyi:** None.

Poster

371. Non-NMDA Receptors

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 371.09/D37

Topic: B.02. Ligand-Gated Ion Channels

Title: Pharmacology of AMPA receptors expressed in cell lines and stem cell-derived neurons

Authors: C. T. BOT[1], *A. R. OBERGRUSSBERGER[2], N. BRINKWIRTH[2], I. RINKE-WEIß[2], N. BECKER[2], T. A. GOETZE[2], P. MUMM[2], S. STOELZLE-FEIX[2], T. STRASSMAIER[1], M. GEORGE[2], A. BRÜGGEMANN[2], N. FERTIG[2]
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Abstract: The vast majority of excitatory neurotransmission is mediated by AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors. The functional receptor exists as a tetramer, either homomers or heteromers, from a repertoire of 4 different subunits, GluA1 - GluA4. It is well known that glutamate is a neurotoxin and it is proposed that overactivation of ionotropic glutamate receptors may underlie many neurodegenerative disorders such as ischemic stroke, epilepsy, Parkinson's and dementia, amongst others. Enhancement of AMPA receptor activation by, for example, BDNF, has been proposed to have beneficial effects of learning and memory and has potential therapeutic value in the treatment of depression, Huntington's and Parkinson's diseases. We have used GluA2 receptors expressed in HEK cells on 3 different automated patch clamp systems recording from either 1, 8 or 384 cells simultaneously. We have compared the glutamate EC_{50} obtained on the different platforms and found that they are similar, ranging from approximately 30 - 70 µM. Responses to glutamate were reproducible using a concentration of 30 or 100 µM, GluA2 could be repetitively activated at least 3 or more times, making the assay suitable for pharmacological characterization. Using pharmacological agents we could either inhibit or enhance the glutamate elicited responses. The inhibitor, CNQX, blocked glutamate responses mediated by GluA2 with an IC_{50} of 600 nM using a glutamate concentration of 100 µM and the concentration response curve for CNQX was dependent on the glutamate concentration. The potentiator, LY404187, was pre-incubated prior to co-application

with 100 µM glutamate giving an EC_{50} of around 500 nM. In addition to GluA2 expressed in HEK cells, we also recorded glutamatergic-enriched cortical neurons derived from induced pluripotent stem cells on an automated patch clamp platform. In these neurons we recorded NaV currents which could be blocked by low concentrations of TTX with an IC_{50} of 12 nM. In addition, glutamate responses were recorded which were potentiated by LY404187.

Disclosures: C.T. Bot: A. Employment/Salary (full or part-time):; Nanion Technologies Inc. **A.R. Obergrussberger:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **N. Brinkwirth:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **I. Rinke-Weiß:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **N. Becker:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **T.A. Goetze:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **P. Mumm:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **S. Stoelzle-Feix:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **T. Strassmaier:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **M. George:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **A. Brüggemann:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **N. Fertig:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH.

Poster

371. Non-NMDA Receptors

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 371.10/D38

Topic: B.02. Ligand-Gated Ion Channels

Title: Susd4, a tether for hect ubiquitin ligases, controls ampa receptor turnover, synaptic transmission and plasticity

Authors: *I. GONZÁLEZ-CALVO[1], K. IYER[1], A. KHAYACHI[1], F. A. GIULIANI[2], J. VINCENT[3], S. M. SIGOILLOT[1], Y. NADJAR[4], A. DUMOULIN[4], A. TRILLER[4], J.-L. BESSEREAU[5], L. RONDI-REIG[3], P. ISOPE[2], F. SELIMI[1]
[1]Cirb-Collège De France, Paris, France; [2]INCI, Univ. de Strasbourg - CNRS, Strasbourg, France; [3]IBPS, Univ. Paris VI, Paris, France; [4]IBENS, Ecole Normale Supérieure, Paris, France; [5]INMG, Univ. Claude Bernard, Lyon, France

Abstract: Brain function and plasticity relies on the tight regulation of the stoichiometry of neurotransmitter receptors and other proteins at synapses. While the molecular mechanisms controlling synaptic incorporation of glutamate receptors are well described, what controls their removal from synapses remains to be found. Here we show that the complement-related transmembrane protein SUSD4 controls synaptic transmission and plasticity of excitatory synapses by tethering ubiquitin ligases of the HECT family and regulating AMPA receptor

turnover. SUSD4 is highly expressed in neurons during postnatal development, a time of intense synaptogenesis and synapse maturation. Affinity purification followed by mass spectrometry revealed that E3 ubiquitin ligases of the NEDD4 family bind to the cytoplasmic tail of SUSD4. Morphological analysis of *Susd4* knockout mice showed no deficit in excitatory synaptogenesis in cerebellar Purkinje cells. However, invalidation of *Susd4* led to increased transmission at Climbing Fiber/Purkinje Cell synapses, lack of climbing fiber-dependent Parallel Fiber long-term depression and facilitation of Parallel fiber long-term potentiation in *Susd4* knockout mice. This phenotype was accompanied by a deficit in motor coordination learning. Immunolabeling experiments and miniature EPSC analysis showed increased and more homogeneous GluA2 levels in Purkinje cells synapses in *Susd4* knockout mice when compared to controls, suggesting that SUSD4 is a tether for the machinery controlling GLUA2 degradation. This chaperone function in targeting HECT ubiquitin ligases could be a general mechanism allowing precise spatiotemporal control of the turnover of specific target proteins in cells.

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Poster

371. Non-NMDA Receptors

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Support: GAUSSI/NSF award DGE-1450032
CSU/CVMBS

Title: The phosphatase PTP-3/LAR regulates transport of AMPARs in the nematode *C. elegans*

Authors: *D. PIERCE[1], B. PULFORD[1], F. J. HOERNDLI[2]
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Abstract: Normal cognition is dependent on AMPAR (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) trafficking, which includes local synaptic trafficking and long-distance transport. Previous work has shown a critical role for the phosphatase leukocyte common antigen-related protein (LAR), in regulating the trafficking of AMPARs to synapses. LAR activity is mediated by a member of the LAR protein tyrosine phosphatase-interacting protein family, Liprin-α. These studies have shown that the localization of LAR to synaptic spines is dependent upon the degradation of Liprin-α by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). However, despite this knowledge we do not know how LAR regulates AMPAR transport. One of our previous studies has shown that kinesin-mediated transport of

AMPARs is controlled by CaMKII. This suggests that LAR might be a downstream target of CaMKII in AMPAR transport regulation. This study aims to identify the function of LAR in long-distance AMPAR transport. Specifically, it will define the site of action of LAR on AMPAR trafficking as well as its role in the CaMKII signaling cascade regulating long-distance transport of synaptic AMPARs. We have previously established an imaging platform enabling the tracking and manipulation of *in vivo* AMPAR transport in real-time using the transparent model *C. elegans*. Here we combine this imaging approach using fluorescently labeled GLR-1 (GluA1 homologue) and genetic analyses to study the role of LAR in long-distance AMPAR transport *in vivo*. First, we measured synaptic SEP::mCherry::GLR-1 at proximal and distal synapses and found that synaptic GLR-1 were decreased at proximal synapses, but increased at distal synapses in LAR loss-of-function mutants. Next, we used time-lapse confocal imaging combined with photobleaching to track transport of single GLR-1 containing vesicles. LAR mutants display decreased transport of single vesicles. Lastly, we used fluorescence recovery after photobleaching (FRAP) and show that LAR mutants have decreased delivery of GLR-1 to synapses. Furthermore, LAR mutants exhibited a decrease in spontaneous reversals, a behavior dependent on functional synaptic GLR-1. Taken together, our results show a critical role of LAR in long-distance synaptic AMPAR transport. However, these results still leave some questions unanswered, for example: how does LAR interact with CaMKII in GLR-1 transport regulation? To better understand this, we will combine *in vivo* transport, LAR structure function analysis and CaMKII genetic epistasis analyses.

Disclosures: D. Pierce: None. **B. Pulford:** None. **F.J. Hoerndli:** None.

Poster

371. Non-NMDA Receptors

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 371.12/D40

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant DA041883

Title: Effects of cornichon homolog-3 (CNIH3) knockdown on hippocampal AMPA receptors and synaptic morphology

Authors: *H. E. FRYE[1], C. TROUSDALE[3], S. B. WILLIAMS[4], J. D. DOUGHERTY[2], E. C. NELSON[5], J. MORON-CONCEPCION[3]
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Abstract: Glutamatergic AMPA receptors (AMPARs) are abundant neurotransmitter receptors in the brain which are tightly regulated by a diverse array of synaptic auxiliary proteins. One AMPAR auxiliary protein whose precise function in the brain remains largely unknown is cornichon homolog-3 (CNIH3). Prior studies of cornichon homologs predominately focus on CNIH2, but a recent genome-wide association study (GWAS) of heroin users identifies single nucleotide polymorphisms (SNPs) in *CNIH3* correlated with protective effects against opioid dependence independent of *CNIH2*. Based on prior studies of CNIH3's effect on AMPAR activity and new evidence that CNIH3 may play a unique role in higher order behaviors, we hypothesize that CNIH3 regulates AMPAR-dependent memory and learning behavior through maintenance of AMPAR interactions with additional regulatory proteins and synaptic activity mediating synaptic morphology. To investigate the specific function of CNIH3, we use a C57BL/6 knock-down (KD) mouse line from the Knockout Mouse Project. *CNIH3* KD animals express 60% less *CNIH3* than wild-type (WT) and *CNIH3* expression is highest in the hippocampus. No compensatory overexpression of *CNIH2* is observed in the KD genotypes. To determine the effects of *CNIH3* KD on AMPAR levels at the synaptic membrane, hippocampal post-synaptic density (PSD) fractions of 6-8-week-old male and female mice are analyzed via western blot analysis. AMPAR subunit levels are not quantifiably changed at the PSD between genotypes, but a significant reduction in the excitatory scaffolding protein PSD-95 is observed in *CNIH3* KD females compared to WT. To investigate potential changes due to *CNIH3* KD in AMPAR protein interactions, co-immunoprecipitation assays targeting AMPAR subunits are performed to measure interactions with additional auxiliary proteins. To assess changes in synaptic integrity that may result from reduced PSD-95 at the synapse, dendritic spine density and morphology are compared between genotypes. Future studies will investigate the behavioral effects of *CNIH3* KD on memory and learning in a Barnes Maze assay, where animal performance is dependent on hippocampal AMPAR function. Sex differences in CNIH3 function are not reported in the present literature, making the discovery of a possible sex-difference in the effects of *CNIH3* KD an important subject for future investigation. Once we understand the cellular mechanisms of hippocampal CNIH3 responsible for mediating behavioral outcomes, we plan to investigate how *CNIH3* SNPs found in the human heroin users can mediate a protective effect on opioid dependence.

Disclosures: H.E. Frye: None. **C. Trousdale:** None. **S.B. Williams:** None. **J.D. Dougherty:** None. **E.C. Nelson:** None. **J. Moron-Concepcion:** None.

Poster

372. Neurotransmitter Release: Vesicle Dynamics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 372.01/D41

Topic: B.05. Neurotransmitter Release

Support: NIH (NINDS/NIA R01NS078165)
Stanley Fahn Award (Parkinson's Disease Foundation) 2018
Michael J. Fox LEAPS Grant 2014
NIH (NINDS R01NS083845-05)

Title: Different molecular species of alpha-synuclein produce distinct effects on synaptic vesicle trafficking *in vivo*

Authors: *A. T. MEDEIROS[1], J. B. SANDERSON[2], H. JIANG[2], D. J. SELKOE[3], T. BARTELS[2], J. R. MORGAN[1]
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Abstract: α-Synuclein is a 14 kDa presynaptic protein that normally regulates synaptic vesicle (SV) trafficking. Although the native forms of α-synuclein are debated, there is strong evidence that neurons generate both monomeric and multimeric α-synuclein under physiological conditions. Altering the balance of these normal molecular species of α-synuclein leads to its aggregation and contributes to neurodegenerative processes in Parkinson's disease (PD), dementia with Lewy bodies (DLB), and other synucleinopathies. Yet how each molecular species of α-synuclein affects synapses and impacts SV trafficking is not understood. We have started to address this by acutely introducing different forms of α-synuclein directly into lamprey giant reticulospinal synapses and determining the effects on SV trafficking. Using detailed ultrastructural analyses, we previously showed that both monomeric and dimeric recombinant α-synuclein severely impaired clathrin-mediated synaptic vesicle recycling. However, while the recombinant α-synuclein monomers inhibited clathrin uncoating, dimeric recombinant α-synuclein inhibited the earlier stage of vesicle fission, providing the first evidence that different molecular species of α-synuclein produce distinct effects on SV trafficking. Here, we focused on the effects of physiological α-synuclein isolated by size-exclusion chromatography (SEC) from healthy human brain, comprising a mixed population of α-synuclein that includes physiological tetramers as well as monomers. Although introduced intra-axonally at low nanomolar concentrations, synapses treated with SEC fractions rich in physiological α-synuclein exhibited a partial depletion of the SV pool, which was compensated by larger diameter SVs and increased number and size of large (>100nm) intracellular vesicles ("cisternae"), suggesting moderate effects on intracellular SV trafficking. However, unlike monomeric and dimeric recombinant α-synuclein, normal brain-derived α-synuclein produced no significant effects on clathrin-mediated synaptic vesicle endocytosis from the plasma membrane. These data provide additional evidence that different molecular species of α-synuclein can produce distinct effects on SV trafficking and suggest that physiological α-synuclein multimers may be partially protective against the deleterious effects of monomers. Ongoing experiments are aimed at identifying the underlying mechanisms and testing the effects of α-synuclein isolated from brains of patients with PD or DLB. Together, these studies should lend insights into the normal synaptic functions of α-synuclein and how these are altered in disease.

Disclosures: A.T. Medeiros: None. **J.B. Sanderson:** None. **H. Jiang:** None. **D.J. Selkoe:** None. **T. Bartels:** None. **J.R. Morgan:** None.

Poster

372. Neurotransmitter Release: Vesicle Dynamics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 372.02/D42

Topic: B.05. Neurotransmitter Release

Support: ISF 1427/12

Title: Synapsin and alpha-synuclein co-regulate synaptic transmission in cultured hippocampal neurons

Authors: *D. GITLER[1], M. ATIAS[1], Y. TEVET[1], A. STAVSKY[1], S. TAL[1], J. KAHN[1], S. ROY[2]
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Abstract: Synaptic transmission is regulated by synapsin and alpha-synuclein (αsyn), the latter being prominently associated with neurological disease. These proteins inversely affect the density of vesicles in the presynaptic terminal and the overall capability of terminals to secrete neurotransmitter. We therefore hypothesized that synapsin and αsyn interact functionally to co-regulate synaptic properties. To test our hypothesis, we directly probed the proximity between synapsin Ia and αsyn and investigated their co-dependence in affecting vesicle recycling. Fluorescence resonance energy transfer (FRET) measurements illustrated that synapsin and αsyn are within molecular-interaction range of each other at rest, but that synaptic activity dissociates them. Moreover, we uncovered that deletion of the synapsins abolishes the inhibitory effect of αsyn on synaptic vesicle recycling, measured using synaptophysin-2XpHluorin and FM1-43. We found that the reduction in the relative size of the recycling pool of vesicles by αsyn was dependent on the presence of synapsins, as was the ability of αsyn to restrict synaptic vesicle mobility between synapses. Finally, the inhibitory effect of αsyn on neurotransmission was reinstated by the reintroduction of exogenous synapsin Ia in synapsin triple-knockout neurons. Our results thus show that key synaptic effects of αsyn are synapsin-dependent, they provide novel insight on the physiological role and interactome of αsyn, and suggest novel opportunities for therapeutic intervention.

Disclosures: D. Gitler: None. **M. Atias:** None. **Y. Tevet:** None. **A. Stavsky:** None. **S. Tal:** None. **J. Kahn:** None. **S. Roy:** None.

Poster

372. Neurotransmitter Release: Vesicle Dynamics

Location: SDCC Halls B-H

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Topic: B.05. Neurotransmitter Release

Support: NIH Grant (NINDS/NIA) R01 NS 078165
Marine Biological Laboratory
Horizon 2020 Grant no. InCure EU Joint Programme, JPND
NIH/NIGMS R01 GM 118933

Title: Hsc70 is an *in vivo* target of excess α-synuclein at synapses

Authors: *M. MCQUILLAN[1], A. T. MEDEIROS[1], S. M. L. BANKS[2], I. TESSARI[3], L. BUBACCO[3], R. SOUSA[4], E. M. LAFER[4], J. R. MORGAN[1]
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Abstract: Parkinson's disease (PD) is characterized by atypical aggregation of α-synuclein in neurons, including at synapses. α-Synuclein is a presynaptically-localized protein that exists physiologically as monomers and multimers. Altering the balance of these normal molecular species leads to aggregation and toxicity in neurons. However, very little is known about how disease-related events, such as overexpression, multimerization or aggregation, affect presynaptic processes. We and others have shown that acute and chronic increases in α-synuclein levels inhibit synaptic vesicle (SV) recycling and/or re-clustering. The underlying mechanisms are presently unknown, prompting the current studies. Using lamprey giant reticulospinal synapses, we show that acute introduction of excess monomeric α-synuclein impairs the uncoating of clathrin-coated vesicles (CCVs) during clathrin-mediated SV recycling. Furthermore, α-synuclein interacts directly *in vitro* with Hsc70, the chaperone protein that serves as the clathrin uncoating ATPase at synapses. Immunofluorescence assays further revealed that increasing the levels of monomeric α-synuclein inhibited stimulation-dependent recruitment of Hsc70 to synapses, indicating that α-synuclein sequesters Hsc70 *in vivo*. Co-injection of exogenous bovine Hsc70 along with α-synuclein rescued the clathrin uncoating and endocytic defects, implicating Hsc70 as a possible ameliorator of α-synuclein induced synaptic defects. Recently, we have begun to examine the effects of α-synuclein multimers at synapses and their potential interactions with Hsc70. When acutely introduced to lamprey synapses, dimeric α-synuclein also inhibited clathrin-mediated SV recycling. However, unlike monomers, dimeric α-synuclein impaired an earlier stage of vesicle fission, while the CCVs were unaffected, indicating that different molecular species of α-synuclein produce distinct effects at synapses.

Further supporting this idea, preliminary data suggest that α-synuclein dimers increase the amount of Hsc70 at unstimulated and stimulated synapses, relative to monomeric α-synuclein. This observation provides additional evidence that α-synuclein targets synaptic Hsc70 *in vivo* and may also explain why CCV uncoating is unaffected under these conditions. Ongoing experiments will determine whether α-synuclein dimers and other multimers functionally interact with Hsc70. Taken together, these data implicate synaptic Hsc70 as a target of excess α-synuclein at synapses and provide mechanistic insight into the α-synuclein induced synaptic defects associated with PD and other synucleinopathies.

Disclosures: M. McQuillan: None. **A.T. Medeiros:** None. **S.M.L. Banks:** None. **I. Tessari:** None. **L. Bubacco:** None. **R. Sousa:** None. **E.M. Lafer:** None. **J.R. Morgan:** None.

Poster

372. Neurotransmitter Release: Vesicle Dynamics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 372.04/D44

Topic: B.05. Neurotransmitter Release

Support: NIH NS082759

Title: Endocytosis is the primary target for short-term overexpression of alpha-synuclein

Authors: X. WU[1], T.-W. LIN[1], W. JANG[2], H.-Y. YUE[1], *J. XU[3]
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Abstract: Multiplication and mutations of alpha-synuclein, a protein primarily located at nerve terminals, are one of the major risk factors in familial and sporadic Parkinson's disease as well as dementia with Lewy bodies. At the cellular level, short-term overexpression or acute oversupply of alpha-synuclein in neurons has been reported to change the cellular excitability by forming non-selective ion pores, affect Ca channel partition by reducing membrane cholesterol, impair neurotransmission by disrupting vesicle clusters, and reduce vesicle supply by inhibiting endocytosis. These findings raise an interesting question as to which process is the initial and more vulnerable target for increased levels of alpha-synuclein. To address this question, we first performed whole-cell patch-clamp recordings at the calyx of Held terminals from postnatal 8-10 days old (P8-10) control mice and transgenic mice that selectively overexpress human A53T alpha-synuclein. Compared to control, calyx terminals from the transgenic mice did not show any change in the resting membrane potential, action potential firing, the amplitude voltage-gated Ca^{2+} channel current, or the voltage-dependence of Ca^{2+} channels. Endocytosis, instead, was significantly reduced in mutant terminals. These results suggest that excess A53T alpha-

synuclein preferentially disturbs endocytosis. To test whether similar effects occur in conventional synapses, we transfected the primary cultures of mouse hippocampal neurons with plasmids to overexpress human wild-type or mutants of alpha-synuclein. By pHluorin imaging, we found that overexpression of wild-type alpha-synuclein or A53T synuclein inhibited endocytosis of vesicular membrane proteins, synaptobrevin and synaptophysin, following exocytosis. Overexpression of A30P alpha-synuclein did not cause significant change to endocytosis. Taken together, we conclude that membrane endocytosis is the initial target regulated by increased levels of alpha-synuclein both in large nerve terminals and small conventional boutons. The mechanisms of such regulation are under investigation.

Disclosures: X. Wu: None. **T. Lin:** None. **W. Jang:** None. **H. Yue:** None. **J. Xu:** None.

Poster

372. Neurotransmitter Release: Vesicle Dynamics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 372.05/D45

Topic: B.05. Neurotransmitter Release

Support: NIH Grant R01NS045873

Title: SAX-7 and MPK-1/Erk promote coordinated locomotion

Authors: *S. SHEORAN[1], M. MOSELEY-ALLDREDGE[2], H. YOO[2], C. O'KEEFE[2], J. RICHMOND[1], L. CHEN[2]
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Abstract: L1CAMs are cell adhesion molecules involved in the development of the nervous system. As transmembrane proteins, L1CAMs transduce extracellular signals into cellular responses that require cross-talk with the cytoskeletal and intracellular signaling networks. Mutations in L1CAMs are directly linked to the neurological L1 syndrome and are implicated in neuropsychiatric disorders such as schizophrenia, the autistic spectrum disorder, and addiction. L1CAMs are conserved in vertebrates as well as invertebrates such as *Caenorhabditis elegans* and *Drosophila melanogaster*. Previous studies in *C. elegans, Drosophila* and mice revealed developmental roles for L1CAM including the axon guidance and dendrite morphogenesis, as well as maintenance of neural integrity. We recently uncovered a non-developmental role for *C. elegans* L1CAM, which is encoded by the *sax-7* gene, in promoting coordinated locomotion. *sax-7* genetically interacts with *rab-3*, *unc-13*, and *snb-1*, genes that function in the synaptic vesicle cycle, resulting in synthetic uncoordinated (Unc) locomotion. To determine the basis of the synthetic Unc phenotype, we performed a genetic suppressor screen and identified loss-of-function mutations in *ksr-1*, which encodes a scaffolding protein that facilitates MPK-1/Erk

activation. In addition to these genetic and behavioral findings, we have conducted electrophysiological and electron microscopy (EM) analyses to examine how SAX-7 and MPK-1/Erk signaling promote locomotion. The preliminary electrophysiology results show changes in evoked synaptic release consistent with the involvement of these genes in the synaptic transmission. These findings are further supported by high pressure freeze EM (HPF-EM) analysis. Together these functional data provide new insights into the interpretation of the behavioral phenotypes and the underlying circuitry involved.

Disclosures: S. Sheoran: None. **M. Moseley-Alldredge:** None. **H. Yoo:** None. **C. O'Keefe:** None. **J. Richmond:** None. **L. Chen:** None.

Poster

372. Neurotransmitter Release: Vesicle Dynamics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 372.06/D46

Topic: B.05. Neurotransmitter Release

Support: NSERC (JSD)
Heart and Stroke (JSD)
NSERC (MBS)
CIHR (MBS)

Title: Acute photoinactivation of a cGMP-dependent protein kinase reveals multiple synaptic functions at the *Drosophila* larval neuromuscular junction

Authors: *J. S. DASON[1], A. M. ALLEN[2,3], O. E. VASQUEZ[3], M. B. SOKOLOWSKI[3]
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Abstract: Kinases are pleiotropic and regulate a wide range of processes in neurons. However, it remains challenging to test the functions of kinases in processes that are tightly coupled owing to the inability to inactivate them with a high spatiotemporal resolution. For example, cGMP-dependent protein kinase (PKG) has been implicated in both synaptic vesicle (SV) exo- and endocytosis. These processes are tightly coupled, thus pharmacological and genetic approaches are unable to distinguish whether PKG affects both processes separately or only one with concomitant effects on the other. Here, we tagged the *Drosophila foraging* (*for*) gene, which encodes a PKG, for fluorescein-assisted light inactivation (FALI). We uncoupled SV exo- and endocytosis using a *shibire* temperature-sensitive dynamin mutant and then inactivated FOR with FALI. We discovered a dual role for presynaptic FOR, where FOR inhibits exocytosis during low frequency stimulation and facilitates endocytosis during high frequency stimulation. Additionally, glial FOR negatively regulates nerve terminal growth and this developmental effect

was independent from FOR's effects on neurotransmission. Our findings demonstrate the multifaceted nature of kinases and the need to inactivate them with high spatiotemporal resolution.

Disclosures: A.M. Allen: None. **O.E. Vasquez:** None. **M.B. Sokolowski:** None.

Poster

372. Neurotransmitter Release: Vesicle Dynamics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 372.07/D47

Topic: B.05. Neurotransmitter Release

Support: Johns Hopkins School of Medicine start-up package

Title: The effect of acute ATP depletion on synaptic vesicle endocytosis visualized at the ultrastructural level

Authors: *Q. GAN[1], S. WATANABE[2]
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Abstract: Following synaptic vesicle fusion at the presynaptic terminal, vesicular membrane and proteins must be retrieved via endocytosis to replenish the vesicle pool. A recent study revealed using the pHluorin assay that local depletion of ATP at presynaptic terminals impairs the rate of vesicle cycling during sustained synaptic activity, possibly by blocking endocytosis. However, due to limitations inherent to the pHluorin approach, it is unknown which exact step in the vesicle cycling process is most sensitive to the availability of ATP. In the present study, we used time-resolved electron microscopy to capture rapid ultrastructural changes in the presynaptic membrane of cultured hippocampal neurons at various time points following stimulation. We combined this method with drug treatments that acutely block ATP synthesis, and found that acute ATP depletion leads to the arrest of spontaneous as well as activity-induced synaptic vesicle endocytosis at early stages. In addition, blocking oxidative phosphorylation alone has a weaker effect than blocking both oxidative phosphorylation and glycolysis. This suggests that anaerobic glycolysis alone might be able to partially meet the energy demand of vesicle cycling. Our results shed new light on the energetics of synaptic vesicle endocytosis, and may have important implications for the pathophysiology of brain ischemia.

Disclosures: Q. Gan: None. **S. Watanabe:** None.

Poster

372. Neurotransmitter Release: Vesicle Dynamics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 372.08/D48

Topic: B.05. Neurotransmitter Release

Title: Differential effects of the neurotoxin taipoxin on distinct forms of endocytosis at central nerve terminals

Authors: *W. JANG, T.-W. LIN, H.-Y. YUE, S. LI, J. XU
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Abstract: Nerve terminals require endocytosis to maintain neurotransmission and synapse integrity. Depending on the intensity of neuronal activity, endocytosis involves different molecular machineries and demonstrates different forms, for example, slow endocytosis, rapid endocytosis, and endocytosis overshoot. To understand the different mechanisms underlying each form of endocytosis, it is important to have genetic and pharmacological tools that selectively inhibit or preserve that form. A snake neurotoxin, taipoxin, has long been known to block neurotransmission by impairing vesicle recycling. However, it remains a question as to whether taipoxin selectively affects any form(s) of endocytosis. Here, using whole-cell membrane capacitance measurements, we studied the effects of acute dialysis of taipoxin at the calyx of Held, a large auditory central synapse accesible to patch clamp technique. In control at 21-24 °C, stimulation with a train of ten 20 ms depolarization pulses at 10 Hz induced vesicle exocytosis followed by rapid endocytosis and slow endocytosis. Dialysis of taipoxin led to severe block of both forms of endocytosis. Taipoxin increased the amount of exocytosis induced by the first stimulation, but reduced it for the subsequent stimulation likely because releasable vesicles were depleted. In control at 33-35°C, stimulation with 500 action potential-like pulses induced excess endocytosis, in which the retrieved membrane exceeds the fused membrane in quantity. Dialysis with taipoxin caused partial inhibition of excess endocytosis. Combination of taipoxin and the nonhydrolyzable ATP analog ATPγS did not produce additional inhibition of excess endocytosis. As we recently found, ATP hydrolysis is required for slow endocytosis and rapid endocytosis, but not for the retrieval of pre-existing membrane that contributes to excess endocytosis. Collectively, our results suggest that taipoxin can be used to isolate endocytosis of pre-existing membrane and dissect mechanisms of endocytosis in future studies.

Disclosures: W. Jang: None. **T. Lin:** None. **H. Yue:** None. **S. Li:** None. **J. Xu:** None.

Poster

372. Neurotransmitter Release: Vesicle Dynamics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 372.09/D49

Topic: B.05. Neurotransmitter Release

Title: Selective cleavage of synaptobrevin by botulinum toxins reveals the prefusion state of the SNARE complex

Authors: *J. BRADY, A. VASIN, M. BYKHOVSKAIA
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Abstract: Neurons release neurotransmitters via the fusion of synaptic vesicles with the neuronal plasma membrane. Vesicle are attached to the membrane via the SNARE complex, a coil-coiled four-helical bundle which includes the vesicle attached protein Synaptobrevin (Syb) zippered onto the membrane associated t-SNARE bundle. It is thought that Syb is partially unraveled in the prefusion SNARE state, however, the specific structure of the partialy zippered prefusion SNARE complex is still debated. The cytosolic protein Complexin (Cpx) interacts with the SNARE complex and inhibits spontaneous vesicle fusion, possibly by preventing the full SNARE zippering. To investigate the pre-fusion state of the SNARE complex, we took advantage of botulinum neurotoxins (BoNT) that cleave unstructured Syb at distinct sites: serotype G, which cleaves Syb at the membrane proximal site (Layer 7), serotype B, which cleaves Syb at a more distal site (Layer 6), and serotype D, which cleaves Syb in the middle of the SNARE bundle in the vicinity of the ionic layer. BoNT mixed with a water soluble fluorescent dye was loaded through a suction pipette into a cut axon at the Drosophila larval neuromuscular junction (NMJ). The spontaneous activity was recorded continuously for thirty minutes from individual boutons at abdominal muscles 6 or 7 with focal macropatch electrodes. To minimize the loading time, we selected the boutons closest to the axon. In the end of each recording, we ascertained that the fluorescent dye reached the bouton. We found that serotype G, in contrast to other serotypes, produced a rapid depression of the spontaneous transmission, suggesting that in the prefusion SNARE state Syb is unraveled up to the Layer 6. To understand the role of Cpx in regulating the prefusion state of the SNARE complex, we repeated these experiments at the cpx-/- Drosophila line. Our results are consistent with the model wherein Cpx promotes a partially unzippered state of Syb.

Disclosures: A. Vasin: None. **M. Bykhovskaia:** None.

Poster

372. Neurotransmitter Release: Vesicle Dynamics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 372.10/D50

Topic: B.05. Neurotransmitter Release

Support: Okinawa Institute of Science and Technology

Title: Isoflurane inhibits presynaptic vesicle fusion machinery, thereby preferentially blocking high frequency excitatory neurotransmission

Authors: *H.-Y. WANG[1], K. EGUCHI[2], T. TAKAHASHI[3]
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Abstract: Volatile anesthetics are widely used to lower the level of consciousness for surgery and animal experiments, but their primary cellular target remains unidentified. At mammalian central synapses, the volatile anesthetic isoflurane attenuates transmitter release. At the calyx of Held in rat brainstem slices, quantal analysis indicated that isoflurane reduces both the release probability and the number of functional release sites. In presynaptic recording, isoflurane is shown to directly inhibit Ca^{2+} channels. Presynaptic membrane capacitance measurements revealed that isoflurane inhibits Ca^{2+} influx-independent component of exocytosis evoked by long pulses. In simultaneous recording of presynaptic and postsynaptic action potentials evoked by repetitive stimulations, isoflurane preferentially impaired fidelity of high-frequency transmission, with no effect on low-frequency transmission. We conclude that the primary target of isoflurane anesthesia is vesicle fusion machinery, which supports high-frequency bursts of postsynaptic action potentials required for the maintenance of consciousness.

Disclosures: H. Wang: None. **K. Eguchi:** None. **T. Takahashi:** None.

Poster

372. Neurotransmitter Release: Vesicle Dynamics

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Program #/Poster #: 372.11/D51

Topic: B.05. Neurotransmitter Release

Support: IARS GF13062
NIH-NIGMS 1 KO8 GM123321-01

Title: Neonatal exposure to anesthesia inhibits key protein regulators of synaptic vesicles docking and fusion

Authors: N. ATLURI, B. FERRARESE, H. OSURU, C. KELLER, R. SICA, Z. ZUO, *N. LUNARDI
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Abstract: Introduction: The effects of general anesthetics (GA) on synaptic vesicle exocytosis remain largely unexplored. In particular, it is not known how GA affects neurotransmitter release proteins during brain development. We previously demonstrated long-lasting decrease in the number of readily releasable vesicles docked at the active zone of CA1-subicular pre-synaptic boutons several days after neonatal GA. Here we test the hypothesis that the observed decrease is due to GA-induced impairment of transmitter release proteins involved in docking and fusion of vesicles to the pre-synaptic plasma membrane.
Methods: On postnatal day 7 (P7, peak of synaptogenesis) rats of both sexes were randomly allocated to receive a) sevoflurane 2.5% and oxygen (O2) 40% for 5 h or b) midazolam 9 mg/kg intraperitoneally, followed by 6 h of nitrous oxide 70%, isoflurane 0.75% and O2 30%. Sham-control rats were administered O2 40%, or intraperitoneal DMSO and O2 30%, respectively. Brains were harvested and the CA1-subiculum collected under a dissecting microscope. Protein and mRNA expression/activation levels of synapsin 1 (SYN 1), its active phosphorylated form (p-SYN 1, a critical effector of vesicle docking), and synaptotagmin 1 (SYT 1, a key facilitator of vesicle fusion) were measured using western blot and quantitative polymerase chain reaction, at 3 developmental time points: 24 h after GA (P8), 5 days after GA (P12) and at P24 (an age that approximates early adolescence in rats). The presence of developmental learning/memory deficits was assessed using the Barnes Maze at P30. Experimenters were blinded to experimental condition.
Results: We found a significant decrease in the levels of SYN 1 (N=12/group, p=0.009 and p=0.008), p-SYN 1(N=12/group, p=0.001 and p=0.03) and SYT 1 (N=12/group, p<0.0001 and p=0.001) at P12 and P24 in rats exposed to midazolam-nitrous oxide-isoflurane. These animals also exhibited a significant decrease in mRNA levels of SYN 1 (N=7/group, p=0.009) and SYT 1 (N=7/group, p=0.0007) at P8. Rats exposed to sevoflurane displayed a significant decrease in SYN 1 at P8 (N=12/group, p=0.02) and P12 (N=12/group, p=0.008). Female rats exposed to midazolam-nitrous oxide-isoflurane (N=9/group) exhibited impairments in long-term at P41 (p=0.03), but not short-term, memory. Female rats exposed to sevoflurane (N=10/group) showed impaired learning during day 3 of task training (p=0.03), and impaired short-term (p=0.01) at P34 and long-term (p=0.01) spatial memory.
Conclusions: Exposure to GA during brain development may depress synaptic vesicle exocytosis by impairing the expression of key pre-synaptic proteins that regulate vesicle docking and fusion.

Disclosures: N. Atluri: None. **B. Ferrarese:** None. **H. Osuru:** None. **C. Keller:** None. **R. Sica:** None. **Z. Zuo:** None. **N. Lunardi:** None.

Poster

372. Neurotransmitter Release: Vesicle Dynamics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 372.12/E1

Topic: B.05. Neurotransmitter Release

Support: National Taiwan University
The Ministry of Science and Technology

Title: Synaptotagmin III regulates exosome export through calcium binding to the C2AB domains

Authors: N.-Y. YU[1], Y.-H. TSAI[1], H.-J. YANG[4], *C.-T. WANG[5,1,2,3,4]
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Abstract: The Synaptotagmin (Syt) protein superfamily, serving as calcium sensor during calcium-dependent exocytosis, consists of two calcium-binding C2 domains (C2A and C2B). Upon calcium binding, Syt-C2AB interacts with SNAREs, thus triggering vesicle fusion. Among Syt isoforms, Syt III particularly consists of six calcium-binding sites. We previously found that through calcium binding to the C2AB domains, Syt III can regulate the fusion pore kinetics of dense-core vesicles (DCVs) and modulate the interaction with a t-SNARE, SNAP-25. Because Syt III is localized mainly to plasma membrane but partially to DCVs, we speculated that in addition to vesicle fusion, Syt III may mediate other cellular functions. Previous studies showed that one type of extracellular vesicles (exosomes) can be released in a calcium-dependent manner. Syt VII, an isoform similar to Syt III, can regulate exosome export, leading to a hypothesis that Syt III may also regulate exosome export. However, there is no evidence for this up to date. In this study, we determined the role of Syt III-C2AB in regulating exosome export. After overexpressing Syt III or Syt III-C2AB* (a mutant harboring the weakened calcium-binding ability in the C2AB domains) in PC12 cells, we detected the changes in subcellular localization of exosomes with the marker, CD9 or CD63. The results from immunofluorescence staining demonstrated that in the absence of high-KCl stimulation, Syt III was evenly distributed in the cytoplasm, but localized to plasma membrane upon high-KCl treatment. Moreover, upon high-KCl stimulation, overexpressing Syt III enhanced exosome export. By comparing with Syt III-overexpressing cells, exosome export was disturbed in cells overexpressing Syt III-C2AB* upon KCl depolarization. Furthermore, by harvesting the KCl-treated medium to isolate exosomes for western analysis, we confirmed that Syt III-C2AB* reduced the amount of exosomes secreted to the medium compared to Syt III. In conclusion, our results suggest that Syt III regulates exosome export through calcium binding to the C2AB domains.

Disclosures: N. Yu: None. **Y. Tsai:** None. **H. Yang:** None. **C. Wang:** None.

Poster

372. Neurotransmitter Release: Vesicle Dynamics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 372.13/E2

Topic: B.05. Neurotransmitter Release

Support: Presbyterian Health Funds
COMAA grant

Title: Age-related cognitive impairment: Role of reduced synaptobrevin-2 levels in the decline of learning and memory

Authors: *F. DEAK[1,2,3]
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Abstract: Introduction: Age related cognitive decline is one of the major disabilities that affect the elderly. It is estimated that number of patients suffering from dementia will reach 10 million in the United States by the year 2030 with significant socio-economic impacts. Synaptic plasticity is a central mechanism in learning and memory and synaptic dysfunction is emerging as the major cause of mild cognitive impairment and dementia. The cellular and molecular changes leading to cognitive decline with age remain elusive. Synaptobrevin-2 (syb2), the major vesicular SNAP receptor protein, highly expressed in the cerebral cortex and hippocampus, is essential for synaptic transmission. We have analyzed syb2 protein levels in mice and found a decrease with age. We hypothesized that reduction of syb2 protein levels with age mediates cognitive decline.
Methods: To investigate the functional consequences of lower syb2 expression we have used adult syb2 heterozygous mice ($syb2^{+/-}$) with reduced syb2 levels. This allowed us to mimic the age-related decrease of syb2 in the brain in order to selectively test its effects on learning and memory. We performed behavioral tests for spatial learning and memory using the radial-arm water maze (RAWM) and a complex cohabitate environment called Intellicage. Neuronal plasticity was assessed by long-term potentiation (LTP) assays on hippocampal slices. We used live fluorescence microscopy to measure the synaptic vesicular (SV) release rate in neuronal cultures.
Results: We found that syb2 knock-out mice express syb2 at about the same level at 12 month-of-age as old (24 mo) wild-type animals. This is 50-60% of the protein levels found in young wild-type littermates (n=3, $p<0.01$, Student t-test). $Syb2^{+/-}$ mice maintained basic spatial memory acquisition for simple place finding (RAWM) but was impaired in spatial memory and learning complex tasks in Intellicage at age of 12 mo compared to WT (n=7-14). $Syb2^{+/-}$ hippocampal

neurons had reduced release probability, reduced synaptic plasticity and impaired LTP in the CA1 region. Syb2$^{+/-}$ neurons also have lower vesicular release rates (~35% reduction at 15 seconds, $p<0.01$ compared to WT). We also obtained results from 24 mo syb2$^{+/-}$ animals, which had impaired learning and memory skills as they perform worse at this age in the radial arm water maze assay.
Conclusions: Our study demonstrates that reduced syb2$^{+/-}$ expression with age is sufficient to cause cognitive impairment.

Disclosures: F. Deak: None.

Poster

372. Neurotransmitter Release: Vesicle Dynamics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 372.14/E3

Topic: B.05. Neurotransmitter Release

Support: NSFC Grant 81625006

Title: Epac interacts with rim1α and acts on presynaptic long-term potentiation in the cerebellum

Authors: *W. XIN-TAI[1], F.-X. XU[2], Y. SHEN[3]
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Abstract: Different forms of plasticity in Purkinje cells (PCs) are important for the cerebellum-related motor learning and brain disorders. cAMP plays a central role in a presynaptic long-term potentiation (LTP) at the parallel fiber-PC synapses. However, the downstream machinery that is involved in this presynaptic LTP is unclear. Here, we investigated the roles of EPAC1 and EPAC2, the cAMP effectors, in 8-Hz stimulation-induced presynaptic LTP at parallel fiber-PC synapses. The deletion of EPAC1 and EPAC2 did not affect the cyto-architecture of the cerebellum. However, both 8-Hz LTP and forskolin-mediated potentiation of parallel fibers neurotransmission were impaired, demonstrating that EPAC contributes to cAMP-dependent potentiation of transmitter release. We also found that synaptic facilitation during short trains was affected by loss of EPAC, which was further corroborated by the reduction of docked vesicle number in EPAC double-knockout mice. We continued to find that EPAC interacted with Rim1α and modulated its activity. Apply EPAC agonist 8-pcpt increased Rab3A-RIM1α-Munc13-1 tripartite complex, and EPAC ablation reduced Rab3A-RIM1α-Munc13-1 tripartite complex in the cerebellar synaptosome fraction. Together, our data demonstrate that EPAC is a novel regulator for
Rim1α and the maintenance of RRP at presynaptic sites, and also associated with presynaptic PC-LTP.

Keywords: EPAC; Rim1α; cAMP; Purkinje cell; cerebellum
X-T W and F-X X contributed equally.

Disclosures: W. Xin-Tai: None. **F. Xu:** None. **Y. Shen:** None.

Poster

372. Neurotransmitter Release: Vesicle Dynamics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 372.15/E4

Topic: B.05. Neurotransmitter Release

Support: Fondazione Cariplo, grant n. 2017-0784

Title: The novel neuronal protein APache is implicated in synaptic vesicle and autophagosome trafficking

Authors: *F. C. GUARNIERI[1,2], A. PICCINI[3], M. BRAMINI[4], E. CASTROFLORIO[4], P. VALENTE[3], B. PINTO[5], B. PARISI[3], J. D. WREN[6], F. VALTORTA[1,2], F. BENFENATI[3,4], S. GIOVEDÌ[3]
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Abstract: Synaptic transmission relies on regulated cycles of synaptic vesicle exocytosis and endocytosis in the presynaptic terminal. To guarantee an efficient release, dysfunctional presynaptic proteins and organelles have to be continuously removed through endosomal sorting and autophagy. Alterations in synaptic vesicle recycling and in the endosomal-autophagic pathway have been extensively linked to neurodevelopmental abnormalities and neurodegeneration, as they directly impact on neuronal survival, synaptic transmission and plasticity. Several proteins have a well-known function in the regulation of synaptic vesicle recycling and of endosome-autophagosome trafficking in the presynaptic terminal, but the identification of new molecular players is desirable to draw a complete picture of these processes and to enhance the possibilities of targeted drug design. We used the algorithm GAMMA (Global Microarray Meta-Analysis) to search for uncharacterized genes with a putative function in synaptic vesicle recycling. We identified APache (*Kiaa1107*) as a novel regulator of clathrin-mediated endocytosis of synaptic vesicles and of autophagosome trafficking. APache is highly enriched in the central nervous system, with the highest levels of mRNA and protein in the cerebral cortex, hippocampus and striatum. Its expression is neuron-specific and developmentally regulated in both mouse brain and primary neurons. APache interacts with the

clathrin adaptor protein complex AP-2 and participates to clathrin-mediated endocytosis. In mature neurons, ultrastructural analysis of presynaptic terminals reveals that APache knockdown induces a significant reduction of synaptic vesicle density and of clathrin-coated vesicles. This is accompanied by an accumulation of enlarged endosomes and autophagic vacuoles at presynaptic terminals. Finally, APache silencing decreases excitatory synaptic transmission and affects early neuronal development *in vitro* and *in vivo*. Until now, genetic mutations have not been identified in the human *KIAA1107* gene. However, our data suggest that future studies should address whether altered levels of APache might contribute to neurodevelopmental and/or neurodegenerative diseases.

Disclosures: F.C. Guarnieri: None. **A. Piccini:** None. **M. Bramini:** None. **E. Castroflorio:** None. **P. Valente:** None. **B. Pinto:** None. **B. Parisi:** None. **J.D. Wren:** None. **F. Valtorta:** None. **F. Benfenati:** None. **S. Giovedì:** None.

Poster

372. Neurotransmitter Release: Vesicle Dynamics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 372.16/E5

Topic: B.05. Neurotransmitter Release

Support: NSFC grant 31761133016, 21790394, 31330024, 31171026, 31327901, 31521062 and 21790390
National Key Research and Development Program of China grant 2016YFA0500401

Title: Dynamin-1 restrains vesicular catecholamine release to a sub-quantal mode in mammalian adrenal chromaffin cells

Authors: *Q. ZHANG, Q. WU, B. LIU, Y. LI, X. WU, F. ZHU, Z. ZHOU
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Abstract: Dynamin 1 (dyn1) is required for clathrin-mediated endocytosis in most secretory cells, including neurons and neuroendocrine cells. In 1996, we first reported two modes of Ca^{2+}-dependent catecholamine release from single large dense-core vesicles: quantal through “full-fusion-like” (FFL) and sub-quantal through "kiss-and-run" (KAR) in adrenal chromaffin cells (ACCs), which have been confirmed by us and others. Quantal size (QS, catecholamine release per exocytotic event) is increased by acute pharmacological manipulation of dyn1, however, the nature of previously-assumed full-quanta and KAR sub-quanta remain hotly debated. Here, using genetic dyn1-knockout (KO) ACCs, we showed that 1) KO increased QS (both average and largest QS) by ≥200% without increasing the vesicle size and vesicular content; 2) the KO-induced increase in QS was rescued by overexpressing WT-dyn1 (but not its deficient mutant or dyn2); 3) the ratio of FFL *versus* KAR events was substantially increased by dyn1-KO; 4)

following a release event, more protein contents were retained in WT *vs* KO vesicles; and 5) the fusion pore size d_p was increased from ≤9 nm to ≥9 nm by dyn1-KO. Thus, most (if not all) Ca^{2+}-induced exocytotic events occur in the sub-quantal/KAR release mode in native ACCs, implying that the dyn1-dependent sub-quantal mode plays an essential role in neurotransmission and hormone signaling.

Disclosures: Q. Zhang: None. **Q. Wu:** None. **B. Liu:** None. **Y. Li:** None. **X. Wu:** None. **F. Zhu:** None. **Z. Zhou:** None.

Poster

372. Neurotransmitter Release: Vesicle Dynamics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 372.17/E6

Topic: B.05. Neurotransmitter Release

Support: NSERC

Title: Characterization of Neuro-2a cells as a model of neuronal catecholamine and ATP co-release

Authors: *B. KIM, D. POBURKO
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Abstract: Sympathetic perivascular nerves co-release several neurotransmitters, including adenosine-5'-triphosphate (ATP) and norepinephrine (NE). Past studies of the molecular mechanisms of ATP and NE co-storage and co-released and whether these events occur at the level of the single sympathetic nerve endings, or varicosities, have been limited by the molecular and spatial resolution of current methods to detect vesicle release. Using immunofluorescence microscopy we previously showed that vesicles containing ATP and NE appear to be distinct in intact blood vessels and cultured superior cervical ganglia neurons. To assess the differential kinetics of vesicle release and the potential colocalization of release sites for ATP and NE containing vesicle, we developed genetically encoded fusion proteins of the vesicular monoamine transporter VMAT2 (SLC18A2) and the vesicular nucleotide transporter VNUT (SLC17A9) with pH-sensitive fluorescent proteins as optical reporters of release of NE and ATP containing vesicles. The constructs are VMAT2-pHuji (a red pH-sensor) and VNUT-pHluorin. We sought a cell line that natively expresses VMAT2 and VNUT containing vesicles as a model in which to characterize these reporters and fundamental aspects of co-release. We examined the dopaminergic Neuro-2a (N2a) cell line as a potential model of catecholamine and ATP co-release as a surrogate for primary sympathetic neurons. We further created pH-insensitive reporters to characterize vesicle pool localization and trafficking. Exogenously expressed constructs traffic to neurites generated in response to differentiation cues, and VMAT2 and

VNUT appear to be expressed in common varicosities but at variable ratios, indicating that they are not strictly localized to the boutons at a constant stoichiometry. Moreover, the release of VMAT2 and VNUT containing vesicles exhibit differential release and recycling kinetics. Thus this cell line represents promising model in which to further identify fundamental aspects of differential trafficking and release of VMAT2 and VNUT containing vesicles, while VMAT-pHuji and VNUT-pHluorin permit simultaneous detection of catecholaminergic and purinergic vesicle release.

Disclosures: B. Kim: None. **D. Poburko:** None.

Poster

372. Neurotransmitter Release: Vesicle Dynamics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 372.18/E7

Topic: B.05. Neurotransmitter Release

Support: NIH Grant R01DC04274
NIH Grant R01EY014043

Title: Endocytosis helps to clear release sites and maintain the pool of releasable vesicles in a mature glutamatergic synapse

Authors: *A. A. DAGOSTIN, H. VON GERSDORFF
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Abstract: Vesicle exocytosis is fundamental for diverse biological events, including neurotransmission. After vesicle fusion and transmitter release, the fused vesicle membrane must be retrieved and recycled in order to maintain vesicle pool sizes and presynaptic exocytic machinery. In this process, dynamin is a key protein for endocytosis, responsible for membrane fission and, ultimately, the first step in vesicle pool replenishment. To study whether the disruption of the endocytic function through dynamin blockade impairs the neurotransmission at high frequencies, we cut brainstem slices from mice containing the Medial Nucleus of the Trapezoid Body (MNTB) and recorded evoked EPSCs from MNTB principal cells after hearing onset (postnatal day P20 to P30). All recordings were performed using an external solution with 1.2 mM Ca^{2+} and at 35°C. To block dynamin function, we preincubated brainstem slices with 30 µM Dyngo 4A for at least 20 minutes prior to recordings. Dyngo 4A is an activity-dependent blocker of dynamin and to ensure its effect, we evoked 150 stimuli at 300 Hz, 50 times. This protocol did not trigger any long term potentiation/depression in controls and was enough to see an effect of the drug. After this conditioning, cells were stimulated with 300Hz trains of 100 stimuli, which yielded EPSCs with amplitudes reduced by >50% in Dyngo 4A compared to control. The reduced EPSC amplitude in the presence of Dyngo 4A may be explained by a

reduction in vesicle pool size (smaller RRP) which becomes clear when we apply the Elmqvist & Quastel extrapolation method to retrieve the RRP data from control and Dyngo 4A recordings: 44 nA and 35 nA, respectively. The effect on EPSC amplitude in the presence of Dyngo 4A may also be explained by a lower vesicle recruitment rate (from 18 ± 2 to 11 ± 3 vesicles/ms - quantal size corrected using a control/kynurenic acid steady state EPSC amplitude ratio) and an inefficient clearance of the active zone from fused vesicular membrane. Even though Dyngo 4A did not change the EPSCs kinetics, the recovery time from depression was longer, with an increase in both the fast and slow time constants of recovery (fast: 30 to 90 ms; slow: 1.8 to 2.7s; control and Dyngo 4A, respectively). These results suggest that dynamin reduces the RRP due to a diminished recovery rate from depression. This may lead to a jamming of the vesicle release site, since the recently fused vesicles are not efficiently cleared anymore from the active zone.

Disclosures: A.A. Dagostin: None. **H. von Gersdorff:** None.

Poster

372. Neurotransmitter Release: Vesicle Dynamics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 372.19/E8

Topic: B.05. Neurotransmitter Release

Support: NIH Grant NS044057

Title: Synaptophysin and synaptogyrin regulate fusion pore kinetics during Ca^{2+}-triggered exocytosis in chromaffin cells

Authors: *Y.-T. HSIAO[1], C.-W. CHANG[1,2], M. B. JACKSON[1]
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Abstract: Synaptophysin (syp) and synaptogyrin (syg) are major secretory vesicle proteins. Syp and syg have similar structures with four transmembrane domains and a cytoplasmic C-terminal tail that can be phosphorylated. Previous studies have shown that both syp and syg regulate exocytosis, vesicle cycling, and synaptic plasticity, but their roles in the membrane fusion steps of Ca^{2+}-triggered exocytosis remain unclear. To investigate this issue, we expressed syp or syg in chromaffin cells from double knockout (DKO) mice that lack both syp and syg, and used amperometry to evaluate changes in dynamic processes during catecholamine release. The frequency of exocytotic events was lower in DKO cells than in wild-type cells. Expressing syp in DKO cells increased the frequency slightly whereas expressing syg increased the frequency about 2-fold. Expression of syg, but not syp, in DKO cells reduced the fraction of kiss-and-run events, suggesting that syg may regulate the mode of exocytosis and favor full-fusion events. Furthermore, the duration of prespike feet in DKO cells was longer than in wild-type cells,

indicating that the absence of syp and syg stabilize the initial fusion pore. Expressing syp or syg in DKO cells greatly decreased the duration, suggesting that syp and syg drive the expansion of the initial fusion pore toward full fusion. Our results demonstrate that both syp and syg influence events in membrane fusion during Ca^{2+}-triggered exocytosis of catecholamine-containing dense-core vesicles, and that syg has stronger effects than syp.

Disclosures: Y. Hsiao: None. **C. Chang:** None. **M.B. Jackson:** None.

Poster

372. Neurotransmitter Release: Vesicle Dynamics

Location: SDCC Halls B-H

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Program #/Poster #: 372.20/E9

Topic: B.05. Neurotransmitter Release

Support: NIH Grant ZIA-NS003009-13
NIH Grant ZIA-NS003105-08

Title: Visualization of membrane pore in live cells reveals a dynamic-pore theory governing fusion and endocytosis

Authors: *W. SHIN, L. GE, G. ARPINO, S. VILLARREAL, E. HAMID, H. LIU, W.-D. ZHAO, P. WEN, H.-C. CHIANG, L.-G. WU
NINDS, NIH, Bethesda, MD

Abstract: Fusion is thought to open a pore to release vesicular cargoes vital for many biological processes, such as exocytosis, intracellular trafficking, fertilization, and viral entry. However, fusion pores have not been observed and thus proved in live cells. Its regulatory mechanisms and functions remain poorly understood. Here we visualized fusion pores in live (neuroendocrine) cells with super-resolution STED microscopy. We discovered extremely dynamic pore behaviors, including opening, expansion, constriction, and closure, where the pore diameter may vary between 0 and 490 nm within 26 ms to seconds (vesicle size: 180-720 nm). These pore dynamics result from competition between pore expansion mediated by F-actin-provided membrane tension and pore constriction/closure mediated by calcium and dynamin. They determine the extent and rate of vesicular cargo release and vesicle retrieval. Our findings provide the missing live-cell evidence proving the fusion-pore hypothesis, and establish a new pore theory accounting for fusion, endocytosis and their regulation.

Disclosures: W. Shin: None. **L. Ge:** None. **G. Arpino:** None. **S. Villarreal:** None. **E. Hamid:** None. **H. Liu:** None. **W. Zhao:** None. **P. Wen:** None. **H. Chiang:** None. **L. Wu:** None.

Poster

373. Epilepsy: Experimental Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 373.01/E10

Topic: B.10. Epilepsy

Support: JSPS KAKENHI Grant Number 15H05719
JSPS KAKENHI Grant Number 16H05438

Title: Influence of a cooling compound, icilin, on penicillin G-induced epileptiform discharges in a rat model: Validation of focal cortical cooling effects by TRPM8 activation

Authors: *H. MORIYAMA[1], S. NOMURA[1], H. KIDA[2], T. INOUE[1], H. IMOTO[1], Y. MARUTA[1], Y. FUJIYAMA[1], D. MITSUSHIMA[2], M. SUZUKI[1]
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Abstract: Focal cortical cooling suppresses penicillin G (PG)-induced epileptiform discharges (EDs) in the cortex. Cooling from 25°C to 15°C activates transient receptor potential melastatin 8 (TRPM8), but involvement of thermosensitive TRP channels in focal cortical cooling for PG-induced EDs has not been widely studied. Therefore, we examined whether TRPM8 activation can suppress PG-induced EDs in the sensorimotor cortex. Icilin, a TRPM8 agonist, was injected after PG injection, and an electrocorticogram (ECoG) and cortical temperature were continuously recorded for 4 h. Spike amplitude, duration, firing rate, and power spectrum density in EDs were measured to evaluate the effects of icilin. PG-induced EDs and increased delta, theta, alpha, and beta power spectra occurred in focal ECoG. Without a change of cortical temperature, icilin dose-dependently suppressed EDs. In particular, 3.0 mM icilin significantly suppressed PG-induced spike amplitude, duration, and firing rate, and improved each band power density in EDs to the level of basal activity in ECoG. These results suggest that TRPM8 activation in epileptic brain regions may be a new approach to treatment for patients with refractory epilepsy.

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Poster

373. Epilepsy: Experimental Therapeutics

Location: SDCC Halls B-H

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Program #/Poster #: 373.02/E11

Topic: B.10. Epilepsy

Support: CONACYT-86784
Fondos Federales INP

Title: Effect of ketogenic diet on KCC2 expression in rat dentate gyrus

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Abstract: The cation chloride cotransporters (NKCC1 and KCC2), which transport chloride within and outside the cell respectively, are responsible for the intracellular chloride concentration. The intracellular chloride concentration determines the strength and polarity of gamma-aminobutyric acid (GABA)-mediated neurotransmission. By the other hand, the ketogenic diet (KD) a high fat and low carbohydrate diet, has been used as a non-pharmacological treatment in refractory epilepsy, although the mechanism of action is unknown. This diet increases β-hydroxybutyrate (a ketone body), which probably modifies NKCC1 activity. The aim of this study was to evaluate the effect of the ketogenic diet on the KCC2 expression in the rat dentate gyrus. Male Sprague-Dawley pups were divided into: control group (n=5) (standard lab-chow diet, ND), and ketogenic diet group (n=5) and fed after weaning and through three months. At the end of the treatment, the body weight and β-hydroxybutyrate blood levels were measured. Rats were sacrificed with pentobarbital over-doses and their tissues fixed via intracardiac perfusion. Brains were removed, post-fixed and cryo-protected, afterwards 50 micron coronal serial sections were obtained and immunostained for KCC2. KCC2 staining images in dentate gyrus of ND and KD fed rats were obtained using Olympus BX51 microscope connected to a digital video camera (MBF Bioscience), and analyzed using NIH ImageJ v1.52a program (USA). The images were obtained in three regions: molecular, granular and polymorphic layer using an objective lens of 20x, the values were expressed as optical density (OD) units. t Student test was performed using SPSS v25 program ($p < 0.05$). The KD group reported higher beta-hydroxybutyrate blood levels than controls. The body weight and KCC2 optical density not showed any significant difference between control and ketogenic groups in any of the regions analyzed. Thus, we conclude that the KD applied over a long period after weaning does not modify the expression of KCC2 in rat dentate gyrus.

Disclosures: L. Granados-Rojas: None. **K. Jerónimo-Cruz:** None. **G. Hernandez-Hernandez:** None. **T. Juárez-Zepeda:** None. **A. Vázquez-Vega:** None. **M. Tapia-Rodríguez:** None. **P. Duran:** None. **L. Carmona-Aparicio:** None.

Poster

373. Epilepsy: Experimental Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 373.03/E12

Topic: B.10. Epilepsy

Support: European Seventh's Framework Program (FP7/2007-2013); Grant agreement No. 602102
German Academic Scholarship Foundation

Title: Molecular imaging reveals pro- and anti-inflammatory effects of fingolimod in a mouse model of epileptogenesis

Authors: *B. J. WOLF[1,2], P. BASCUÑANA[1], I. LEITER[1], L. B. N. LANGER[1], T. L. ROSS[1], F. M. BENGEL[1], J. P. BANKSTAHL[1], M. BANKSTAHL[2]
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Abstract: Neuroinflammation is a hallmark of epileptogenesis and has been considered as target for anti-epileptogenic treatment. The sphingosine-1-phosphate analog fingolimod (FTY720) is used for anti-inflammatory treatment in multiple sclerosis. Here, we used molecular imaging targeting the translocator protein (TSPO), which is over-expressed in activated microglia, to quantify effects of FTY720 during epileptogenesis.
Status epilepticus (SE) was induced by injecting kainate into the right hippocampus of male NMRI mice. Six hours after SE induction, mice received either 0.3 mg/kg FTY720 (SE n=8; sham n=5) or vehicle injections (SE n=18; sham n=8) once daily for 6d. Static [^{18}F]GE180 TSPO PET scans were performed 1 and 2 weeks after SE. Subsequently, mice were sacrificed for immunohistochemistry.
TSPO signal in post-SE mice was increased over sham by up to 90% in the kainate injection area, up to 49% in the ipsilateral ventral hippocampus, thalamus and cortex and up to 39% on the contralateral side in dorsal and ventral hippocampus, peaking at 2 weeks after SE ($p<0.05$). FTY720 treatment further elevated TSPO PET signal at 7 days post SE by 17% in the ipsilateral hippocampus vs vehicle-treated mice ($p<0.05$). Conversely, TSPO signal was 19% lower in the contralateral cortex and ventral hippocampus ($p<0.05$) after 2 weeks of FT720 treatment. Atlas-based results were confirmed by statistical parametric mapping (SPM). Immunohistochemical analyses at 2 weeks post-SE revealed reduced astrocyte activation in the contralateral hippocampus after treatment, but no neuroprotection.
[^{18}F]GE180 PET identified regional microglia activation during epileptogenesis. FTY720

treatment evoked a localized increase in TSPO expression in the kainate injection area, and a reduction in areas distant to the injection site. Stimulation as well as reduction of local inflammation by FTY720 may underlie reported anti-eplieptogenic properties of the treatment.

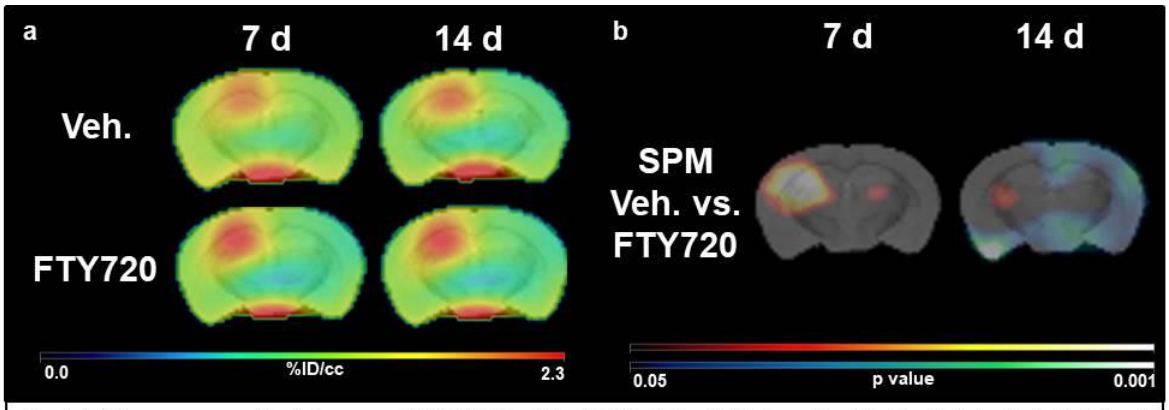

Fig.1: (a) Average coronal brain images of [^{18}F]GE180 uptake (%ID/cc) 7 and 14 days after SE of vehicle treated (Veh.) and FTY720 treated animals (b) Statistical parametric mapping analysis of [^{18}F]GE180 uptake during epileptogenesis using SPM12 software. Coronal t-maps resulting from comparisons of [^{18}F]GE180 uptake between vehicle treated and FTY720 treated animals after 7 and 14 days after SE. (student's t-test, $p<0.05$, minimum cluster size of 50 voxels).

Disclosures: B.J. Wolf: None. **P. Bascuñana:** None. **I. Leiter:** None. **L.B.N. Langer:** None. **T.L. Ross:** None. **F.M. Bengel:** None. **J.P. Bankstahl:** None. **M. Bankstahl:** None.

Poster

373. Epilepsy: Experimental Therapeutics

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Topic: B.10. Epilepsy

Support: BT/PR12754/INF/122/2/2016

Title: Antioxidative and antiapoptotic effect of dehydroepiandrosterone on iron-induced epilepsy model

Authors: *C. PRAKASH, M. MISHRA, P. KUMAR, D. SHARMA
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Abstract: Epilepsy is a complex neurological disorder categorized by recurring seizures. Despite recent advancements in pharmacological interventions, there is no effective treatment available for epilepsy. Although, many antiepileptic drugs are available which suppress seizures but have severe side effects. Oxidative stress is underlying mechanism in the initiation and progression of epilepsy. Enhanced oxidative stress is also implicated in apoptotic cell death. Hence, identification of safe antiepileptic agent with neuroprotective properties is of great importance.

Thus, we assessed exogenous treatment of dehydroepiandrosterone (DHEA) in iron-induced epilepsy. The present study is focused on the antioxidative and antiapoptotic effect of DHEA. Male Wistar rats were made epileptic by injecting intracortical injection of $FeCl_3$ and received i.p. injection of DHEA at 30 mg/kg body wt./day for 7, 14 and 21 days. Epileptogenesis was confirmed by monitoring EEG. Neuronal degeneration and apoptosis were assessed by cresyl violet staining and TUNEL assay. Oxidative stress was assessed in tissue homogenate by biochemical analysis of lipid peroxidation. The activities of antioxidant enzymes (superoxide dismutase, glutathione-s-transferase, glutathione peroxidase and glutathione reductase) were also measured. Additionally, the expression of antioxidant enzymes and cleaved caspase-3 was analysed by RT-PCR and western blots respectively. In results, DHEA suppresses seizure, neuronal degeneration, oxidative stress and enhances antioxidant defense in epileptic rats. Moreover, DHEA upregulates the mRNA expression of antioxidant enzymes while downregulates protein expression of caspase-3. In conclusion, the present study indicates that DHEA exerts as a potent neuroprotective anent against iron-induced epilepsy via upregulation of antioxidant enzymes and reversal of caspase-dependent apoptosis.

Disclosures: C. Prakash: None. **M. Mishra:** None. **P. Kumar:** None. **D. Sharma:** None.

Poster

373. Epilepsy: Experimental Therapeutics

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Program #/Poster #: 373.05/E14

Topic: B.10. Epilepsy

Support: CSIR, India vide project MLP 0204
DST-INSPIRE fellowship vide letter no. DST/INSPIRE Fellowship/2013/820

Title: Exploring the role of mycophenolate mofetil on neurohistopathological changes and gene expression in rat model of lithium pilocarpine induced spontaneous recurrent seizures

Authors: *A. G. MAZUMDER, V. PATIAL, D. SINGH
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Abstract: Neuroinflammatory processes remain a key factor for the progression of epileptogenesis particularly in case of temporal lobe epilepsy (TLE). Earlier reports have shown that cytokines released during inflammation binds to its receptor site and activate several pathogenic pathways that play a crucial role in neuroanatomical, molecular and biochemical changes resulting in epilepsy. Mycophenolate mofetil, commonly used as an immunosuppressant, has shown neuroprotective effects in several preclinical and clinical studies. In our preliminary studies, mycophenolate mofetil showed a reduction in seizure severity and aggression-like behavior.[1] The present work was designed to study the effect of mycophenolate

mofetil on neurohistopathological changes and hippocampal mRNA expression in a rat model of lithium-pilocarpine-induced epileptic spontaneous recurrent seizures. Male wistar rats randomly divided into different groups were subjected to lithium pilocarpine induced chronic seizure method, followed by 28 days post treatment with mycophenolate mofetil at different doses. Following seizure and behavioral recordings, rats were decapitate, histopathological parameters were assessed using various staining procedures such as Nissl stain, Timm stain and hippocampal mRNA expression studies were studied using quantatitative real time-PCR. The results showed that treatment with the mycophenolate mofetil distinctively reduced neuronal death and mossy fiber sprouting along the hippocampus, considered as a characteristic neurohistopathological feature of TLE. It also caused attenuation of amplified gene expression. Our findings concluded that mycophenolate mofetil, apart from being antiepileptic, also reduced neurohistopathological changes and aberrant gene expression in the hippocampus of epileptic rats.

References:

1.Investigating the antiepileptic potential of mycophenolate mofetil in a rat model of temporal lobe epilepsy. In: Neuroscience, 2017, the 47th Annual Meeting of the Society for Neuroscience (SfN), Washington DC, USA, 2017 (Poster no: 665.09/N1).

Disclosures: A.G. Mazumder: None. **V. Patial:** None. **D. Singh:** None.

Poster

373. Epilepsy: Experimental Therapeutics

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Program #/Poster #: 373.06/E15

Topic: B.10. Epilepsy

Support: NIH / NINDS U01 NS090414: SUDEP Research Alliance

Title: The ketogenic diet increases serotonin levels in the mouse brain

Authors: *F. A. TERAN, Y. KIM, G. B. RICHERSON
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Abstract: Sudden unexpected death in epilepsy (SUDEP) is a major cause of mortality in people with drug-resistant epilepsy. We recently demonstrated that SUDEP in a mouse model of Dravet Syndrome (DS) is due to seizure-induced respiratory arrest. Our preliminary studies show that SUDEP in DS mice is greatly reduced with a ketogenic diet (KD), an effect that is independent of ketosis but is correlated with higher brain levels of serotonin (5-HT). Defects in the 5-HT system have been linked to both respiratory dysfunction and SUDEP. We hypothesize the rescue effect of the KD is not due to ketosis, but rather to a specific dietary component that alters 5-HT metabolism. To assess the effect of a KD on 5-HT metabolism in the mouse brain, C57BL/6J

(WT) mouse littermates were placed either on a KD or a conventional diet for five days starting soon after weaning (P20). Brain levels of 5-HT, 5-hydroxyindole acetic acid (5-HIAA), L-tryptophan (Trp), dopamine (DA), homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC) and norepinephrine (NE) were measured from whole brain tissue using HPLC-ECD. In addition, extracellular 5-HT and DA were measured *in vivo* using microdialysis. Guide cannulae were implanted stereotaxically under anesthesia in the amygdala, a region implicated in seizure propagation and postictal apnea, of WT mice. Implanted animals were placed in a freely moving collection system and allowed to recover for at least 20 hours. Basal monoamine dialysate samples were collected for three hours, after which mice were administered decanoic acid (C10), a medium chain fatty acid found in KDs and known to be anticonvulsant, or vehicle via oral gavage, and samples were collected for three more hours. To measure the releasable pool of 5-HT and DA, 3,4-methylenedioxymethamphetamine (MDMA) was injected i.p. to cause heteroexchange release of 5-HT and DA and additional samples were collected. Total brain 5-HT, 5-HIAA, Trp and NE were 1.3 to 1.5-fold higher in KD-fed mice compared to littermates fed a control diet ($p<0.05$ with Mann-Whitney U-tests; n=8 per group). Preliminary data show that intragastric administration of C10 (n=3) or vehicle (n=2) had no effect on baseline extracellular 5-HT or DA levels over 3 hours. However, treatment with C10 led to a 300-fold increase in MDMA-induced release of 5-HT, but not DA, from the amygdala compared to control diet. Our preliminary results indicate that a KD and C10 both alter 5-HT metabolism. C10, and possibly other components of KDs, may be protective in part by increasing brain 5-HT. Future experiments include testing effects of 5-HT depletion on mortality in KD-treated DS mice.

Disclosures: F.A. Teran: None. **Y. Kim:** None. **G.B. Richerson:** None.

Poster

373. Epilepsy: Experimental Therapeutics

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Program #/Poster #: 373.07/E16

Topic: B.10. Epilepsy

Support: SIP-IPN Grant 20181861

Title: Phenylamides as anticonvulsants

Authors: *S. E. MEZA TOLEDO, A. L. SILVA-RAMÍREZ, N. ALVAREZ-JUÁREZ, E. BURGUEÑO TAPIA, M. SUÁREZ QUEZADA
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Abstract: In an effort to discover novel anticonvulsants, we prepared and characterized several phenylamides and studied their anticonvulsant activity against seizures induced by

pentylenetetrazol in CD-1 mice. Compounds 2-hydroxy-2-(3',5'-bis(trifluoromethylphenyl)) propionamide (**1**), 2-hydroxy-2-(3'-trifluoromethylphenyl) propionamide (**2**), 2-hydroxy-2-(3',5'-bis(trifluoromethylphenyl)) butyramide (**3**) and 3-hydroxy-3-(3',5'-bis(trifluoromethylphenyl)) butyramide (**4**) were prepared using condensation reactions and characterized through infrared spectrophotometry and nuclear magnetic resonance spectroscopy. Each of the four compounds exhibited an anticonvulsant activity in mice greater than that of sodium valproate, a widely-used antiepileptic, against seizures induced by pentylenetetrazol. Compounds **1**, **3** and **4** were equipotent with phenobarbital. Isobologram plots showed that phenylamides and phenobarbital exhibit an additive anticonvulsant effect against seizures induced by pentylenetetrazol. This study shows that phenylamides represent a new class of anticonvulsant compounds worthy of further development for potential antiepileptic therapy.

Disclosures: S.E. Meza Toledo: None. **A.L. Silva-Ramírez:** None. **N. Alvarez-Juárez:** None. **E. Burgueño Tapia:** None. **M. Suárez Quezada:** None.

Poster

373. Epilepsy: Experimental Therapeutics

Location: SDCC Halls B-H

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Program #/Poster #: 373.08/E17

Topic: B.10. Epilepsy

Support: startup funds

Title: Sulfasalazine as a treatment for acquired epilepsy

Authors: O. ALCOREZA[1], *S. L. CAMPBELL[2], H. SONTHEIMER[3]
[1]Virginia Tech. Carilion Res. Inst., Blacksburg, VA; [2]Animal and Poultry Sciences/ Sch. of Neurosci., Virginia Polytechnic Inst. and State Universit, Blacksburg, VA; [3]Sch. of Med. and Res. Inst., Virginia Tech. Sch. of Neurosci., Roanoke, VA

Abstract: Epilepsy affects approximately 2.2 million Americans, with 150,000 new cases being diagnosed each year. Despite some success managing epilepsy, current therapeutics offer no benefit to 1-in-3 patients. Previous studies from our lab revealed that primary brain tumors release glutamate and induce electroencephalographic (EEG)-confirmed behavior seizures in adult mice implanted with human-derived glioma cells. These studies identified increased expression of system xc (SXC), a cysteine/glutamate exchanger, on glioma cells as a major contributor to elevated glutamate levels in tumor-implanted mice. Inhibition of SXC via sulfasalazine (SAS), a FDA-approved anti-inflammatory drug, decreased glutamate release and EEG-confirmed behavior seizures.
SXC is normally expressed on glial cells and gliosis is a prominent feature of many forms of epilepsy. Additionally, recent studies revealed that astroglial dysfunction, leading to pathological

changes in the extracellular environment and neuronal metabolism, may play a critical role in the initiation of seizures and development of epilepsy. We therefore hypothesize that gliosis may cause an increase in SXC expression leading to enhanced glutamate release in acquired epilepsies and that treatment with SAS can decrease seizure occurrence in mouse models of epilepsy. To test this, we used the kainic acid (KA)-induced model of acquired epilepsy, which present with gliosis, to characterize changes in the expression of SXC. Our preliminary data reveal that animals treated with KA showed increase protein expression of SXC in the hippocampus and that treatment with SAS decreases the expression of SXC. Using the beta-1 integrin knockout (β1KO) mouse model, which is characterized by widespread chronic astrogliosis and spontaneous seizures, our preliminary data demonstrate elevated SXC protein expression in the cortex of β1KOs and a decrease in seizure activity after SAS treatment. These results suggest that SXC may play a role in the pathogenesis of acquired epilepsies and that further studies on SXC and the effects of SAS is warranted as a potential novel therapeutic modality. As most treatments for epilepsy involves modifying neuronal excitatory or inhibitory mechanisms, SXC provides an unexplored glial target for decreasing glutamate release in acquired epilepsies.

Disclosures: O. Alcoreza: None. **S.L. Campbell:** None. **H. Sontheimer:** None.

Poster

373. Epilepsy: Experimental Therapeutics

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Program #/Poster #: 373.09/E18

Topic: B.10. Epilepsy

Support: NIH/NINDS MAPK Epilepsy R56 NS083527

Title: Dusp4: An endogenous anti-epileptogenic inhibitor of map kinase signaling

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

Abstract: BACKGROUND: One third of epileptic patients are resistant to current drugs, many of which produce significant side effects. To develop improved therapeutics, the pathways that underlie epileptic areas of the neocortex need to be better understood. Using high-throughput genomic studies of human epileptic neocortical tissue, we identified the mitogen activated protein kinase (MAPK) pathway as highly upregulated in epileptic regions and have further shown that inhibition of MAPK reduces epileptic spiking in an animal model. Here, we evaluated these genes within the MAPK family for those that could be novel therapeutic targets. **OBJECTIVES**: To characterize genes involved in MAPK signaling in human neocortical epilepsy as a means to develop novel anti-epileptogenic therapeutics.

METHODS: We identified genes within the MAPK signaling pathway that were differentially expressed in high versus low spiking human cortex studied with long-term intracranial recordings. From 20 pairs of high and low spiking we used ontological methods to identify those genes within the MAPK pathway and then performed a clustering analysis to identify subgroups. In situ hybridizations were then used to localize specific genes and examine their spatial patterns of expression within human epileptic neocortex. Mechanistic studies in SY5Y and human tissues were performed to define roles of candidate genes.
RESULTS: About one third of the differentially expressed genes in high spiking regions were in the MAPK pathway. Clustering of the MAPK genes revealed a larger group contained known MAPK genes previously linked to epileptic regions. Another cluster contained an ERK1/2 inhibitor, DUSP4. In situ hybridization studies revealed that DUSP4 was activated in focal cortical areas where most MAPK genes were shut down, suggesting that DUSP4 turns off MAPK signaling. Repeated depolarization of human neuronal-like cells showed that while DUSP4 mRNA is not induced with activity, DUSP4 protein is dramatically induced and blocks MAPK signaling genes. Human tissue studies confirmed that regions with high levels of DUSP4 protein had lower levels of MAPK signaling genes.
CONCLUSION: DUSP4 is an endogenous inhibitor of MAPK signaling that is activity-dependent and spatially restricted in human epileptic brain regions in a way that could create local boundaries of reduced epileptogenicity. Since MAPK activation may be an important driver of epileptogenesis, treatments that augment DUSP4 expression could be novel inhibitors of the epileptogenic process.

Disclosures: A. Kirchner: None. **S. Bagla:** None. **F. Dachet:** None. **J.A. Loeb:** None.

Poster

373. Epilepsy: Experimental Therapeutics

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Program #/Poster #: 373.10/E19

Topic: B.10. Epilepsy

Support: DoD/MRICD Contract W81XWH-16-C-0140

Title: Assessment of antiseizure and neuroprotective effects of novel compounds in a delayed-treatment rat model of organophosphate (OP) exposure

Authors: *J. SPAMPANATO, M. SMOLIK, F. DUDEK
Univ. of Utah, Salt Lake City, UT

Abstract: Exposure to organophosphates (OP), or organophosphate nerve agents, results in status epilepticus (SE) and neuronal damage in the central nervous system. SE is can be lethal; however, early control of seizure activity minimizes mortality, as well as neuronal damage.

Unfortunately, in the event of a mass release in a civilian setting or on the battlefield, treatment of casualties is likely to be delayed to periods longer than typically acceptable for current medications that can be administered without hospital support. Therefore, there is a pressing need for anti-seizure treatments that can be administered with a significant delay and in a pre-hospital setting. Now in our second year, the CounterACT Neurotherapeutic Screening (CNS) Program has screened 9 externally submitted and 8 internally submitted test compounds. For screening, male, Sprague Dawley rats (150-200 g) were implanted with electrodes for recording of the electroencephalogram (EEG). On treatment day, SE was induced by administration of diisopropyl fluorophosphate (DFP). One hour after the start of SE, rats were co-administered midazolam (MDZ) and test compound. EEG was recorded for 24 hours at which time the rats were perfused, brains were sectioned and labeled with Fluoro-Jade B. Neuropathology was assessed as the number of Fluoro-Jade B positive neurons in 10 regions: dorsal CA1, dorsal CA3, hilus, ventral CA1, ventral CA3, amygdala, thalamus, and the parietal, entorhinal and piriform cortices. Here we present a summary of the program to date. Of the coded, externally submitted compounds, we have seen significant anti-seizure effects in the presence and absence of MDZ for two compounds. These compounds reduced both seizure power and seizure duration. Each of these compounds also reduced cell death in one or more brain areas in the presence of MDZ. Tested in the absence of MDZ, both compounds were less effective in reducing seizures and cell death. These data can be compared to ganaxolone which had a minimal effect on seizures and bumetanide which had no effect on seizures. These data demonstrate that OP-induced SE can be reversed even when treated at a long-delay and this delayed treatment can significantly reduce cell death.

Disclosures: J. Spampanato: None. **M. Smolik:** None. **F. Dudek:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Epitel, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Epitel, Inc.. F. Consulting Fees (e.g., advisory boards); Epitel, Inc..

Poster

373. Epilepsy: Experimental Therapeutics

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Program #/Poster #: 373.11/E20

Topic: B.10. Epilepsy

Support: National Counsil of Science and Technology, scholarship No. 622940

Title: Transcranial focal stimulation augments the effects of anticonvulsant drugs in rats

Authors: D. PEREZ-PEREZ[1,2], *L. L. ROCHA[3], W. BESIO[4], J. SOTELO[5]
[1]Natl. Autonomous Univ. of Mexico, Mexico, Mexico; [2]Plan of Combined Studies in Med.,

Mexico, Mexico; [3]CINVESTAV, Mexico, Mexico; [4]Univ. of Rhode Island, Kingston, RI; [5]Natl. Inst. of Neurol. and Neurosurg. of Mexico, Mexico, Mexico

Abstract: Combined therapy in patients with epilepsy is mainstream in the treatment of this condition. It is necessary to find alternative strategies with less adverse effects. On the other hand, transcranial focal stimulation (TFS) has shown anticonvulsant effects that are increased when combined with diazepam. Nevertheless, it is not known if this capability can be translated to other antiepileptic drugs. The aim of the present study was to evaluate the effects of TFS alone and combined with phenytoin (PHT, a sodium channel blocker) or phenobarbital (PB, a GABA receptor agonist), in a model of generalized seizures induced by 3-mercaptopropionic acid (3MP). Male Wistar rats of 250-300 g initial weight, were habituated for 7 days by daily administration of saline solution (SS, 0.9%, 1ml/kg, ip) and 2 minutes of kindly handle. The head of the animals was shaved at the end of the last manipulation. One day after the end of habituation, the rats were administered with PHT (75 mg/kg, ip), PB (15 mg/kg, ip), or SS (1 ml/kg, ip). Fifty-three minutes later, they received TFS (charge-balanced biphasic squared pulses at 200 us of duration, 300 Hz, and 100 mV) or manipulation without stimulation, for 2 minutes. Five minutes after TFS, the rats received 3MP (37.5 mg/kg, ip) and the behavioral changes were evaluated for 30 minutes. Each experimental condition was evaluated in 8 animals. All rats from the Sham group (100%) presented the following behavioral changes: myoclonic jerks, clonus, wild jump, tonic component followed by clonic seizures, with a latency of 284 ± 6.7, 303 ± 6.35, 512 ± 34.6, 684 ± 62.4, and 705 ± 61.5 s, respectively. Animals treated with PHT showed similar incidence of behavioral changes when compared with the Sham group, except for a reduction in wild jump (20%, p=0.05), and tonic component (0%, p=0.007). For PB, the changes were not different from the Sham group except for a lower incidence of the tonic component (28%, p=0.03). TFS did not induce significant changes in the behavioral seizures compared with the Sham group, except for a reduction in the incidence of continuous seizures (40%, p=0.04). However, animals receiving TFS combined with PHT or PB presented a reduction in the incidence of clonic seizures (60% for PHT and 50% for PB), in contrast to the groups receiving the antiepileptic drugs alone. None of the therapeutic strategies modified the seizure latency for the different behavioral changes evaluated. In conclusion, TFS augmented the anticonvulsant effects of PHT and PB in motor generalized seizures.

Disclosures: D. Perez-Perez: None. **L.L. Rocha:** None. **W. Besio:** None. **J. Sotelo:** None.

Poster

373. Epilepsy: Experimental Therapeutics

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Topic: B.10. Epilepsy

Support: NIH Grant 5R01NS024067
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FACES: Finding a Cure for Epilepsy and Seizures

Title: Cannabidiol decreases the excitability of pyramidal neurons and increases inhibitory drive in hippocampal area CA1

Authors: *S. CHAMBERLAND[1], E. C. ROSENBERG[1], O. DEVINSKY[2], R. W. TSIEN[1]
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Abstract: Cannabis has been used for several centuries in the treatment of several diseases. The therapeutic potential of cannabinoids has recently resurfaced. Cannabidiol (CBD), a non-psychoactive cannabinoid, has recently been shown to decrease epileptic seizures in a double-blind placebo-controlled clinical study (Devinsky et al., 2017, 2018). However, the effects of cannabidiol on neuronal activity and neuronal network remain obscure. We used whole-cell and cell-attached recordings in acute hippocampal slices to understand how CBD (10 - 20 μM) modulates neuronal activity.

Our results indicate that CBD reduces neuronal activity through three mechanisms in the CA1 hippocampus. First, CBD increased synaptic inhibition in CA1 pyramidal neurons. Second, CBD decreased the firing rate of CA1 pyramidal neurons at depolarized membrane potentials and capped their maximal firing rate. In contrast, CBD had no effect on CA1 pyramidal cell resting membrane potential or spontaneous firing. In addition, CBD did not affect the action potential waveform. Third, in contrast to pyramidal neurons, GABAergic interneurons recorded in the stratum oriens and in the alveus demonstrated a cell-type specific effect. While fast-spiking interneurons increased their firing rate in response to CBD, the firing rate of regular-spiking interneurons was decreased.

Overall, our results show that CBD acts via multiple functional mechanisms to tone down principal neuronal activity. These mechanisms may contribute to the anti-epileptic properties of CBD. We are currently investigating CBD's cell type-specific mechanism of action and how these effect are summed at the network level to regulate the propagation of activity in the hippocampus.

Disclosures: S. Chamberland: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); GW Pharmaceuticals. **E.C. Rosenberg:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); GW Pharmaceuticals. **O. Devinsky:** None. **R.W. Tsien:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); GW Pharmaceuticals.

Poster

373. Epilepsy: Experimental Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 373.13/E22

Topic: B.10. Epilepsy

Support: GW Pharmaceuticals

Title: An investigation of the discriminative stimulus and reinforcing effects of cannabidiol in rats

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Abstract: Cannabidiol (CBD) is being developed for treating severe, orphan, early-onset, treatment-resistant epilepsy syndromes. We have investigated CBD's discriminative stimulus in rats trained to discriminate between midazolam and saline, and CBD's reinforcing effect by intravenous self-administration (IVSA) in heroin-trained rats. Freely fed female, Lister hooded rats were trained to discriminate midazolam (0.5mg/kg ip) from saline in a 2-choice operant test. Lever-pressing was reinforced by sweetened milk rewards obtained on a FR5 schedule for responses on either lever. Once the rats were competent in the task (>75% correct responding for midazolam and saline), rats were tested with CBD (20, 75, 150 mg/kg po), midazolam (0.5, 1.0, 1.5mg/kg po), alprazolam (0.125, 0.25, 0.5, 1.0mg/kg po). Mildly food-restricted, male, Sprague-Dawley rats were initially trained to lever-press for food rewards before being surgically implanted with in-dwelling jugular catheters. Rats were allowed to self-administer heroin (15µg/kg/inj) on a fixed ratio (FR3) schedule of reinforcement in 2hr training sessions. After establishment of consistent heroin self-administration, the rats were subjected to saline extinction. The reinforcing effects of CBD (20, 100, 500µg/kg/inj), diazepam (1, 3, 4.5 or 10µg/kg/inj) and midazolam (0.3, 1, 1.5, 2.25 or 3µg/kg/inj) were then evaluated on a FR3 schedule in 2hr sessions. Results are mean ± SEM. In drug-discrimination, all CBD doses generalised to saline (maximum generalisation to the ip midazolam cue: 11±5% @ 20mg/kg). Midazolam and diazepam both dose-dependently generalised to midazolam (91±5%, 89±7%, respectively). Heroin maintained self-administration in rats (17.6±0.5 inj/session, n=39) at levels significantly greater ($p<0.001$) than saline (3.7±0.2 inj/session, n=39). CBD (100µg/kg/inj) [6.9±1.8 inj/session, n=8] maintained self-administration at levels significantly greater than saline ($p<0.05$). Diazepam (3µg/kg/inj) [7.0±2.1 inj/session, n=8] and midazolam (1.5µg/kg/inj) [7.3±1.3 inj/session, n=16] also served as positive reinforcers ($p<0.05$). This study is the first to systematically investigate the discriminative and reinforcing potential of CBD. The results reveal that CBD did not produce benzodiazepine-like psychoactive effects in rats. CBD served as a

weak reinforcer at a single dose in heroin-trained rats. Diazepam and midazolam also served as weak reinforcers at a single dose.

Disclosures: D.J. Heal: 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.; GW Pharmaceuticals. **S. Holland:** None. **J. Gosden:** None. **R.A. Gray:** None. **S. Smith:** None.

Poster

373. Epilepsy: Experimental Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 373.14/E23

Topic: B.10. Epilepsy

Title: Screening for anti-epileptic compounds with an *in vitro* epilepsy model based on the human ipsc-derived microbrain® 3D platform

Authors: *O. GUICHERIT[1], C. CARROMEU[1], S. DEA[1], N. SODHI[1], F. ZANELLA[1], C. ANDERSEN[1], B. C. GAY[2], J. M. ANDRESEN[2], G. R. STEWART[2]
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Abstract: Epilepsy is one of the most prevalent neurological disorders, with over 50 million sufferers worldwide. Current anti-epileptic dugs (AEDs) only treat symptoms and carry significant risk for drug interactions and adverse effects. Moreover, up to one-third of all epileptic patients are refractory to AEDs. This significant unmet medical need creates an urgent demand for safer and more effective AEDs. Towards that end, we set out to develop an *in vitro* model based on the StemoniX human induced pluripotent stem cell (iPSC)-derived microBrain® 3D Platform to identify drugs with potential anti-epileptic activity. The microBrain® 3D 384-well screening plates contain neural spheroids comprised of a physiologically relevant mixture of fully differentiated cortical-like neurons and astrocytes from a single human donor source of iPSCs. The homogeneous spheroids show large, consistent, spontaneous and synchronous calcium oscillations, and are responsive to a variety of pharmacological neuromodulators. For drug screening, the microBrain® 3D spheroids were first characterized with known neuromodulators and AEDs alone. Next, a collection of 120 targeted Pairnomix proprietary compounds was evaluated. These compounds were selected based on their demonstrated ability to modulate the *in vitro* activity of ion channels implicated in epilepsy. The library comprises compounds with different mechanisms of action: sodium channel inhibitors, potassium channel activators, $GABA_A$ receptor channel activators, and glutamate receptor channel inhibitors. This 120-drug panel was screened against the microBrain® 3D platform at 3 different concentrations (0.1, 1, 10 µM) across two replicates. Hit compounds with substantial inhibitory activity in the assay were then re-tested in serial dilution to confirm activity and derive an IC_{50} and maximal

effectiveness from concentration-response curves. Testing was done in the presence and absence of a seizure-inducing compound (4-Aminopyridine). More than half the compounds showed robust inhibition of the spontaneous calcium oscillations consistent with their known ion channel activity and potential as novel AEDs. The assay also showed a large dynamic range, with many compounds reaching over 99% inhibition in a concentration-dependent fashion. Finally, none of the test compounds demonstrated overt toxicity. Overall, the microBrain® 3D platform generated remarkably consistent and reproducible results, establishing the microBrain® 3D platform as a useful and robust cell-based platform for high-throughput drug screening.

Disclosures: O. Guicherit: A. Employment/Salary (full or part-time):; StemoniX. **C. Carromeu:** A. Employment/Salary (full or part-time):; StemoniX. **S. Dea:** A. Employment/Salary (full or part-time):; stemonix. **N. Sodhi:** A. Employment/Salary (full or part-time):; Stemonix. **F. Zanella:** A. Employment/Salary (full or part-time):; stemonix. **C. Andersen:** A. Employment/Salary (full or part-time):; stemonix. **B.C. Gay:** A. Employment/Salary (full or part-time):; Pairnomix. **J.M. Andresen:** A. Employment/Salary (full or part-time):; Pairnomix. **G.R. Stewart:** A. Employment/Salary (full or part-time):; Pairnomix.

Poster

373. Epilepsy: Experimental Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 373.15/E24

Topic: B.10. Epilepsy

Title: Morphological and functional evaluation of compound activity using human iPSC - derived neuronal 3D cultures

Authors: *C. ANDERSEN[1], O. SIRENKO[2], G. CHANDY[2], M. HAMMER[2], C. CRITTENDEN[2], S. VARGAS-HURLSTON[2], F. PARHAM[3], K. RYAN[3], R. GORDON[1], F. ZANELLA[1], O. GUICHERIT[1], C. CARROMEU[1]
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Abstract: There is an increasing interest in using more complex, biologically relevant, and predictive cell-based assays for assay development and compound screening. StemoniX microBrain® 3D Assay Ready Platform is a high throughput 3D culture platform that more closely resembles the tissue development and constitution of native human brain tissue. In this platform, human iPSC-derived neuronal spheroids are composed of a physiologically relevant co-culture of functionally active cortical glutamatergic and GABAergic neurons and astrocytes. This balanced cellular mix allows the development of a neural network enriched in synapses, creating a highly functional neuronal circuitry. The neuronal cells in the microBrain 3D spheroids are physiologically active, with spontaneous synchronized, readily detectable calcium

oscillations.
We used fast kinetic fluorescence imaging on the FLIPR® Tetra System to measure the patterns and frequencies of the Ca2+oscillations of neuro-spheroids as monitored by changes in intracellular Ca2+ levels with calcium-sensitive dyes. A set of known neuromodulators was tested, including agonists and antagonists of NMDA, GABA and AMPA receptors; kainic acid, analgesic, and anti-epileptic drugs. Changes were observed as inhibitions or activations of the oscillation patterns, matching the expected effect of the correspondent neuromodulator. In addition, we tested a set of known neurotoxic compounds including selected pesticides and flame retardants and demonstrated sensitivity of the assay to the effects of compounds. Assay was optimized for HTS in 384-well plates and allows for the characterization of oscillation profiles in neural spheroid by using multi-parametric analysis with ScreenWorks Peak Pro Software. The automatically measured read-outs include the oscillation rate, peak frequency, peak width, amplitude, and waveform irregularities. In addition, the potential impact of treatment on cell viability and mitochondrial integrity was evaluated by high content imaging using ImageXpress® Micro Confocal High-Content Imaging System. We determined EC50s for the impact of different compounds on the Ca2+ oscillation rates or cell viabilities. In conclusion, we demonstrated that functional and morphological assays using 3D neuronal spheroids formed with human iPSC-derived cells can be used for evaluation of drug candidates and neurotoxicity assessment.

Disclosures: C. Andersen: A. Employment/Salary (full or part-time):; Stemonix. **O. Sirenko:** None. **G. Chandy:** None. **M. Hammer:** None. **C. Crittenden:** None. **S. Vargas-Hurlston:** None. **F. Parham:** None. **K. Ryan:** None. **R. Gordon:** None. **F. Zanella:** None. **O. Guicherit:** None. **C. Carromeu:** None.

Poster

374. LTP: Kinases and Intracellular Signaling

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 374.01/E25

Topic: B.07. Synaptic Plasticity

Support: The Henry and Marilyn Taub Foundation
Zuckerman gift for normal brain aging
Parkinson's Disease Foundation

Title: ATF4 is downregulated during synaptic plasticity potentially to maintain depression or reset potentiation

Authors: *F. AMAR[1], C. CORONA[2], J. LIU[3], E. GRAEFF[4], L. GREENE[3], M. L. SHELANSKI[1]
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Abstract: Activating transcription factor 4 (ATF4/CREB2), in addition to its well-studied role in stress responses, has also been proposed to play other important physiologic functions in the nervous system including regulation of learning and memory. ATF4 has been proposed to both impair and enhance neuronal plasticity and memory, though the studies that have directly regulated ATF4 rather than regulating it through upstream activators or dominant negative inhibitors all indicate that ATF4 is required for normal learning and memory. Our hypothesis is that ATF4 is a homeostatic regulator of synaptic function and that either paucity or excess could affect learning and memory. To further shed light on the possible role of ATF4 in these functions, we examined how ATF4 is modulated during synaptic plasticity. Long-term potentiation (LTP) and long-term depression (LTD) are the underlying molecular mechanism for learning and memory. Thus, we investigated whether either chemically induced LTP or LTD (cLTD, cLTD) modulates ATF4 levels in neurons. Using cultured hippocampal and cortical neurons we found that glutamate-induced LTP reduced ATF4 protein for up to 4 hours and NMDA-induced LTD rapidly induced downregulation over 24 hours. LTP-induced ATF4 depletion required NMDA receptor activation and the initiation of the MEK, pERK pathway upstream of eiF2α phosphorylation. The NMDAR antagonists AP5 and MK801, but not the other ionotropic or metabotropic glutamate receptor antagonists, blocked cLTP and cLTD-induced ATF4 down-regulation as did MEK inhibitor U0126. This cLTP and cLTD-induced ATF4 down-regulation was followed by a decrease in the actin stabilizing protein Cdc42, a protein known to be regulated by ATF4 via RhoGDIα. Interestingly, ATF4 overexpression did not affect initiation of cLTP but appeared to prevent the return of mEPSPs to baseline levels at 24 hours post induction which is normally observed in cells with endogenous levels of ATF4. Together our findings indicate that NMDA receptor dependent cLTP is accompanied by a rapid depletion of ATF4 protein levels that may be necessary to reset the system and allow subsequent rounds of learning and memory.

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Poster

374. LTP: Kinases and Intracellular Signaling

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 374.02/E26

Topic: B.07. Synaptic Plasticity

Support: the Henry and Marilyn Taub Foundation
Zuckerman gift for normal brain aging

Title: Generation of a ATF4 conditional KO mouse line for elucidating the role of ATF4 in learning and memory

Authors: *C. CORONA[1], F. AMAR[3], C.-S. LIN[2], L. GREENE[4], M. L. SHELANSKI[3]
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Abstract: Activating Transcription Factor 4 (ATF4) is a member of the ATF/cAMP responsive element binding protein (CREB) family which is involved in learning and memory. In this regard, we previously reported that specific long-term hippocampal ATF4 down-regulation induces deficits in synaptic plasticity and memory accompanied by a reduction in glutamatergic functionality. In addition, under the same conditions, our electrophysiological data show that ATF4 down-regulation significantly increases the frequency of spontaneous action potentials, an effect mediated by the reduction of the Rho GTPase Cell Division Cycle 42 (Cdc42) and, consequently, membrane $GABA_B$ receptor levels. Because of the conditions of long-term down-regulation of ATF4 employed in our previous studies (4-6 weeks *in vivo*, 14 days *in vitro*), the precise short-term role of ATF4 in the cascade of events involved in learning and memory are still unclear. For instance, it remains to be seen whether short-term modulation of ATF4 that does not impact synaptic structure will improve or impair synaptic plasticity, as well as whether behavioral protocols that stimulate learning and memory formation require ATF4 in order to be effective. Given the challenges in using ATF4 KO mice (adult animals are blind and show severe skeletal abnormalities) to answer these questions, we created a region-specific inducible model of ATF4 down-regulation by generating an ATF4 floxed mouse line and crossing it with a line with CRE expressed under the CAMKII promoter. Our preliminary data with the offspring show that five consecutive days of Tamoxifen injections (75mg/kg body weight) followed by a five days waiting period is sufficient to induce an 80% down-regulation of ATF4 protein in the forebrain but not in the cerebellum. This selectively induced down regulation of ATF4 in the adult mouse does not appear to cause any visual or skeletal deficits. These mice are a novel system for investigating the role of ATF4 in learning and memory processes in a time controlled manner. This mouse will also be useful for studying the role of ATF4 in neurodegenerative diseases such as Alzheimer's disease as one can modulate ATF4 before or after AD related pathologies are apparent.

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Poster

374. LTP: Kinases and Intracellular Signaling

Location: SDCC Halls B-H

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Program #/Poster #: 374.03/E27

Topic: B.07. Synaptic Plasticity

Support: Intramural funding, Rajiv Gandhi Centre for Biotechnology
Kerala State Council for Science, Technology and Environment, India
Council of Scientific and Industrial Research, Govt. of India
Department of Biotechnology, Govt. of India

Title: Intracellular regulation of calcium-calmodulin dependent protein kinase type II function by NMDA receptor subunit GluN2B

Authors: *L. K[1], M. M[1], A. R C[1], R. RAJU[2], A. ANIRUDHAN[2], O. R V[1]
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Abstract: Ca^{2+}/calmodulin dependent protein kinase type II (CaMKII) is enriched in the post synaptic density (PSD) of excitatory synapses. It participates in cellular events such as long-term potentiation (LTP), neurogenesis and excitotoxicity. CaMKII is activated by calcium entry through N-methyl-D-aspartate receptor (NMDAR). Upon binding of Ca^{2+}-calmodulin complex, CaMKII undergoes autophosphorylation at Thr^{286} making it autonomously active. Active CaMKII can translocate to PSD and bind with NMDAR subunit GluN2B. This interaction is implicated in the induction of LTP and also in pathological signaling. CaMKII can engage in two types of binding to GluN2B depending upon the extent of calcium stimulation. Brief stimulation causes reversible binding (S-site binding) and longer stimulation results in persistent binding (T-site binding) intracellularly (Bayer *et al*, 2006, *J. Neursci*., 4, 1164-1174). GluN2B binding to the T-site significantly modulates CaMKII catalysis, leading to a reduced but stable activity (Cheriyan *et al*, 2011, *PLoS One.* 6: e16495). In addition, dephosphorylation of phospho-Thr^{286} of α-CaMKII was also inhibited by binding to GluN2B *in vitro* which is mediated by His^{282} and Glu^{96} of α-CaMKII (Mayadevi *et al*, 2016, *PLos One*, 11(9): e0162011). The modulation of activity and protection of dephosphorylation of CaMKII by GluN2B might play important roles in memory formation or in excitotoxicity. To investigate the existence of the regulation of α-CaMKII by GluN2B under intracellular conditions, experiments were conducted by giving brief and extended stimulation using calcium and the ionophore, ionomycin, to HEK-293 cells in which GFP-α-CaMKII and GluN2B sequence were ectopically expressed. Subsequently, the phosphorylation status of α-CaMKII-Thr^{286} was analyzed as an indicator of CaMKII activity. We found that α-CaMKII-Thr^{286} was phosphorylated upon short stimulation which was reversed by subsequent incubation in the absence of calcium. The reversal was mediated by phosphatases as it was prevented by okadaic acid. However Thr^{286}-phosphorylation persisted under conditions in which CaMKII-GluN2B association also persisted such as upon extended stimulation. This appears to be due to the regulated dephosphorylation of α-CaMKII when it is bound to GluN2B. Mutants such as H282A-α-CaMKII that are defective in the GluN2B-mediated regulation are being studied using this system. Our data demonstrates the regulation of CaMKII dephosphorylation by GluN2B under intracellular conditions.

Disclosures: L. K: None. **M. M:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Mayadevi M is

designated as an inventor in a US patent application for the stable cell line used in this study. **A. R c:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Arunkumar, R. C. is designated as an inventor in a US patent application for the stable cell line used in this study. **R. Raju:** None. **A. Anirudhan:** None. **O. R v:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Omkumar R V is designated as an inventor in a US patent application for the stable cell line used in this study.

Poster

374. LTP: Kinases and Intracellular Signaling

Location: SDCC Halls B-H

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Program #/Poster #: 374.04/E28

Topic: B.07. Synaptic Plasticity

Support: NSF CRCNS Grant 1753700 to TA
NSF CRCNS Grant 1515686 to KTB
Carver Chair in Neuroscience to TA
Office of Naval Research MURI N00014-10-1-0198 to KTB
NIH/NIAAA R01 AA18060 to KTB, TA

Title: Dissecting the contribution of beta-adrenergic signaling to hippocampal synaptic plasticity using biased ligands

Authors: *M. S. SHETTY[1,2], K. T. BLACKWELL[3], T. ABEL[1,2]
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Abstract: Different forms of activity-dependent synaptic plasticity, involving diverse signaling mechanisms, underlie learning and memory. These synaptic plasticity processes are subject to modulation by a variety of neurotransmitters. The noradrenergic system, activated by emotional arousal, stress and novelty, is one of the critical modulators of synaptic plasticity and memory in the hippocampus. In particular, the β-adrenergic receptor (β-AR)-mediated signaling has been highly implicated. Recent evidence suggests that the activation of β-ARs can recruit multiple independent arms of downstream signaling cascades such as Gsα-mediated, Giα-mediated or β-arrestin-mediated pathways, all of which converge on the activation of MAPK (ERK1/2). This diversity leads to the question of which downstream signaling pathways mediate β-adrenergic facilitation of specific forms of synaptic plasticity. We have begun addressing this question by using a computational model of these signaling pathways to predict the outcome of a variety of stimulation paradigms, both with and without β-adrenergic activation in the model. The predictions from the simulations are then tested using acute hippocampal slice electrophysiology experiments combined with pharmacological manipulations using biased ligands. The results

show that carvedilol, a biased β-AR ligand that recruits β-arrestin and MAPK activation but does not increase cAMP levels, fails to induce persistent long-term potentiation (LTP) when combined with a low-frequency stimulation protocol (LFS; 5Hz, 3 min). The same LFS leads to persistent LTP when combined with the application of isoproterenol, a full agonist of β-AR. These results confirm predictions from the computational model. We are now testing the effect of carvedilol co-application on the short-lasting LTP induced by a single 100 Hz stimulation, which as predicted by the model, should not lead to LTP enhancement. We also will be testing the effects of other ligands, such as carazolol that leads to MAPK activation independent of cAMP- or β-arrestin-mediated pathways and ICI-118551, a complete antagonist of the β-AR. These findings will help dissect the β-AR signaling mechanisms underlying specific forms of synaptic plasticity.

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Poster

374. LTP: Kinases and Intracellular Signaling

Location: SDCC Halls B-H

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Program #/Poster #: 374.05/E29

Topic: B.07. Synaptic Plasticity

Support: NIMH Grant R37 MH057068
NIMH Grant R01 DA034970

Title: Multiple compensatory mechanisms in tamoxifen-inducible/CreERT2 conditional PKMζ knockout mice

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Abstract: PKMζ is a brain-specific, atypical PKC isoform that has been proposed to maintain LTP and long-term memory through its persistent kinase activity. During long-term potentiation (LTP) and long-term memory induction, new PKMζ is synthesized from a dedicated PKMζ mRNA, generated from an alternate internal promoter within the PKCζ/PKMζ gene *(Prkcz).* Using a pharmacogenetic approach, a recent study (Tsokas et al., 2016) demonstrated that another atypical PKC, PKCι/λ, the gene product most closely related to PKMζ,

compensates for the absence of PKMζ in constitutive PKCζ/PKMζ knockout (KO) mice, thus explaining the findings that LTP and long-term memory can be maintained in the absence of PKMζ in these mice (Volk et al., 2013). To further examine potential mechanisms for compensation of PKMζ during LTP and long-term memory, we generated tamoxifen-inducible conditional knockout (cKO) mice of PKCζ/PKMζ by crossing PKCζ/PKMζ$^{fl/fl}$ mice with CaMKII-CreERT2 mice (PKCζ/PKMζ$^{fl/fl}$;CreER). The PKMζ-cKO mice received daily intraperitoneal injections of tamoxifen for 5 days; control mice were either wild-type mice injected with tamoxifen or PKCζ/PKMζ$^{fl/fl}$;CreER mice injected with vehicle. Three days after the final injection, the mice were sacrificed and their dorsal hippocampi were excised and CA1 region assayed for PKMζ mRNA, total PKMζ- and PKCι/λ-specific antisera, as well as two markers of PKMζ activity, phospho-Thr555/Thr563 PKCζ/λ antisera recognizing the atypical PKC site phosphorylated by mTORC2 and/or autophosphorylation, and phospho-atypical PKC activation loop antisera (phospho-PKCζ/λ Thr410/403). The results reveal that after knockdown of PKMζ, cKO mice compensate by increasing the activity of the remaining PKMζ, as indicated by large increases in both the phospho-Thr555/Thr563 and phospho-Thr410/403 immunostaining of the residual PKMζ. In addition, PKMζ cKO mice show increased expression of total PKCι/λ, similar to the compensatory mechanism of constitutive PKMζ KO mice. Thus, both enhanced activation of residual PKMζ and increased PKCι/λ compensate for the partial loss of PKMζ in cKO mice. Future experiments will examine whether these compensatory mechanisms contribute to the maintenance of LTP and long-term memory in PKMζ cKO mice.

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Poster

374. LTP: Kinases and Intracellular Signaling

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Program #/Poster #: 374.06/E30

Topic: B.07. Synaptic Plasticity

Support: NIH Grant MH101703
NIH Grant NS057128

Title: P18 is critical for lysosomal Ragulator-Rag complex assembling and synaptic plasticity in hippocampal neurons

Authors: *J. SUN[1], Y. LIU[2], X. HAO[1], W. LIN[1], B. DIEP[2], E. CHIANG[2], M. BAUDRY[2], X. BI[1]
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Abstract: Accumulating evidence indicates that the lysosomal Ragulator complex is essential for full activation of the mechanistic target of rapamycin complex 1 (mTORC1). A recent crystal structure study revealed that p18 (aka LAMTOR1) forms a “ribbon” around the other 4 members of the Ragulator to stabilize the complex and provide contact points for Rag GTPases, which recruit and activate mTORC1 on lysosomes. Yet, the precise function of p18 in CNS has rarely been studied. We report here that in hippocampal neurons, p18 is essential for lysosomal localization of other Ragulator members and Rag GTPases, as p18 knockdown (KD) markedly reduced the lysosomal localization of LAMTOR4 and RagA. We further showed that lysosomal localization of the Ragulator-Rag complex is essential for mTORC1 activation in hippocampal neurons. We previously reported that Ube3a, an E3 ligase that plays important roles in brain development and function, ubiquinates p18, resulting in its proteasomal degradation, and Ube3a deficiency in hippocampus of Angelman syndrome (AS) mice produces increased lysosomal localization of p18. We found that increased p18 levels in AS mice are associated with increased lysosomal localization of the Ragulator-Rag complex and mTORC1, but not mTORC2. Our results also support the idea that the Ragulator functions not only as a platform but also as a Rag GTPase GEF to facilitate mTORC1 activation. Furthermore, our in vivo experiments showed that p18 KD in AS mice, in addition to “normalizing” mTORC1/mTORC2 activation, restored TBS-induced LTP, increased the number of mature dendritic spines in hippocampus and restored hippocampus-dependent learning. Intriguingly, p18 KD in WT mice impaired LTP, reduced the number of mature spines, and reduced context-dependent learning performance. Immunostaining results of Arc in CA1 dendritic field showed that levels of Arc were significantly higher in control siRNA-injected AS mice, as compared to control siRNA-injected WT mice; p18 KD significantly reduced Arc expression in both WT and AS hippocampal slices. In addition, p18 KD induced a larger reduction in Arc expression in WT than in AS mice, which may contribute to reduced LTP in p18 siRNA WT group. Collectively, our results indicate that an optimal level of lysosome-anchored Ragulator-Rag complex is required for normal activation of mTORC1, which is critical for functional and structural synaptic plasticity as well as learning and memory.

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Poster

374. LTP: Kinases and Intracellular Signaling

Location: SDCC Halls B-H

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Program #/Poster #: 374.07/E31

Topic: B.07. Synaptic Plasticity

Support: PRIN2015 to FG
MinSan 2013 to FG

Title: Rabphilin 3A modulates structural and functional synaptic plasticity through interaction with GluN2A/PSD-95 complex

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Abstract: We previously reported that Rabphilin3A (Rph3A) plays a key role in the stabilization of GluN2A-containing NMDA receptors at synapses through the formation of a ternary complex with GluN2A and PSD-95. In particular, perturbing GluN2A/Rph3A interaction induces a decrease of NMDAR-mediated currents and dendritic spine density (*Stanic et al., Nat. Comm., 2015*). Based on these previous observations, the two main goals of the present study are: i. investigating the role of Rph3A in functional and structural synaptic plasticity at hippocampal synapses and ii. elucidating the molecular mechanisms regulating Rph3A binding to NMDA receptors and its consequent recruitment at the postsynaptic density (PSD). Different experimental protocols were used to induce synaptic plasticity *in vitro and in vivo* in presence or absence of agents modulating Rph3A function. Following treatments, Rph3A interaction with NMDA receptor complex, its localization at the excitatory synapse, synaptic plasticity and dendritic spine morphology were evaluated by using a vast array of biochemical, imaging and electrophysiological analyses. Electronic microscopy experiments revealed that about 50% of spines display Rph3A in resting conditions in hippocampal neurons. Interestingly, Rph3A-positive spines show increased PSD length and thickness and an increase of spine volume. Knock-down of Rph3A by shRNA greatly impedes the insertion of GluA1-containing AMPA receptors at the synapse following induction of chemical long-term potentiation (cLTP). Accordingly, inhibition of Rph3A activity leads to failure in LTP induction by high-frequency stimulation and prevents LTP-dependent structural modifications of spines. Overall, our study demonstrate that Rph3A is necessary to trigger functional and structural synaptic plasticity at the excitatory glutamatergic synapse.

Disclosures: F. Gardoni: None. **J. Stanic:** None. **L. Franchini:** None. **M. Mellone:** None. **S. Musardo:** None. **E. Zianni:** None. **G. Olivero:** None. **A. Pittaluga:** None. **C. Racca:** None. **C. Bellone:** None. **E. Marcello:** None. **M. Di Luca:** None.

Poster

374. LTP: Kinases and Intracellular Signaling

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 374.08/E32

Topic: B.07. Synaptic Plasticity

Support: JSPS KAKENHI 26430063
JSPS KAKENHI 17H03557
AMED Grant 17bk010477h0001

Title: Drebrin binds to CaMKIIβ and forms activity-dependent tripartite interaction between drebrin, CaMKII and F-actin in dendritic spines

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Abstract: Drebrin increases F-actin stability by elongating the helical crossover of F-actin. Drebrin is highly concentrated in dendritic spines and exit from it in an activity-dependent manner. In this study, we identified Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) β isoform as a novel drebrin-binding protein using a yeast two-hybrid system. We analyzed the drebrin-binding region of CaMKIIβ by immunoprecipitation from HEK293 cells transfected with Drebrin-FLAG and HA-CaMKIIβ 1-280 or 281-542. Anti-FLAG immunoprecipitations showed that drebrin interacts with CaMKIIβ 1-280, but not CaMKIIβ 281-542. A non-F-actin binding CaMKIIβ isoform CaMKIIβe could bind to drebrin, suggesting that CaMKIIβ directly binds to drebrin but not through their actin-binding activity. Then, we investigated the drebrin–CaMKIIβ relationship in dendritic spines using rat hippocampal neurons. CaMKIIβ was localized at dendritc spines in the control neurons but not in the drebrin knockdown (KD) neurons. Although CaMKIIα did not bind to drebrin, the spine-localization of CaMKIIα was also weakened in the drebrin-KD neurons, suggesting that drebrin stabilizes CaMKIIα&β hetero-oligomer in dendritic spines by interacting with CaMKIIβ. Fluorescence recovery after photobleaching analysis showed that drebrin KD increased the stable fraction of CaMKIIβ, indicating the presence of drebrin-independent, more stable CaMKIIβ. Super-resolution microscopy (N-STORM) elucidated that CaMKIIβ is widespread in a dendritic spine and partially co-localizes with drebrin in the inner region of dendritic spines during the resting state. NMDA receptor activation decreased CaMKIIβ colocalization with drebrin while it increased CaMKIIβ colocalization with PSD. In parallel with this change, NMDA-receptor activation induced drebrin exodus from dendritic spines. Previous study shows that CaMKIIβ detaches from F-actin by autophosphorylation. Together, we conclude that drebrin stabilizes CaMKIIβ in the inner region of dendritic spines as drebrin/CaMKII/F-actin tripartite complex, and this interaction is regulated by neuronal activity.

Disclosures: T. Shirao: None. **H. Yamazaki:** None. **Y. Sekino:** None.

Poster

374. LTP: Kinases and Intracellular Signaling

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Program #/Poster #: 374.09/E33

Topic: B.07. Synaptic Plasticity

Support: NSF Grant IOS1121886
NIH Grant R25GM109442

Title: Increased sucrose experience prevents sucrose-induced increases in glutamate AMPA receptor phosphorylation in dorsal hippocampal neurons

Authors: N. BARNETT[1], A. P. ROSS[1], A. D. FAULKNER[2], R. C. HANNAPEL[1], *M. B. PARENT[1]
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Abstract: Evidence in humans suggests that meal-related memory influences later eating behavior. Memory can serve as a vital mechanism for controlling eating behavior because it provides a record of recent intake that likely outlasts most physiological signals generated by ingestion. We have proposed that dorsal (dHC) and ventral hippocampal (vHC) neurons, which are critical for memory, limit energy intake during the postprandial period. In support, our lab found that temporarily inactivating dHC or vHC neurons after the consumption of a sucrose meal decreased the latency to eat the next sucrose meal and increased the size of that meal. If dHC or vHC neurons control intake through a process that requires memory, then ingestion should increase events necessary for synaptic plasticity in dHC and vHC during the postprandial period. To test this, we determined whether ingesting a sucrose solution induces phosphorylation of AMPAR GluA1 subunits at serine 831 ($pSer^{831}$) and serine 845 ($pSer^{845}$) residues. We also determined whether increasing the amount of previous experience with the sucrose solution, which would be expected to decrease the mnemonic demands associated with that meal, would also attenuate sucrose-induced phosphorylation. Specifically, we exposed male Sprague-Dawley rats to a sucrose solution for 10 min/day for 3, 5 or 10 days and their brains were harvested 90 min after their last sucrose bout. Quantitative immunoblotting of dHC and vHC membrane fractions demonstrated that sucrose ingestion increased postprandial dHC $pSer^{831}$ and that increased sucrose experience prevented this effect and decreased vHC $pSer^{831}$. Sucrose ingestion did not affect $pSer^{845}$ in either dHC or vHC. The sucrose-induced increase in dHC GluA1 $pSer^{831}$ is noteworthy because previous research has shown that learning produces a comparable elevation of dHC GluA1 $pSer^{831}$, which results in increased glutamate AMPA receptor conductance and augmented synaptic strength. These findings indicate that ingestion activates

proteins necessary for synaptic plasticity and memory in dHC, which is consistent with the hypothesis that dHC neurons form a memory of a meal.

Disclosures: N. Barnett: None. **A.P. Ross:** None. **A.D. Faulkner:** None. **R.C. Hannapel:** None. **M.B. Parent:** None.

Poster

374. LTP: Kinases and Intracellular Signaling

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Program #/Poster #: 374.10/E34

Topic: B.07. Synaptic Plasticity

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Title: LTP-induced translocation of MAP2 into dendritic spines

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Abstract: Long-term potentiation (LTP) is a cellular model for information storage which involves synaptic reorganization of signaling and regulatory proteins leading to structural and functional plasticity. Microtubule-associated protein 2 (MAP2) is a microtubule binding protein required for the regulation of microtubule dynamics and stability. Using biochemical, cell biological, and imaging approaches, we found that a subpopulation of MAP2 in dendritic shaft rapidly translocated to dendritic spines in response to chemical LTP (cLTP) stimulation. Spine translocation of MAP2 was dependent on NMDA receptor activation and intracellular calcium concentration, while either chemical long-term depression or KCl-mediated depolarization failed to induce MAP2 spine translocation. Bath application of independent Ras-MAPK inhibitors significantly reduced the number of neurons with MAP2-positive spines, whereas other pharmacological inhibitors against a variety of key synaptic protein kinases including CaMKII, PKC, and PKA did not affect the cLTP-induced MAP2 spine translocation in cultured rat hippocampal neurons. Live cell confocal imaging showed that the spine translocation of MAP2 coupled with cLTP-induced spine enlargement. Coherently, surface AMPA receptor levels were increased in neurons with MAP2-positive spines compared to those with MAP2-negative spines following cLTP stimulation. Furthermore, immuno-electron microscopy combined with electrical LTP stimulation revealed synaptic localization of MAP2 in dendritic spines of CA1

hippocampal neurons in acute brain slices. Together, these results suggest a novel role of MAP2 in synaptic plasticity in addition to its well-established function of microtubule regulation in dendritic compartment.

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Poster

374. LTP: Kinases and Intracellular Signaling

Location: SDCC Halls B-H

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Topic: B.07. Synaptic Plasticity

Support: NIH/NICHD R01 HD083828-01
NIH/NINDS R21 NS100047-01

Title: The impact of PKA-dependent phosphorylation of GluN2B at Ser1166 on hippocampal plasticity and cognition

Authors: *M. W. PORCH[1], J.-Y. HWANG[1], A. E. CHAVEZ[2], R. S. ZUKIN[1]
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Abstract: NMDA receptors (NMDARs) are glutamate-gated ion channels that are enriched at excitatory synapses, where they are strategically positioned to play a crucial role in regulation of synaptic function. A unique feature of NMDARs is their high permeability to Ca^{2+}. Ca^{2+} influx through NMDARs is essential for synaptogenesis, plasticity of neural circuitry, and higher cognitive functions, such as learning and memory. Emerging evidence reveals that PKA signaling represents a fundamental mechanism by which NMDAR-mediated Ca^{2+} influx is modulated in neurons. We recently identified serine 1166 (Ser1166) in GluN2B to be the molecular target of PKA phosphorylation relevant to PKA-dependent NMDAR Ca^{2+} permeability. Whereas the impact of Ser1166 on NMDAR Ca^{2+} permeability is well-established, its role in NMDAR-dependent synaptic plasticity and cognition is, as yet, unclear. To address this issue, we generated a mouse in which we knocked in GluN2B containing a single point mutation, S1166A, by means of CRISPR/cas technology. Whereas basal synaptic transmission and synaptic plasticity in the form of high frequency stimulation induced LTP (HFS-LTP) was normal in the knockin mice, theta-burst stimulation-induced LTP (TBS LTP) at CA1 synapses was greatly diminished in slices from knock-in vs. wild-type mice. A distinguishing feature of spaced TBS- *vs*. condensed HFS-LTP at CA1 synapses is a requirement for the transient synaptic incorporation of GluA2-lacking AMPARs during the induction phase of LTP, which are subsequently replaced by GluA2-containing AMPA receptors. A hypothesis under consideration is that Ser1166 is critical for synaptic incorporation of Ca^{2+} permeable (but not Ca^{2+}-

impermeable) AMPARs. We found that, upon induction of TBS-LTP, AMPARs EPSPs are inwardly rectifying and that the LTP is blocked by inhibition of Ca^{2+} permeable AMPARs with IEM-1460 and philanthotoxin 74. TBS LTP in KI mice was rescued by rolipram, which acts as a PKA activator and leads to the insertion of Ca^{2+}-permeable AMPARs to rescue LTP, and by a transient increase in extracellular Ca^{2+}. We further showed that visual cognition, assessed by means of the novel object recognition task, was markedly impaired in knockin *vs*. wild-type mice. Thus, loss of a single site within the GluN2B subunit not only eliminates PKA-induced Ca^{2+} signaling in spines, but greatly diminishes Ca^{2+}-permeable AMPAR-dependent synaptic plasticity in the form of TBS-LTP and hippocampal-based memory in the form of visual recognition.

Disclosures: M.W. Porch: None. **J. Hwang:** None. **A.E. Chavez:** None. **R.S. Zukin:** None.

Poster

374. LTP: Kinases and Intracellular Signaling

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 374.12/E36

Topic: B.07. Synaptic Plasticity

Support: NIMH 1R01MH109719

Title: Characterization of PP1 isoforms in CA1 post-synaptic plasticity

Authors: *K. F. FOLEY[1,2], J. ZHANG[2], H. HOU[2], A. C. NAIRN[3], H. XIA[1,2]
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Abstract: Protein phosphatase 1 (PP1) is one of the primary regulators of post-synaptic plasticity, attenuating long-term potentiation (LTP) and required for NMDA receptor-induced long-term depression (LTD). In addition to the electrophysiological characterization of PP1 activity, behavioral studies have demonstrated that PP1 activity can constrain learning and memory. Likewise, its inhibition can prolong memory and facilitate LTP. However, there are three separate PP1 genes in mammals, all of which are expressed in neural tissue. Previous studies have made little distinction between these isoforms, using indiscriminate pharmacological inhibition. Although they share a common, conserved catalytic domain, there is little homology in their N- and C-termini, leading to differences in localization and protein binding. For example, PP1α and PP1γ1 are enriched in dendritic spines whereas PP1β is enriched in dendrites. To elaborate the differences between PP1 isoforms, we selectively knockout each isoform in mice and perform acute slice recordings in CA1 of the hippocampus. While PP1β KO during early development is lethal, PP1α and PP1γ KO mice are viable. Surprisingly, PP1α and PP1γ KO mice show no significant change in CA1 LTP following high frequency stimulation. In

line with differences in localization, these findings suggest a possible compensation between PP1α and PP1γ while PP1β is necessary for development. Further work is needed to delineate the possible differential roles of PP1α, β, and γ in synaptic functions.

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Poster

374. LTP: Kinases and Intracellular Signaling

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Topic: B.07. Synaptic Plasticity

Support: San Diego Fellowship
AFOSR Grant FA9550-18-1-0051

Title: Spatiotemporal analysis of calcium transients in dendritic spines

Authors: *M. BELL[1], T. BARTOL[2], T. J. SEJNOWSKI[2], P. RANGAMANI[1]
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Abstract: Dendritic spines are small subcompartments along dendrites that act as epicenters of synaptic communication and signaling activity. These spines have several characteristic shapes, and while it is known that this shape is associated with their function, this relationship is not well understood. In this work, we investigate the relationship between the shape and size of both the spine head and spine apparatus in governing calcium dynamics. Calcium is a vital secondary messenger in the spine that triggers various signaling cascades leading to long term potentiation or depression, and spine volume changes. To elucidate this relationship, we developed a spatial continuum model of calcium dynamics through various flux sources including N-methyl-D-aspartate receptors (NMDAR), voltage sensitive calcium channels (VSCC), and various ion pumps. With this model, we show that i) size and shape of the spine regulate calcium dynamics. ii) Membrane fluxes nonlinearly impact calcium dynamics both temporally and spatially. iii) The spine apparatus rescales calcium by acting as a sink. iv) Buffering proteins are required for physiological temporal dynamics that match experimental and computational calcium models.

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Poster

374. LTP: Kinases and Intracellular Signaling

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Topic: B.07. Synaptic Plasticity

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Dept. of Veterans Affairs Grant 1I01BX002316
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NIH T32 AG00096-34

Title: Mechanisms of hippocampal long-term potentiation (LTP) are sexually dimorphic in rodents

Authors: A. A. LE[1], W. WANG[2], C. D. COX[1], J. C. LAUTERBORN[1], E. R. LEVIN[3], G. LYNCH[1], *C. M. GALL[1]
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Abstract: Men are generally better than women in learning spatial relationships whereas the reverse holds for semantic information, but the neurobiological bases for these differences are not understood. We will describe a striking sexual dimorphism in synaptic mechanisms of memory encoding in hippocampal field CA1, a region critical for spatial learning. Studies of acute hippocampal slices from adult rats and mice showed that theta-burst stimulation (TBS)-induced long-term potentiation (LTP) in the Schaffer-Commissural (S-C) projections to CA1 depends upon endogenous estrogen and estrogen receptor α (ERα) in gonadally intact, adult females but not in males. Antagonists for ERß or GPER1 receptors did not disrupt S-C LTP in either sex. Experiments with mutant mice that express only nuclear or membrane forms of ERα (NOER or MOER mice, respectively) confirmed that LTP depends on membrane ERα in females. Quantitative immunofluorescence revealed that the sex differences extend to activity-induced kinase signaling at S-C synapses: TBS driven phosphorylation of ERK1/2 and Src requires ERα in females only. Downstream actin signaling initiated by RhoA, TrkB, and ß1-integrin was comparable between the sexes, except for the expected dependency on ERα in females. Finally, spatial learning activated synaptic ERK1/2 and Src in both sexes but in females this response and long-term memory required ERα. In all, induction of memory-related synaptic modifications by naturalistic patterns of afferent activity involves an ERα-kinase link in females that is not utilized by males. We propose that the addition of this step alters LTP parameters and thereby elevates the threshold for encoding of certain forms of memory. Potential advantages associated with a higher threshold will be discussed.

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Poster

374. LTP: Kinases and Intracellular Signaling

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Topic: B.07. Synaptic Plasticity

Support: NIH NS040701
AHA 16PRE29880006
NIH F31NS096815-02
NSF DBI-1337573
NIH S10 RR023381
NIH/NCATS UL1 TR001082

Title: AKAP150 palmitoylation regulates synaptic incorporation of Ca^{2+}-permeable AMPARs basally and during LTP

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Abstract: Synaptic incorporation of Ca^{2+}-permeable AMPA-type glutamate receptors (CP-AMPARs) containing GluA1, but lacking GluA2, subunits contributes to multiple forms of synaptic plasticity, including long-term potentiation (LTP). A-kinase anchoring protein (AKAP) 150 scaffolds kinases and phosphatases to AMPARs to regulate GluA1 phosphorylation and trafficking. AKAP150 targeting to cellular membranes is modulated through its palmitoylation on two Cys residues. Here, we developed a palmitoylation-deficient knock-in mouse to show that AKAP150 palmitoylation regulates CP-AMPAR incorporation at hippocampal CA1 synapses. Using a combination of biochemical, super-resolution fluorescence imaging, and electrophysiological approaches, we found that palmitoylation promotes AKAP150 association with the postsynaptic density (PSD) and limits basal CP-AMPAR synaptic incorporation. In addition, we found that AKAP150 palmitoylation is required for LTP induced with weaker stimuli that recruit CP-AMPARs but not stronger stimuli that recruit predominantly GluA2-containing AMPARs. Thus, AKAP150 palmitoylation controls its postsynaptic localization to maintain proper basal and activity-dependent regulation of synaptic AMPAR subunit composition.

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Poster

374. LTP: Kinases and Intracellular Signaling

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Topic: B.07. Synaptic Plasticity

Title: Competitive tuning of Ca2+/calmodulin activated proteins provides a homeostatic effect in synaptic plasticity

Authors: *M. C. PHARRIS, N. M. PATEL, D. R. ROMANO, T. L. KINZER-URSEM
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Abstract: Synaptic plasticity depends on NMDA receptor-mediated calcium ion (Ca^{2+}) flux. Intracellular Ca^{2+} binds to the Ca^{2+}-sensor calmodulin (CaM), which modulates downstream effector proteins including kinases, phosphatases, and ion channels, whose differential activation leads to either potentiation or depression of synaptic strength. The activation of individual downstream CaM binding proteins (CBPs) is in-part a function of the frequency of Ca^{2+} flux, such that each CBP is preferentially "tuned" to different Ca^{2+} input signals. Tuning of CBP activation may depend on a variety of mechanisms such as feedback loops and spatial effects. Here, we will explore an additional mechanism called "competitive tuning" in which competition among CBPs for binding to Ca^{2+}/CaM is sufficient to recreate *in silico* the observed *in vivo* Ca^{2+} frequency-dependence of several CBPs. For example, the CBP calcineurin preferentially binds CaM at low frequencies (<10Hz) in a competitive model of many explicitly-defined CBPs, in agreement with experimental results.
One prediction of competitive tuning is that CaM-binding of Ca^{2+}/CaM-dependent kinase II (CaMKII) decreases with decreasing concentration of the CaM buffer neurogranin. This counter-intuitive result highlights how the dynamics of CBP activation may strongly depend on the identity and concentration of the proteins that constitute the competitive pool.
We hypothesize that although perturbations may decrease activation of one CBP such as CaMKII, a separate but functionally similar competitor could compensate, providing a chemostatic effect. We explore this mechanism by first quantifying the effect of parameter perturbations on competitive tuning. We also extend our model to include phosphatase activity and AMPA receptor phosphorylation (AMPARp); a hallmark of synaptic plasticity. With this extended model we proceed to examine the effect of perturbations in the concentration of the CaM buffer neurogranin. Neurogranin knockout simulations show a realignment in competition for CaM that decreases Ca^{2+}/CaM-dependent kinase II activation, but interestingly, overall AMPARp levels are maintained by a concomitant increase in adenylate cyclase 8 (AC8)

activation. Our results provide further evidence that competitive tuning is an important mechanism in the regulation of synaptic plasticity.

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Poster

374. LTP: Kinases and Intracellular Signaling

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Topic: B.07. Synaptic Plasticity

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Max Planck Florida Institute

Title: Integration of spatiotemporally distinct signals by PKCα during synaptic plasticity

Authors: *L. A. COLGAN[1], M. HU[1], J. A. MISLER[1], P. PARRA-BUENO[1], C. M. MORAN[1], M. LEITGES[2], R. YASUDA[1]
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Abstract: The Protein kinase C (PKC) family of enzymes has long been established as critical for synaptic plasticity. However, it is unknown whether Ca^{2+}-dependent PKC isozymes are activated in dendritic spines during plasticity, and if so, how this synaptic activity is encoded by PKC. Here, using newly-developed, isozyme-specific sensors, we demonstrate that classic isozymes are activated to varying degrees and with unique kinetics. PKCα is activated robustly and rapidly in stimulated spines and is the only isozyme required for structural plasticity. This specificity, depends on a PDZ-binding domain present only in PKCα. The activation of PKCα during plasticity requires both NMDAR Ca^{2+}-flux and autocrine BDNF-TrkB signaling, two pathways that differ by orders of magnitude in their spatiotemporal scales of signaling. Our results suggest that by integrating these two signals, PKCα combines a measure of recent, nearby synaptic activity with local synaptic input, enabling complex cellular computations such as heterosynaptic facilitation of plasticity necessary for efficient hippocampal-dependent learning.

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Poster

374. LTP: Kinases and Intracellular Signaling

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Topic: B.07. Synaptic Plasticity

Support: Research Council of Norway, grant 248828
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Title: Unified biochemical model for long-term synaptic plasticity in the cortex - What are the essential pathways?

Authors: *T. MÄKI-MARTTUNEN[1], K. T. BLACKWELL[2], A. G. EDWARDS[3]
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Abstract: Despite the advances made in mechanistic modelling of long-term potentiation (LTP) and depression (LTD) in hippocampus (Ajay & Bhalla 2004, Eur J Neurosci 20(10):2671-80), cerebellum (Antunes & de Schutter 2012, J Neurosci 32(27):9288-9300), and basal ganglia (Lindskog et al. 2006, PLoS Comput Biol 2(9): e119), the signaling pathways underlying long-term plasticity in the neocortex remain less understood. Nevertheless, plasticity in neocortical synapses is crucial to many tasks performed by prefrontal and sensory cortices (Carcea & Froemke 2013, Prog Brain Res 207:65-90), and accordingly, a number of brain disorders have been associated with deficits in neocortical plasticity (Battaglia et al. 2007, Biol Psychiatry 62(12):1405-1412; Çavuş et al. 2012, Biol Psychiatry 71(6):512-520). Remarkably, computational models of Hebbian learning have been widely adopted in *in silico* studies of neocortical circuits. These models, however, typically fail to describe the subcellular mechanisms underlying the plasticity and thus prevent from drawing conclusions on the genetic and metabolic deficits underlying the brain disorders. In this work, we review the molecular mechanisms proposed for long-term plasticity in the neocortex. In particular, we identify the proteins and signaling molecules that act at the post-synaptic domain of pyramidal cells to induce and maintain LTP or LTD. The dependence of these pathways on protein synthesis is not explored. Figure 1 shows the composition of known major pathways and their mutual dependencies. These pathways are similar to the pathways that are important for hippocampal plasticity, although the dopaminergic signaling cascades may play a more central role in cortical LTP/LTD than in hippocampal LTP/LTD.

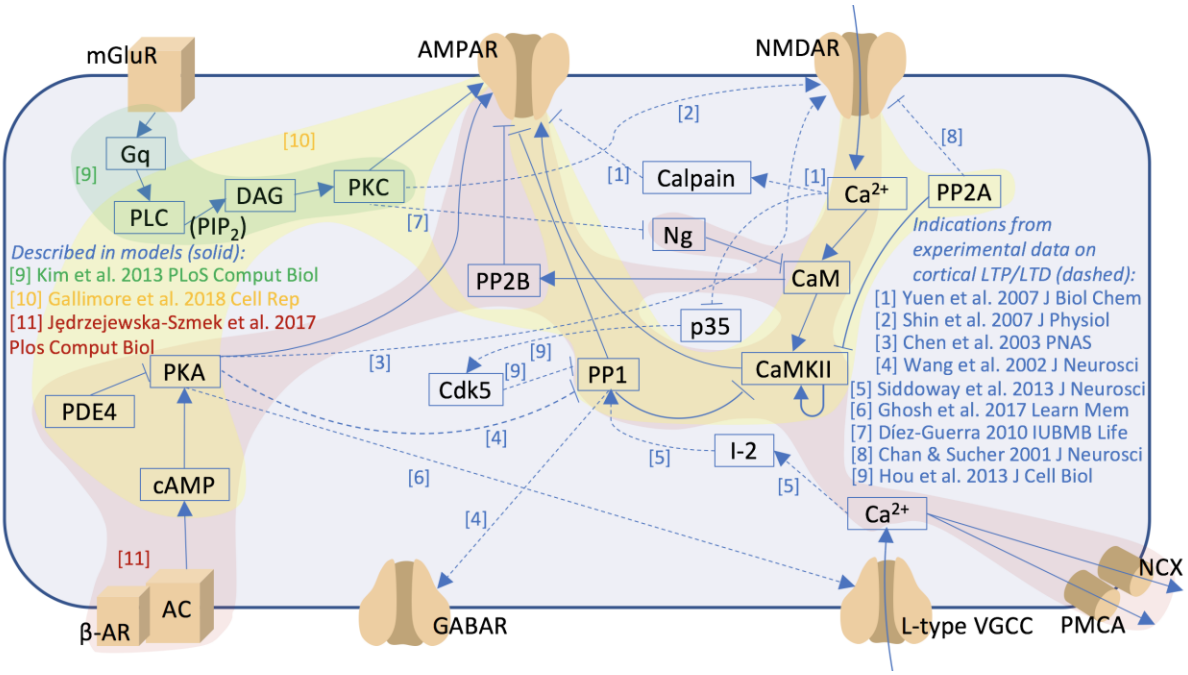

Figure 1. Signaling pathways leading to LTP/LTD in neocortical synapses (at the post-synaptic domain). Solid connections represent actions that have been previously described in computational models of other brain regions (see colored background), whereas dashed lines represent actions not yet described in models.

Disclosures: T. Mäki-Marttunen: None. **K.T. Blackwell:** None. **A.G. Edwards:** None.

Poster

374. LTP: Kinases and Intracellular Signaling

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 374.19/E43

Topic: B.07. Synaptic Plasticity

Support: HHMI Gilliam Fellowship

Title: Adult-selective deletion of KIBRA reduces long-term potentiation and increases sharp wave ripples

Authors: *M. L. MENDOZA, L. D. QUIGLEY, R. PENDRY, B. E. PFEIFFER, L. J. VOLK
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Abstract: Our capacity to learn and remember changes dynamically throughout our lifespan, however the molecular, synaptic, and circuit basis for age-related changes in cognition remain poorly understood. Synaptic plasticity is widely accepted as a key cellular mechanism underlying cognitive functions such as learning and memory. At the molecular level, dynamic AMPA receptor (AMPAR) trafficking is critical for many forms of synaptic plasticity. AMPAR trafficking is regulated by multiple proteins including the synaptically-localized scaffold protein known as KIBRA. Human genetic evidence suggest that common KIBRA polymorphisms are associated with natural variation in human memory performance. Interestingly, though KIBRA is expressed throughout development and adulthood, deficits in synaptic plasticity do not emerge until early adulthood in constitutive KIBRA knockout mice. Whether the loss of KIBRA facilitates synaptic maturation during juvenile development, or adult synapses are selectively vulnerability to a loss of KIBRA remains to be addressed. Thus, to determine if adult neurons are selectively vulnerable to a loss of KIBRA, we generated an inducible knockout mouse by breeding *Kibra* $^{floxed/floxed}$ mice with CaMKII Cre^{ERT2} mice. Here, we demonstrate that reducing KIBRA expression selectively in adult mice reduces long-term potentiation (LTP), but surprisingly has no affect on long-term depression (LTD). Furthermore, we observed no difference in basal synaptic transmission. Next, we sought to examine how the aforementioned deficits in LTP influence hippocampal circuit function. By utilizing *in vivo* electrophysiology in freely behaving mice, our preliminary data indicate that reducing KIBRA expression selectively in adult mice increases the frequency of hippocampal sharp wave ripples. In ongoing studies we are investigating circuit function during adolescent development in KIBRA KO mice as well as molecular mechanisms by which KIBRA influences LTP.

Disclosures: M.L. Mendoza: None. **L.D. Quigley:** None. **R. Pendry:** None. **B.E. Pfeiffer:** None. **L.J. Volk:** None.

Poster

374. LTP: Kinases and Intracellular Signaling

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 374.20/E44

Topic: B.07. Synaptic Plasticity

Support: MH095248
MH113189

Title: Evidence for a sex difference in the requirement of PKA to induce long-term potentiation in the hippocampus

Authors: *A. JAIN, C. S. WOOLLEY
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Abstract: The involvement of cAMP-activated protein kinase A (PKA) in long-term potentiation (LTP) of CA3-CA1 synapses in the hippocampus has been studied extensively. While most studies indicate little role for PKA in early LTP, some studies have shown that early LTP can be PKA sensitive. For example, LTP induced by pairing postsynaptic depolarization with presynaptic stimulation was reduced by ~30% in the presence of a PKA inhibitor (Otmakhova et al., J Neurosci 2000; 20:4446-51). Separately, our lab has identified a sex difference in the requirement for PKA in synaptic potentiation induced by acute estradiol (E2) application to hippocampal slices; E2-induced potentiation was abolished by PKA inhibition in females, but was unaffected in males. In the current study, we asked whether the sex difference in PKA involvement in synaptic potentiation generalizes to LTP.
We recorded excitatory postsynaptic currents (EPSCs) in acute hippocampal slices from adult male and female rats with or without the cell-permeant PKA inhibitor mPKI (0.5 μM) in the bath. After recording 10-15 min of baseline EPSCs, we induced LTP by pairing postsynaptic depolarization to 0 mV with 200 presynaptic stimulations delivered at 1.4 Hz and continued recording for 30-70 min. We calculated the magnitude of potentiation within each cell by averaging the amplitude of all EPSCs recorded following LTP induction compared to all EPSCs recorded during the baseline period. In the absence of the PKA inhibitor, the magnitude of LTP was similar in males and females. Overall, EPSC amplitude increased by 145 ± 30% above baseline in males and by 121 ± 15% above baseline in females (unpaired t-test, $t = 1.14$, $df = 14$, $p = 0.27$). In contrast, LTP induced in the presence of the PKA inhibitor was starkly different between the sexes. In males, PKA inhibition attenuated LTP to 82 ± 36% above baseline, whereas in females, PKA inhibition essentially abolished LTP, to only 14 ± 16% above baseline (two-way ANOVA, sex x mPKI interaction, $F(1,21) = 5.6$, $p = 0.03$). Although still preliminary, these results suggest a sex difference in the involvement of PKA in the mechanisms that underlie LTP: Whereas PKA contributes to pairing-induced LTP in males, as has been reported previously, PKA appears to be required for pairing-induced LTP in females. Further experiments will be done to confirm these results and to extend male-female comparisons of PKA sensitivity to other forms of LTP. If confirmed, a sex difference in the PKA requirement for LTP will add to a growing literature demonstrating latent sex differences in mechanisms of synaptic modulation in which males and females achieve similar functional endpoints through distinct underlying mechanisms.

Disclosures: A. Jain: None. **C.S. Woolley:** None.

Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 375.01/E45

Topic: B.08. Intrinsic Membrane Properties

Support: NSF grant DMS-1715808

Title: Modification of ion channel gene expression regulates the expression of multiple ionic currents

Authors: *J. P. GOLOWASCH[1], D. H. DAUDELIN[1], H. G. ROTSTEIN[2], D. J. SCHULZ[3]
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Abstract: The nervous system must maintain relatively stable properties while adapting to changes in the environment. The wide variety of inputs and perturbations that neurons receive during their lifetime is thought to make neurons of the same type express the same genes to different levels (Marder & Taylor, 2011), while maintaining an identical phenotype. Ionic current variability in identical cells is widespread (Marder, 2011, Schulz 2006) and is thought to be constrained by the co-regulated expression of subsets of ion channels (Golowasch, 2014). The result is that ionic currents and maximal conductances appear correlated in populations of identical cells, which has been observed in multiple cell types of various animal species (Temporal et al. 2011, Tobin et al, 2009, Trinh et al, 2017). Thus far, only theoretical work has shown potential mechanisms that generate such correlated currents (O'Leary et al 2014). However, direct manipulation of the genes involved to test the hypothesis that co-regulation of ion channels is the underlying mechanism has been limited (MacLean et al, 2003). Consistent with this hypothesis, we predict that up- or down-regulation of one ion channel should result in up- or down-regulation of other channels whose mRNA or ionic currents have been shown to correlate with it (Temporal et al. 2011, Khorkova and Golowasch 2007, Schulz et al. 2006). We probe these mechanisms by tampering with the expression levels of individual ion channels in neurons of the crab *Cancer borealis* stomatogastric ganglion and studying how the unperturbed ion channels of the cells react. For this, we inject mRNA that codes for specific channels directly into neurons to boost their expression or alternatively inject double stranded RNA (dsRNA) to depress expression. We co-inject mRNA or dsRNA with Green Fluorescent Protein (GFP) mRNA to track expression. We have confirmed that expression of GFP alone does not significantly affect ionic current levels after 2 days (n=9). Preliminary results (n = 11) show that 2 days after *Shal* dsRNA is injected expression, both the transient K^+ current, I_A (coded by *Shal*, P=0.011), and the high-threshold, voltage-gated outward potassium currents, I_{HTK} (P=0.024), decrease significantly (paired t-tests) relative to control measurements taken at the time of dsRNA injection. This leads to the conclusion that a regulatory mechanism is in place in these neurons that tracks the levels of certain ion channels, and that at least one other current known to be correlated with the target channel in unperturbed cells is similarly affected. We predict that overexpression will have the reciprocal effects, and also that multiple channels are affected.

Disclosures: J.P. Golowasch: None. **D.H. Daudelin:** None. **H.G. Rotstein:** None. **D.J. Schulz:** None.

Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 375.02/E46

Topic: B.08. Intrinsic Membrane Properties

Support: Michael Smith Foundation for Health Research Post-doctoral Award
Canadian Institutes for Health Research Post-doctoral fellowship

Title: Frequency-dependent coupling between neuronal activity and mitochondrial Ca^{2+} dynamics *in situ*

Authors: *C. J. GROTEN[1], A. NELSON[1], B. A. MACVICAR[2]
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Abstract: Alterations in mitochondrial energy production in neurons have dramatic consequences for brain health. Despite this, the mechanisms by which mitochondria influence/respond to neuronal function have not been fully examined in native brain tissue. As a first step towards understanding potentially unique aspects of this relationship, we examined a role for mitochondrial Ca^{2+} dynamics in the CNS. While this property has long been associated with pathological changes in ATP/ROS production and apoptotic signalling, its significance for brain function is unclear. To address this, I utilized two-photon microscopy and examined the relationship between action potential frequency and cytosolic Ca^{2+}/mitochondrial Ca^{2+} in neurons from cortical brain slices. Current-clamp recordings from pyramidal neurons expressing the mitochondrial Ca^{2+} reporter mitoRGECO1.0 revealed a firing frequency threshold (5 Hz) above which a long-lasting change in the Ca^{2+} content of somato-dendritic mitochondria was observed. These responses were reduced by blocking voltage-gated Ca^{2+} channels or the mitochondrial Ca^{2+} uniporter (using Ru360). Consistent with these findings, Ru360 substantially enhanced the magnitude/duration of cytosolic Ca^{2+} signals only at firing frequencies above 5 Hz. Collectively, our results reveal that dramatic changes in mitochondrial [Ca^{2+}] occur *in situ* in a non-pathological setting. Our findings suggest that the link between spike frequency and mitochondrial [Ca^{2+}] changes could regulate both cellular energetics and cytosolic [Ca^{2+}] signalling during heightened neuronal excitability.

Disclosures: C.J. Groten: None. **A. Nelson:** None. **B.A. MacVicar:** None.

Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 375.03/E47

Topic: B.08. Intrinsic Membrane Properties

Support: R.D. Weigel Research Grant
NSF IOS 1354932

Title: Differential current accumulation from antidromic action potentials modulates stimulus encoding

Authors: *M. DEMAEGD, W. STEIN
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Abstract: Information propagation in neurons is often presumed unidirectional from encoding sites in the dendrites through the axon to the synaptic terminals. However, antidromic action potentials originating from 'ectopic' initiation sites are common in many nervous systems. These action potentials can invade dendritic encoding regions or the axon initial segment and alter the neuron's sensitivity to incoming stimuli. While this effect is well described, little is known about the underlying mechanisms.

We are studying the effects of antidromic action potentials on stimulus encoding using the single cell muscle tendon organ AGR (anterior gastric receptor) in the crab stomatogastric nervous system. AGR initiates ectopic action potentials in its axon, the frequency of which is under direct neuromodulatory control by higher order chemosensory neurons. As ectopic action potentials invade AGR's peripheral dendrites antidromically, there is a frequency-dependent decrease in AGR's sensitivity to changes in muscle tension. We hypothesize that these effects on stimulus encoding are due to slow ionic currents that accumulate differentially with changes in ectopic frequency.

To predict which intrinsic ionic currents may mediate changes in stimulus encoding, we designed a computational model with axon and dendritic compartments using Hodgkin and Huxley equations. The model suggests that fast Sodium and Potassium channels are insufficient to cause frequency-dependent changes in stimulus encoding from invading action potentials. In contrast, slower currents could accumulate differentially with antidromic frequency and introduce frequency-dependent membrane excitability changes via modulation of membrane potential and total conductance. Strength and sign of excitability changes depended on current direction and time constant of activation. Hyperpolarizing currents and slower time constants resulted in the greatest reduction in membrane excitability. These effects were facilitated when time constants became progressively slower with hyperpolarization.

Our model thus predicts that invading action potentials modulate stimulus encoding via current

accumulation at the stimulus encoding site. We are now testing this prediction using electrophysiological and molecular techniques using AGR's sensory response as a measure.

Disclosures: M. Demaegd: None. **W. Stein:** None.

Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 375.04/E48

Topic: B.08. Intrinsic Membrane Properties

Support: ANR-14-CE13-0003
Ecole des Neurosciences de Paris

Title: The electrical impact of axon initial segment plasticity

Authors: *S. GOETHALS, R. BRETTE
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Abstract: In most vertebrate neurons, action potentials are triggered at the distal end of the axon initial segment (AIS). They are then transmitted to the soma where they are regenerated and further propagated in the dendritic tree. The exact AIS start position and length is highly variable across and within neuron types. Moreover, recent studies have reported that the AIS undergoes structural plasticity. Changes in electrical activity can trigger a relocation or elongation/shortening of the AIS. In view of the crucial role of the AIS in spike initiation, these observations raise the question of the function of AIS geometry. What is the specific effect of AIS plasticity on electrical function? Experimentally, that effect is not straightforward to understand because AIS plasticity goes along with other intrinsic changes such as channel expression and phosphorylation. Simulations have also shown a variety of effects, with no clear understanding of the conditions for each of them. So we analyzed theoretically the effect of changes in position, length and sodium channel density on electrical function. We start by studying analytically the impact of AIS geometry on excitability in a simple axon model. First, we look at the impact of changes in AIS geometry on the somatic voltage threshold. Considering only Na channels in the AIS, our theoretical results suggest that the threshold depends mainly on the middle position of the AIS and on the total number of Na channels. In particular, shifting the AIS away from the soma decreases the threshold and in consequence increases excitability. Second, we show that if we add a hyperpolarizing current at the AIS (e.g. Kv channels), the effect of shifting the AIS is reversed. Third, we examine the impact of AIS geometry on the current that backpropagates to the soma. We find that this impact is determined by different parameters. The backpropagating spike is mainly determined by the AIS start position, and by the Na channel density in the AIS. These theoretical results agree with simulations of an action

potential model. Although previous studies have focused on changes in intrinsic excitability, we point out that the electrical effect of AIS plasticity is multidimensional. In particular, different geometrical factors affect the spike threshold and the backpropagation to the soma.

Disclosures: S. Goethals: None. **R. Brette:** None.

Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 375.05/E49

Topic: B.08. Intrinsic Membrane Properties

Support: KAKENHI 18K06514

Title: Burst firing of hippocampal mossy fibers by local blockade of axonal potassium channels

Authors: *H. KAMIYA
Dept. of Neurobio., Hokkaido Univ. Grad. Sch. of Med., Sapporo, Japan

Abstract: A potassium channel blocker 4-aminopyridine (4-AP) has been shown to exert pronounced convulsive action and generates burst discharges when applied to hippocampal slices. However, it remains unclear how a blockade of potassium channels leads to generation of burst discharges. An intriguing possibility is ectopic spiking from the sites different from those for physiological spike initiation at the axon initial segment, as suggested for other experimental models of epileptogenesis in the hippocampus. To test for possible ectopic spike generation at distal axon by 4-AP application, direct recordings from exceptionally large mossy fiber terminals in mouse hippocampal slices were made with loose-patch clamp technique. To localize the action of 4-AP on distal axon, focal perfusion around the recording site were used in combination with recordings from mossy fiber terminals. Focal application of 4-AP on distal portion of mossy fibers reliably induced burst discharges of the mossy fiber terminals. Numerical simulation based on a realistic mossy fiber model suggested that local blockade of axonal potassium channels prolonged duration of action potentials and thereby caused reverberating spiking activities at distal axons and subsequent antidromic propagation towards the soma. Taken together, it was suggested that local blockade of voltage dependent potassium channels in distal axons by application of 4-AP is sufficient to cause a hyperexcitable state of hippocampal mossy fiber axons and initiate bursting activities of the CA3 neuronal network.

Disclosures: H. Kamiya: None.

Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 375.06/E50

Topic: B.08. Intrinsic Membrane Properties

Support: NIH P30 GM110702
FONCYT, BID 1728 OC.AR. PICT-2016-1728
UBACYT 2014-2017 #20120130101305BA
BID 1728 OC.AR. PICT 2015-2594

Title: Pedunculopontine intrinsic gamma oscillations are blocked by histone deacetylase inhibitors

Authors: *E. E. GARCIA-RILL[1], V. BISAGNO[2], F. J. URBANO[3]
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Abstract: Epigenetic mechanisms (i.e., histone post-translational modification and DNA methylation) play a role in regulation of gene expression in response to a wide range of environmental stimuli. Histone deacetylases (HDACs) are epigenetic regulators that regulate the histone tail, chromatin states, protein-DNA interaction, and transcription.HDACs and their inhibitors are promising candidates for treating cancer and several neurodegenerative disorders. However, the mechanisms of action of HDAC inhibitors need to be elucidated. Indeed, there are potential neurological side effects by modulating HDAC functions. More research is needed to design new and more specific HDAC inhibitors with the lowest range of neurological side effects. The pedunculopontine nucleus (PPN) is an element of the reticular activating system that regulates arousal. We previously described intrinsic gamma oscillations in PPN neurons that are subserved by voltage-dependent, high threshold N- and P/Q-type Ca2+ channels. In the present study, we investigated whether PPN intrinsic gamma oscillations are affected by inhibition of histone deacetylation *in vitro*. Our results on 150 PPN neurons showed that, a) acute in vitro exposure to the HDAC Class I and II inhibitor trichostatin A (TSA, 1uM) eliminated high threshold, voltage-dependent Ca2+ channel-mediated oscillations in the gamma band range, but not lower frequencies ($p<0.05$), b) pre-incubation with TSA (1uM, 90-120 min) led to a similar decrease specifically in gamma band oscillations (One-way ANOVA, $p<0.05$), c) a significant reduction in Ca2+ currents (I_{Ca}) was elicited by TSA, d) a HDAC Class I inhibitor MS275 (500 nM), and a HDAC Class IIb inhibitor tubastatin A (150-500 nM), both failed to affect intrinsic gamma oscillations in PPN neurons ($p>0.05$). These results suggest that there is a specific effect on gamma band oscillations when histone deacetylation is blocked and that these phenomena might underlie the negative side effects of HDAC inhibitors on arousal.

Disclosures: E.E. Garcia-Rill: None. **V. Bisagno:** None. **F.J. Urbano:** None.

Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 375.07/E51

Topic: B.06. Synaptic Transmission

Support: NIH ES090089 to J.L.Y.

Title: a7 nAChR in hippocampal theta generation

Authors: *Z. GU, J. L. YAKEL
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Abstract: We have recently found that hippocampal muscarinic acetylcholine receptors (mAChRs) are important for theta generation in the entorhinal hippocampal network in freely moving mice. Here we show that the alpha 7 nicotinic AChRs (α7 nAChR) also play an important role in theta generation. Local hippocampal infusion of selective antagonists of the α7 nAChR partially reduced hippocampal theta power in freely moving mice, similar to the effect of mAChR antagonists. There was no significant additive effect when the two antagonists were combined. Our *in vitro* studies in cultured slices suggest that mAChR activation promotes transient theta generation, while α7 nAChR activation may prime the network to future theta generation by the same inputs. With receptor knockout in neuronal subpopulations, we found that α7 nAChRs expressed in interneurons play an important role in theta generation, while we previously found that m1 mAChRs expressed in pyramidal neurons played the major role in theta generation. These results suggest that neuronal subpopulations recruit different cholinergic receptors to support theta generation. Currently we are trying to identify the interneuron subpopulations that are involved in α7 nAChR-dependent theta generation.

Disclosures: Z. Gu: None. **J.L. Yakel:** None.

Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 375.08/F1

Topic: B.06. Synaptic Transmission

Title: Computational modeling of synaptic plasticity induced by repeated dysinhibition

Authors: I. GUERREIRO[1], Z. GU[2], J. L. YAKEL[3], *B. S. GUTKIN[4,5]
[1]Group for Neural Theory, LNC INSEMR U960, Ecole Normale Superieure, Paris, France; [2]Neurobio. Lab., NIEHS/NIH, Rtp, NC; [3]Natl. Inst. of Envrn. Hlth. Sci., Research Triangle Park, NC; [4]Group for Neural Theory, LNC INSERM U960, Ecole Normale Superieure, Paris, France; [5]Inst. for Cognitive Neurosci., NRU Higher Sch. of Econ., Moscow, Russian Federation

Abstract: There are more than 20 subtypes of interneurons in the hippocampus, targeting different subcellular domains of the principal cells, and providing diverse modulation of synaptic integration and neuronal firing patterns. It was shown experimentally that repeated inhibition of hippocampal CA1 somatostatin-positive interneurons can induce lasting potentiation of Schaffer collateral to CA1 EPSCs, suggesting that in addition to acutely gating the generation of action potentials, repeated dendritic disinhibition also plays a role in the induction of synaptic plasticity. We used a biophysically realistic computational model to examine mechanistically how inhibitory inputs to hippocampal pyramidal neurons can modulate the plasticity of excitatory synapses.
We found that reduced GABA release (disinhibition) could lead to increased NMDAR activation and intracellular calcium concentration, which could in turn upregulate AMPAR permeability. Repeated disinhibition of the excitatory synapses could lead to larger and longer lasting increase of the AMPAR permeability, i.e. synaptic plasticity.
We also found experimentally that repeated cholinergic activation can also induce synaptic plasticity through a7 nAChRs expressed in interneurons. Currently we are exploring the interneuron subtypes that may be involved in a7 nAChR-dependent synaptic plasticity, and how can they modulate disinhibition of excitatory synapses.

Disclosures: I. Guerreiro: None. **Z. Gu:** None. **J.L. Yakel:** None. **B.S. Gutkin:** None.

Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 375.09/F2

Topic: B.08. Intrinsic Membrane Properties

Support: NIH R01 MH115832

Title: Intrinsic mechanisms of frequency selectivity in the proximal dendrites of CA1 pyramidal neurons

Authors: *C. L. COMBE[1], C. C. CANAVIER[2], S. GASPARINI[1]
[1]Neurosci. Ctr., [2]Neurosci. Ctr. and Dept. of Cell Biol. and Anat., Louisiana State Univ. Hlth. Sci. Ctr., New Orleans, LA

Abstract: Gamma oscillations, one of multiple bands of synchronous activity, are thought to play a role in learning and memory. Multisite recordings within the rat hippocampal formation indicate that different behavioral tasks are associated with synchronized activity between areas CA3 and CA1 at either slow (25-50 Hz) or fast (65-100 Hz) gamma. Slow gamma is phase-locked to activity in area CA3 and presumably driven by the Schaffer collaterals.
We used a combination of computational modeling and *in vitro* slice electrophysiology to test the hypothesis that the intrinsic properties of CA1 pyramidal neurons prime them to "listen" to different bands of gamma frequency input from CA3, depending on physiological conditions, and to identify the mechanisms involved. Both approaches demonstrated that, under control conditions, in response to temporally precise input at Schaffer collaterals, CA1 pyramidal neurons fire preferentially in the slow gamma range regardless of whether the input is at fast or slow gamma frequencies, suggesting frequency selectivity in CA1 output with respect to CA3 input. In response to 10 pulse-trains, cells fired an average of 8.8 ± 0.1 spikes in response to 40 Hz stimulation and 5.3 ± 0.1 spikes at 100 Hz stimulation ($p < 0.0005$, $n = 16$). In addition, phase-locking was more precise for slow gamma than fast gamma input: the vector strengths, *r*, from the cumulative data across all neurons were 0.899 (0.888-0.910) at 40 Hz and 0.577 (0.537-0.616) at 100 Hz; 95% confidence intervals in parentheses. Synaptic depression did not play a role in frequency selectivity at Schaffer collateral inputs, as subthreshold EPSPs showed only patterns of summation at both slow and fast gamma frequencies. The frequency selectivity was absent when stimulation was achieved through trains of 2 ms-current injections in the soma (9.9 ± 0.1 spikes at 40 Hz and 9.2 ± 0.3 at 100 Hz, $p = 0.221$, $n = 10$). The frequency selectivity was also greatly attenuated when the Ca^{2+}-activated K^+ (SK) current was removed from the model or blocked *in vitro* with apamin (the number of action potentials generated by 100 Hz input significantly increased from 5.5 ± 0.2 in control to 6.7 ± 0.3 in apamin, exact $p = 0.002$, $n = 10$), or by the broad-spectrum cholinergic agonist carbachol (control, 5.3 ± 0.1; carbachol 6.4 ± 0.2; exact $p = 0.016$, $n = 7$). Perfusion of slices with $BaCl_2$ (100 µM) to block A-type K^+ channels tightened this frequency selectivity (at 100 Hz, control, 5.3 ± 0.1; $BaCl_2$ 4.5 ± 0.1, exact $p = 0.008$, $n = 8$). We propose that these intrinsic mechanisms provide a means by which CA1 phase locks to CA3 at different gamma frequencies preferentially *in vivo*, as physiological conditions change with behavioral demands.

Disclosures: C.L. Combe: None. **C.C. Canavier:** None. **S. Gasparini:** None.

Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 375.10/F3

Topic: B.08. Intrinsic Membrane Properties

Title: Data-driven biophysical models of hippocampal CA3 pyramidal cell-types to investigate cellular mechanisms of sharp-wave generation

Authors: *D. L. HUNT[1], N. P. SPRUSTON[2], D. LINARO[3]
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Abstract: Hippocampal sharp-waves are network synchronization events originating in the CA3 region. They are vital to memory function, yet little is known about how they are generated. Our previous work has revealed that the hippocampal CA3 region consists of two principal cell-types, including classically described regular-spiking pyramidal cells adorned with postsynaptic spiny structures known as thorny excrescences, and intrinsically bursting pyramids that lack these specialized spines (i.e., athorny). We investigated the functional roles of these two cell types by recording their activity patterns during sharp-waves *in vivo*. The data suggest a novel cellular mechanism for sharp-wave initiation by cell-type-specific bursting of athorny pyramids, which promotes the synchronized generation of single spikes in a population of thorny pyramids underlying the sharp-wave local field potential. Understanding how thorny and athorny cells integrate their synaptic inputs to produce regular-spiking and burst-firing output *in vivo* would contribute to a more detailed mechanistic understanding of sharp-wave generation. Here we adopted a modeling approach to gain insight into the biophysical mechanisms of cell-type-specific synaptic integration. To this end, we have developed biophysically detailed multi-compartmental models based on high-resolution morphological reconstructions and *in vitro* patch-clamp electrophysiology (subthreshold and suprathreshold waveform features extracted from the membrane potential of the cells in response to somatic current injection) from both CA3 cell-types. To recapitulate the cell-type-specific intrinsic properties, we implemented a multi-objective optimization framework in conjunction with a genetic algorithm to tune the parameters of the cell-type-specific models. The genetic algorithm evolves a population of models exhibiting values of the features that closely match the experimental data from *in vitro* experiments. Subsequently, we inferred the structure of synaptic input during sharp-waves by finding activation patterns that mimic the distribution of inter-spike-intervals observed in CA3 principal cells recorded *in vivo*. These models can be used to make testable predictions concerning the relationship between the biophysical properties of principal neurons, their synaptic connectivity in the circuit, and their firing patterns during sharp waves *in vivo*.

Disclosures: D.L. Hunt: None. **N.P. Spruston:** None. **D. Linaro:** None.

Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

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Program #/Poster #: 375.11/F4

Topic: B.08. Intrinsic Membrane Properties

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Title: Interacting resonances in neuronal systems

Authors: *H. G. ROTSTEIN[1], A. STETSENKO[2], T. ITO[2], S. LI[3], E. STARK[4]
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Abstract: To elucidate the microcircuits that take part in neuronal oscillations, it is crucial to understand how the intrinsic properties of the participating neurons (e.g., ionic currents, morphology) interact with the network properties (excitation/inhibition balance) to produce the rhythms. Several neuron types exhibit membrane potential resonance (MPR) generated by a combination of restorative and regenerative ionic currents. Because these currents may be heterogeneously distributed in space and active at different voltage ranges, neurons may exhibit more than one type of MPR. The consequences of this multiplicity of MPRs for spiking activity are not well understood. It is also not well understood how biophysical (e.g. channel-mediated), morphological (e.g. compartmental/spatial) and network filtering properties interact. We address these issues theoretically in the context of the hippocampal area CA1 microcircuit using mathematical modeling and numerical simulations. Pyramidal neurons (PYR) have been shown to exhibit MPR in the theta (4-10 Hz) range by two different mechanisms (Hu et al., 2002), one primarily somatic (I_M and I_{Nap}) and the other primarily dendritic (I_h). PV^+ interneurons (INT) have been reported to be low-pass filters (Zemankovics et al., 2010) or to exhibit MPR at gamma frequencies (~40 Hz) by unknown mechanisms (Pike et al., 2000). The minimal model we use includes these two cell types with AMPA excitation (PYR to INT), $GABA_A$ inhibition (INT to PYR) and periodic inputs. INT gamma resonance was generated by I_{Ks} (slow-potassium) and amplified by either I_{Nap} or I_{Kir}. In PYR, the spatial separation of the two resonances in multi-compartment models allows the I_h-based rebound spiking to be more robust, as compared to single-compartment models. The PYR MPR fails to be communicated to the spiking regime upon direct PYR activation, independently of the INT model employed, consistent with previous observations in vivo (Stark et al., 2013). When INT are modeled as low-pass filters, direct INT activation creates a PYR spiking low-pass filter by post-inhibitory rebound mechanisms. In

contrast, when INT are modeled as gamma band-pass filters, direct INT activation generates a theta-band spiking resonance in the PYR as a result of the interplay of the PYR low-pass and the INT high-pass filtering properties in the theta frequency range. The results of this work have direct implications for the generation of network resonance in periodically forced PYR-INT networks involving the interplay of filtering properties at different levels of organization, and suggest a novel network-based mechanism for spiking-regime theta-band resonance.

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Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

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Topic: B.08. Intrinsic Membrane Properties

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Title: Monitoring dendritic excitability during behavior using neuropixels silicon probes

Authors: *A. ROTH, N. A. STEINMETZ, V. CHEREPANOVA, M. CARANDINI, K. D. HARRIS, M. HAUSSER
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Abstract: Action potentials backpropagate into the dendrites of many types of neurons, providing a report of neuronal output to dendritic synapses and shaping local plasticity rules for updating their weights. The spatial extent of backpropagation both shows systematic variation across neuronal types, and can change rapidly in individual neurons due to changes in synaptic input, brain state and/or neuromodulation. While action potential backpropagation has been characterized in many experiments in vitro and in vivo, its modulation during behavior has not been well studied. Backpropagating action potentials can also be associated with local dendritic electrogenesis, for example during triggering of calcium spikes in layer 5 neocortical pyramidal neurons, with its own conditions and consequences for synaptic and intrinsic plasticity, and thus for the learning rules implemented in single neurons and neuronal networks.
We are addressing these problems using Neuropixels silicon probes sampling the extracellular potential simultaneously from 384 densely-spaced recording sites along a linear shank running parallel to the apical dendrite of layer 5 pyramidal neurons. This configuration allows the extracellular signature of action potential backpropagation to be recorded at high spatial and temporal resolution during behavior. Recordings were performed in visual cortex of awake,

head-fixed mice exposed to sparse noise visual stimuli or darkness, while their forepaws rested on a wheel whose movements were recorded. After spike sorting based on classical signals of putatively somatic origin, the dendritic signatures of well-isolated units were examined in a spatiotemporal spike-triggered average of the extracellular potential. Units with a soma in neocortical layer 5 whose average extracellular signature in this plot could be followed over the largest spatial extent towards the surface of the cortex were selected for further analysis.
In these units, the average velocity of the signal propagating from the soma towards the pia was in the range of 0.4 to 1.2 m/s, consistent with the propagation velocity of action potentials in neocortical pyramidal neurons at physiological temperature. The space constant of decay of these signals, when fitted with a single exponential, was on the order of 100 µm, but often exhibited a smaller-amplitude long-range component. We are currently using this approach to monitor active dendritic events during behavior, as well as track the changes in dendritic excitability that may accompany learning.
A.R. and N.A.S. contributed equally to this work.

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Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

Location: SDCC Halls B-H

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Program #/Poster #: 375.14/F7

Topic: B.08. Intrinsic Membrane Properties

Title: Fear learning modulates neural and dendritic activity in the auditory cortex

Authors: *L. GODENZINI, L. M. PALMER
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Abstract: Neural activity underlying sensory perception in the neocortex is constantly modified by learning. For example, learning induces a shift in network activity, enhancing feedback signalling. Despite its importance, how single neurons integrate such changes in feedback input during learning is currently unknown. Here, this is addressed by investigating somatic and dendritic activity in the auditory cortex following fear learning. In differential fear conditioning, mice robustly learn to discriminate between two auditory stimuli, previously presented with (CS+) or without (CS-) a mild foot shock. Using patch clamp electrophysiology in vivo, we identified changes in neuronal excitability of layer 2/3 pyramidal neurons in the auditory cortex, following fear learning. Despite no difference in subthreshold auditory evoked activity, action potential generation in response to the CS+ was significantly greater compared to the CS-. To investigate whether dendrites in the auditory cortex also alter their activity following fear learning, dendritic activity was assessed using two-photon Ca^{2+} imaging in layer 2/3 pyramidal

neurons sparsely expressing the genetic Ca^{2+} indicator, GCaMP6f. Here, tuft dendrites differentially responded to the auditory stimuli presented during fear conditioning. A subset of dendrites were active only during the presentation of either CS+ or CS-, whereas, other dendrites were active during both conditions. Taken together, these results suggest that dendrites in the upper cortical layers of the auditory cortex are differentially involved in driving neural signalling during auditory fear learning.

Disclosures: L. Godenzini: None. **L.M. Palmer:** None.

Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

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Program #/Poster #: 375.15/F8

Topic: B.06. Synaptic Transmission

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EB022903

Title: Voltage and calcium signals in dendrites of medium spiny neurons

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Abstract: The medium spiny neuron (MSN) is a key neuron in the basal ganglia circuit, involved in several major neurological disorders including Parkinson's disease, Huntington's disease and attention deficit hyperactivity disorder (ADHD). MSNs exhibit two neural states. The "Down state" (hyperpolarized membrane potential) alternates with the "UP state" (depolarized membrane potential) in accordance to the temporal availability of glutamatergic synaptic inputs impinging onto their spiny dendrites. To understand the functional roles of MSNs, it is necessary to study dendritic physiological properties. Intrinsic membrane properties of MSN dendrites are poorly understood because glass electrode recordings are not tolerated well by thin dendritic branches. Here, we performed simultaneous somatic whole-cell recording with dendritic imaging to focus on dendritic properties. We injected voltage sensitive dyes (JPW-3028 and JPW-4008) or Ca^{2+} indicators (OGB1 and Fluo-5N) into MSNs. Dendritic membrane potential changes were induced by backpropagating action potentials (bAPs) or by local application of glutamate on dendrite. Our results reveal an increasing dendritic AP peak latency depending on the distance from the soma. The amplitudes of action potential-induced dendritic Ca^{2+} influxes decrease with distance from the soma suggesting a distance dependent attenuation of bAPs in dendrites of MSNs. Next, we characterized the voltage waveforms of glutamate evoked plateau potentials in dendrite and cell body simultaneously. Invariably, the dendritic plateaus preceded somatic plateau potentials and the initiation phase of the dendritic UP state

(plateau) was steeper than that in the soma, consistent with a local initiation of the dendritic plateau. Detailed characterization of voltage and Ca^{2+} signals is expected to reveal the intrinsic dendritic properties and bring us closer to the functional roles of MSNs in the basal ganglia circuits. Support , NIH MH109091 and EB022903 grants.

Disclosures: J. Jang: None. **S.D. Antic:** None.

Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

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Program #/Poster #: 375.16/F9

Topic: B.06. Synaptic Transmission

Support: R01MH109471

Title: Estrous cycle-dependent sex differences in rat dorsal striatal medium spiny neuron excitability

Authors: *J. WILLETT, A. JOHNSON, O. PATEL, D. DORRIS, J. MEITZEN
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Abstract: The neuroendocrine environment in which the brain operates is both dynamic and differs by sex. How this unstable neuroendocrine state affects neuron properties has been significantly neglected in neuroscience research. Behavioral data across humans and rodents indicate that natural changes in steroid sex hormone exposure affect sensorimotor and cognitive function in both normal and pathological contexts. These behaviors are critically mediated by the dorsal striatum: a well-conserved constituent of the basal ganglia that is instrumental for forebrain function, various forms of learning, and sensorimotor performance. In the dorsal striatum, medium spiny neurons (MSNs) are the predominant and primary output neurons. As such, MSNs are fundamental components of the circuits which underlie striatal-mediated behaviors. Importantly, MSNs express membrane-associated estrogen receptors and demonstrate estrogen sensitivity. However, the effects of cyclical hormone changes across the estrous cycle on the basic electrophysiological properties of MSNs have not been investigated. Here, I test the hypothesis that dorsal striatal MSN intrinsic excitability is a dynamic property that is modulated in adult females across the estrous cycle via the associated changes in steroid sex hormone levels. I performed whole-cell patch clamp recordings on male, diestrus female, proestrus female, and estrus female MSNs in acute brain slices obtained from adult rat dorsal striatum. Assessment and analysis of the electrophysiological properties indicate that the properties that govern cellular excitability differ over the course of the estrous cycle for female MSNs. Overall, given the estrous-dependent sex differences in the normal and pathological behavioral output of

circuits involving the dorsal striatum, understanding the nature of neuroendocrine modulation of MSN function is an important research goal.

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Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

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Program #/Poster #: 375.17/F10

Topic: B.06. Synaptic Transmission

Support: R01MH109471

Title: Estrous cycle-induced sex differences in medium spiny neuron excitatory synaptic transmission and intrinsic excitability in adult rat nucleus accumbens core

Authors: *S. PROANO, H. MORRIS, L. KUNZ, J. MEITZEN
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Abstract: Naturally occurring hormone cycles in adult female humans and rodents create a dynamic neuroendocrine environment. These cycles include the menstrual cycle in humans, and its counterpart in rodents, the estrous cycle. These hormone fluctuations induce sex differences in the phenotypes of many behaviors, including those related to motivation, and associated disorders such as depression and addiction. This suggests that the neural substrate instrumental for these behaviors, including the nucleus accumbens core (AcbC), likewise differs between estrous cycle phases. It is unknown if the electrophysiological properties of AcbC output neurons, medium spiny neurons (MSNs), change between estrous cycle phases. This is a critical knowledge gap given that MSN electrophysiological properties are instrumental for determining AcbC output to efferent targets. Here we test whether the intrinsic electrophysiological properties of adult rat AcbC MSNs differs across female estrous cycle phases and to males. We recorded MSNs using whole cell patch-clamp technique in two experiments: the first using gonad-intact adult males and females in differing phases of the estrous cycle, and the second using gonadectomized males and females wherein estrous cycle was eliminated. MSN intrinsic electrophysiological and excitatory synaptic input properties robustly changed between female estrous cycle phases and males. Sex differences in MSN electrophysiology disappeared when the estrous cycle was eliminated. These novel findings indicate that AcbC MSN electrophysiological properties change across the estrous cycle, providing a new framework for understanding how biological sex and hormone cyclicity regulate motivated behaviors and other AcbC functions and disorders.

Disclosures: S. Proano: None. **H. Morris:** None. **L. Kunz:** None. **J. Meitzen:** None.

Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

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Topic: B.06. Synaptic Transmission

Support: RO1MH109471

Title: Effect of neonatal hormone exposure on electrophysiological properties of striatal medium spiny neurons in prepubertal rats

Authors: *J. CAO, J. MEITZEN
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Abstract: Steroid sex hormones and genetic sex regulate the phenotypes of motivated behaviors and relevant disorders. Growing evidence indicates that sex differences can exist in many brain areas other than those directly involved in reproduction, which includes the dorsal striatum (caudate/putamen) and nucleus accumbens. This striatal regions show robust sex differences in gene expression, neuromodulator action (including dopamine and 17β-estradiol), and relevant sensorimotor behaviors and pathologies such as the responsiveness to drugs of abuse.
Our laboratory has demonstrated that the electrophysiological properties of rat striatal medium spiny neurons (MSNs) vary by sex, including female MSNs exhibiting increased intrinsic excitability compared with male MSNs in prepubertal rat dorsal striatum. These data suggest that neonatal masculinization via sex hormone action may regulate MSN electrophysiological properties in dorsal striatum, similar to previously published findings that neonatal estradiol exposure masculinizes rat nucleus accumbens core MSNs. Here we test the hypothesis that neonatal estradiol exposure also regulates the electrophysiological properties of dorsal striatal MSNs. To accomplish this, we exposed female and/or male rats to either estradiol, agonists of estrogen receptors α, β, or GPER-1, or vehicle control, on the first two days after birth. We then recorded the electrophysiological properties of MSNs on PND 16-23. MSN electrophysiological properties were found to be largely mature at that developmental stage. Further data analysis is ongoing, and encompasses action potential, intrinsic excitability, passive membrane properties and mEPSC properties. The results from this experiment will demonstrate whether neonatal hormone exposure will eliminate the sex difference of increased intrinsic excitability in female, and the underlying hormone. These data will help elucidate the underlying mechanisms by which sex differences in striatal neuron properties are generated.

Disclosures: J. Cao: None. **J. Meitzen:** None.

Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

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Title: Response propagation through photoreceptors that mediate high-acuity vision

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Abstract: Humans and many other primates have the highest visual acuities observed in mammals (Veilleux and Kirk, 2014). This performance originates in the fovea, where the image is resolved at a fine grain by cone photoreceptors that are uniquely slender and tightly packed. Light has direct access to this photoreceptor array due to the lateral displacement of downstream cells. Foveal cones drive these cells by extending axons, called Henle fibers, that can reach half a millimeter in length (Drasdo et al., 2007; Perry and Cowey, 1988). High visual acuity depends on the effective propagation of graded electrical responses through the elongated foveal cones. However, computational modeling suggests that frequencies important to vision are attenuated several-fold by propagation (Hsu et al., 1998). We have now addressed this topic experimentally using macaque cones. Our principal approach was to make simultaneous patch-clamp recordings from the inner segment (IS) and presynaptic terminal of single cones that were dissociated from the retina, injecting currents of various waveforms into the IS and recording the membrane voltage at both sites. We found that peripheral cones had IS and terminal responses that were nearly indistinguishable, consistent with their stout shapes and short (~40 micron) axons. With regard to foveal cones, even the longest displayed effective propagation—for a stimulus at 60 Hz, IS and terminal responses differed by <20% in amplitude and phase lag. Although voltage-gated ion channels produced dynamic nonlinearities such as resonance and adaptation, blocking them did not diminish propagation. Indeed, passive compartmental models built according to the responses and morphologies of recorded cones showed propagation with little attenuation. These models indicated that one key property is a cytoplasmic resistivity that is >1 order of magnitude lower than values reported for cones of other species (Lasater et al., 1989). The characteristics of

dissociated cones were similar to those of cones that we examined in the intact retina using single-site (IS) recordings. We conclude that electrotonic conduction is sufficient for high-fidelity, graded signaling that is robust to the extreme morphological variation of primate cones and is suitable for the sharp acuity of foveal vision.

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Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

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Title: Oscillatory activity in local cortical networks during drug-induced loss of consciousness

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Abstract: Understanding the neural correlates of consciousness remains a fundamental task in neuroscience for both clinical and scientific reasons. In particular, changes in the membrane potential of cortical neurons have been associated with different states of consciousness, such as wakefulness, sleep, and general anesthesia. We used automated, *in vivo* whole-cell recordings to observe the membrane potential of neurons in the mice barrel cortex before and after drug-induced loss of consciousness. We tested the effects of two clinically relevant anesthetic drugs: dexmedetomidine and ketamine. Dexmedetomidine is an alpha-2 noradrenergic antagonist, and ketamine is an NMDAR blocker. Both drugs were infused systemically via an indwelling

intraperitoneal cannula after baseline recordings of active and quiet wakefulness. The point of loss consciousness was estimated through a probabilistic function of the motion flow obtained from video recordings of the mice spontaneous behavior. Before drug dosing, the membrane potential was characterized by slow fluctuations in the membrane potential, about one per second, interspersed with persistent depolarizations typically accompanied by spiking and coincident with periods of active wakefulness. After dosing, both drugs abolished the persistent depolarizations, but the slow fluctuations remained and became more periodical as compared to those observed during quiet wakefulness. Simultaneous recordings of pairs of neurons revealed that these fluctuations were synchronous within hundredths of micrometers. Previous studies using gaseous anesthetics have shown that the quiescence periods during loss of consciousness can be as long as one second. This is not the case during ketamine and dexmedetomidine-induce loss of consciousness. However, our results are consistent with previous observations of locally synchronous slow oscillations in human cortex during propofol-induced loss of consciousness. Therefore, both prolonged quiescence of the membrane potential and oscillatory entrainment of the local cortical network seem to be incompatible with a normal state of alert consciousness, and the actions of drugs with different pharmacological targets can result in loss of consciousness through either of these two mechanisms.

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Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

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Title: Dynamics of ketamine-induced loss of consciousness and return of consciousness across primate neocortex

Authors: *J. J. BALLESTEROS[1,4], J. BRISCOE[1], S. R. PATEL[2], P. HUANG[1], E. N. BROWN[4,1], E. N. ESKANDAR[3,5], Y. ISHIZAWA[1]
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Abstract: Anesthetic-induced altered states of consciousness are thought to be associated with highly structured oscillations in the brain. Although many general anesthetics act as $GABA_A$ allosteric modulators, ketamine is a NMDA antagonist and has unique EEG profiles. Here we have investigated the neuronal dynamics of the ketamine-induced loss of consciousness (LOC) and return of consciousness (ROC) in primates. We recorded local field potentials (LFPs) and single neuron activity in a somatosensory (S1) and ventral premotor (PMv) network in two macaque monkeys using microelectrode arrays. The animals' behavioral response was analyzed using a state space model (Fig. 1A). Ketamine was infused at 100 µg/kg/min for 60 min through a vascular port. During wakefulness, the spectrograms showed prominent beta oscillations in both S1 and PMv (Fig. 1B, C). Following the start of ketamine infusion, the beta oscillations appeared to be disrupted before LOC . LOC was then identified during a gradual increase in the high beta-gamma oscillations and the appearance of alpha oscillations. The slow-delta oscillations did not increase following LOC, contrary to the effects of propofol and dexmedetomidine. ROC was observed during a gradual decrease in the gamma power and an increase in the beta power. Neither LOC nor ROC appeared to correspond with a distinctive neural change . ROPAP was also identified in the half of ketamine sessions . Ketamine significantly increased the average spike firing rate in both S1 and PMv (Fig.1D). Our results demonstrate that ketamine-induced LOC is identified during a gradual increase of gamma oscillations. The slow-delta and alpha-theta oscillations only increase as ketamine infusion continues, suggesting an association with deeper anesthetic level or unarousability. Contrary to the distinctive dynamics during propofol-induced LOC and ROC, ketamine-induced LOC and ROC are both identified during a gradual change of the oscillatory profile, suggesting that general anesthetics of distinct molecular mechanisms may induce unconsciousness through unique mechanisms.

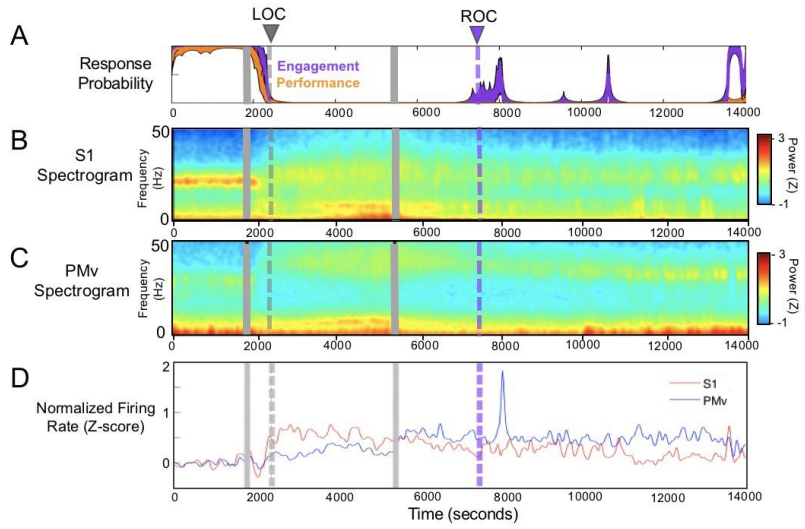

Figure 1. Neuronal dynamics changes in S1 and PMv during ketamine induction and recovery. **A**. Task response probability. *LOC* was defined as the time at which the probability of any response was less than 0.3 (gray arrow and dotted lines), *ROC* as the first time, since being unconscious, at which the probability of any response was greater than 0.3 (purple arrow and dotted lines), and *ROPAP* as the first time at which the probability of a correct response was greater than 0.9 during recovery. ROPAP was not observed during this recording session. **B**. Spectrograms in S1. **C.** Spectrograms in PMv. **D**. Average firing rate in S1 (red) and PMv (blue). Ketamine was infused at 100 µg/kg/min from 1800 sec to 5400 sec (gray lines).

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Poster

375. Intrinsic Membrane Properties: Neural Oscillators and Dendritic Properties

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Topic: B.06. Synaptic Transmission

Title: Similitudes and differences in the functional properties of membrane proteins between species

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Abstract: While it is immediately recognized that species display significant differences in their morphology and at deeper structural levels for example in the brain organization, it is often thought that functional properties of membrane proteins such as ligand gated or voltage gated ion channels are essentially closely related. This mode of thinking is easily understood when considering fundamental properties of, for example, voltage gated ion channels which are at the origin of the action potential and the strong homologies observed between the squid axon and the human nerve. This reasoning bias can have, however, a significant impact in the drug discovery. Since any compound aimed at clinical trials requires safety experiments conducted in several species (i.e. rodents, rabbits or dogs), the question of similitude and/or differences should be considered.

Whereas it is often not possible to examine with appropriate details the function of membrane proteins expressed in specific neurons or cells, the rapidly increasing knowledge of genomes offers new alternatives to challenge the question of species differences. Moreover, with the combination of efficient data base analysis with plasmid synthesis and expression in a recombinant system, such as the *Xenopus* oocytes, it is now possible to examine the functional properties of a specific protein across many different animal species.

Although it was already documented that functional properties of a given protein can be altered by the modification of a single base in the genomic sequence, such as observed in genetically transmissible diseases, comparison of functional properties of specific receptors including NMDA, $5HT_3$, nAChRs, $GABA_A$ are further underlining the relevance of appropriate testing and how this could save important time and cost in the drug development pathway.

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Poster

376. Glial Mechanisms in Neurological Disorders

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 376.01/F16

Topic: B.11. Glial Mechanisms

Support: CIHR CGS-D
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Title: Astrocyte thrombospondin-1 expression is altered in Fragile X syndrome following treatment with exogenous UTP

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Abstract: The neurological symptoms of Fragile X syndrome (FXS) arise due to aberrant glial-neural communication, driven in part by dysregulation of astrocyte-secreted synaptic proteins. In FXS, expression of the astrocyte-secreted protein thrombospondin-1 (TSP-1) is decreased, thereby impairing the structure and density of immature excitatory synapses. TSP-1 expression is thought to be regulated through UTP-induced activation of purinergic/pyrimidinergic P2Y receptors; however, the impact of pyrimidinergic signaling on TSP-1 expression in FXS remains unstudied. We therefore cultured cortical astrocytes from postnatal transgenic *Fmr1* knockout ($Fmr1^{-/-}$) and wildtype ($Fmr1^{+/+}$) mice, and investigated their response to treatment with 0.1uM-100uM exogenous UTP. Western blotting and immunocytochemistry revealed significant increases in intracellular TSP-1 expression in both wildtype and *Fmr1* knockout astrocytes following UTP treatment. Wildtype astrocytes displayed a linear dose-response relationship between TSP-1 expression and UTP concentration, while knockout astrocytes exhibited maximal TSP-1 expression following each UTP dose, suggesting differential P2Y receptor activation between genotypes. This upregulation of TSP-1 was not simply a consequence of UTP-driven hypertrophy, as astrocyte size remained consistent between naïve and UTP-treated astrocytes of both genotypes. Furthermore, UTP treatment produced contrasting patterns of TSP-1 release; *Fmr1* knockout astrocytes secreted greater quantities of TSP-1 than their wildtype counterparts. These results suggest that pyrimidinergic signaling may be differentially regulated in FXS astrocytes, resulting in elevated production and release of TSP-1 following stimulation with UTP. Future directions include co-treatment with exogenous UTP plus P2Y receptor antagonists, as well as quantification of baseline physiological UTP levels in FXS astrocytes using mass spectrometry. By further exploring the relationship between pyrimidinergic signaling and TSP-1 expression, we can begin to elucidate the therapeutic relevance of this pathway to FXS.

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

Poster

376. Glial Mechanisms in Neurological Disorders

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Title: Aberrant calcium channel expression is associated with reactive astrocytosis in optic nerve head astrocytes

Authors: *A. K. GHOSH[1,2], V. R. RAO[2], Y. NAUMCHUK[3,4], V. B. MAKSYMENKO[4], E. B. STUBBS, Jr.[2,5], S. KAJA[2,3,5]
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Abstract: Glaucoma is a progressive optic neuropathy that manifests with a triad of characteristic pathological changes, including optic nerve head remodeling, damage to the optic nerve, and retinal ganglion cell loss. Optic nerve head astrocytes (ONHAs) are the primary cell type in the optic nerve head; in response to noxious stimuli, ONHAs undergo reactive astrocytosis (RA), a physiological response characterized by increased expression of glial fibrillary acidic protein (GFAP), as well as increased proliferative and migratory potential. RA of ONHAs underlies optic nerve head remodeling in glaucoma.The molecular mechanisms underlying RA remain largely unknown; however, aberrant expression of plasma-membrane calcium channels has previously been reported to contribute to RA in the CNS (Burgos *et al*. Glia 2007, 55:1437-48). Therefore, the goal of the present study was to determine the expression of multiple types of plasma membrane calcium channels in ONHAs during RA to further elucidate their potential roles in glaucoma.RA was induced by two methods, by exposure either to hyperbaric pressure (25 - 30 mmHg above ambient pressure for 16 - 20 hours) using a custom-built pressure chamber or mechanical strain (10% equibiaxial stretch for 16 hours) using a Flexcell® FX-5000 Tension System (Flexcell International). Induction of RA was confirmed by increased GFAP immunoreactivity. Expression of the pore-forming subunits of voltage gated ion channels (pan-$Ca_V\alpha$) was increased 2.2-fold during RA ($P < 0.05$). We determined that this increase was largely mediated by the aberrant expression of $Ca_V2.1$ (P/Q-type) calcium channels. We detected strong immunoreactivity for mechanosensitive Piezo1 channels. Piezo1-specific

immunoreactivity was present exclusively near the plasma membrane, and increased 2.1-fold ($P < 0.001$) during RA. A similar increase in Piezo1 mRNA were observed ($P < 0.01$). A detailed electrophysiological analysis of aberrant calcium channel currents in ONHAs is currently underway. Our data are in accordance with earlier reports that have reported upregulation of voltage-gated calcium channels in hippocampal astrocytes during astrocytosis (Burgos *et al*.), and a study finding increased Piezo1 mRNA levels in the optic nerve head of glaucomatous dba/2J mice (Choi *et al*. Mol Vision 2015, 21:749-66). Our data tentatively suggest a conserved mechanism underlying RA and implicate mechanosensitive and voltage-gated plasma membrane calcium channels in the pathophysiology of glaucoma.

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Poster

376. Glial Mechanisms in Neurological Disorders

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Topic: B.11. Glial Mechanisms

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Title: Scutellarin promotes microglia mediated astrogliosis in ischemia injury in rats and astrocytic reaction *in vitro*

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Abstract: Scutellarin, an anti-inflammatory agent, has been reported to effectively suppress microglia activation in rats with middle cerebral artery occlusion (MCAO) and in BV-2 microglia. We have shown that scutellarin decreased the production of proinflammatory cytokines and reactive oxygen species in activated microglia. Robust microglia activation was followed by astrogliosis. We reported recently that scutellarin also promoted astrocytic reaction but through activated microglia. However, the effects of scutellarin on reactive astrocytes have not been fully explored. This study was aimed to determine more specifically the outcomes of scutellarin treatment in reactive astrocytes that play an important role in tissue repair. Expression

of brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and insulin-like growth factor-1 (IGF-1), and cell cycle markers cyclin-D1/B1 was assessed by immunofluorescence staining and western blot in reactive astrocytes in scutellarin injected MCAO rats. The above-mentioned proteins were also investigated in TNC1 and primary astrocytes, treated respectively with conditioned medium from BV-2 microglia with or without pretreatment of scutellarin and lipopolysaccharide (LPS) in vitro. The migration and morphological change of astrocytes induced by microglial conditioned medium was detected by the transwell chamber assay and electron microscopy respectively. Robust astrocyte reaction was observed in the penumbral region in ischemic cerebral cortex after MCAO with treatment of scutellarin. Reactive astrocytes significantly increased in number and exhibited hypertrophy bearing long extending processes. Expression of BDNF, NT-3, IGF-1, and cyclin-D1/B1 was markedly augmented following the treatment of scutellarin in MCAO rats. In addition, TNC1/primary astrocytes responded vigorously to micrglial conditioned medium treated with scutellarin + LPS as shown by enhanced expression of all the above markers by Western and immunofluorescence analysis. Migration of TNC1 in conditioned medium was significantly decreased. Furthermore, hypertrophic TNC1 astrocytes in conditioned medium showed abundant microfilaments admixed with microtubules by electron microscopy. The results suggest scutellarin is neuroprotective which can amplify astrogliosis but through activated microglia, and concurrently induces morphological and functional changes in reactive astrocytes.

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Poster

376. Glial Mechanisms in Neurological Disorders

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Support: KAKENHI 25460093
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Title: Fstl1 is involved in polyI:C-induced neuronal impairment

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Abstract: Viral infection in perinatal period may affect early brain development, which increases the risk for psychiatric disorders such as schizophrenia. Our previous findings showed that polyriboioinic-polyribocytidylic acid (polyI:C) treatment in neonatal mice, which mimics virus infection by inducing innate immune system, leads to behavioral abnormality in adulthood. Furthermore, polyI:C treatment induces the expression of interferon-induced transmembrane protein 3 (Ifitm3) in astrocytes but not neurons. Ifitm3 may mediate polyI:C-induced neuronal impairment through the induction of humoral factors secreted from astrocytes. However, it remains unknown which molecular entity leads to the neuronal impairment. To answer this question, we screened humoral factors secreted from astrocytes by proteomic approach. As a result, we identified Follistatin like-1 (Fstl1) as a candidate astroglial factor. Astrocyte condition medium (ACM) from secondary cultured astrocytes treated with polyI:C contained a significantly higher amount of Fstl1 protein than the level in control ACM. Increase in Fstl1 level by polyI:C treatment was not observed in astrocytes from Ifitm3 KO mice. Treatment of recombinant Fstl1 on primary cultured neurons induced an impairment of neurite elongation, whereas, knockdown of Fstl1 in astrocytes diminished the neuronal impairment caused by treatment with ACM. These results suggest that astrocyte-derived Fstl1 plays a crucial role in polyI:C-induced neuronal impairment.

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Poster

376. Glial Mechanisms in Neurological Disorders

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Topic: B.11. Glial Mechanisms

Support: NIH/NINDS (R01NS103940)

Title: Immediate early astrocytes (ieAstrocytes): A transition state between quiescent and reactive astrocytes during neuroinflammation

Authors: *Y. KIHARA[1], A. GROVES[2], D. JONNALAGADDA[3], R. RIVERA[3], G. KENNEDY[3], M. R. MAYFORD[4], J. CHUN[3]
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Abstract: Multiple sclerosis (MS) is an autoimmune disease characterized by neuroinflammation, demyelination and neurodegeneration in the central nervous system (CNS). Peripheral immune cells, including T lymphocytes, play pivotal roles in MS pathogenesis.

However, not all cell types involved in neuroinflammation are known. Here, we identify a subtype of astrocytes, which we named ***ieAstrocytes*** ("immediate early astrocytes"), as being early responders during experimental autoimmune encephalomyelitis (EAE). An unbiased screen for cellular activation during EAE was developed using TetTag c-Fos reporter mice, that labeled c-Fos-activation-experienced cellular nuclei with a stable GFP, fused with histone H2B. A four-dimensional (temporal and spatial) cellular c-Fos activation map in the spinal cord revealed an expansion of GFP signals during EAE. Immunohistochemistry revealed that over 95% of GFP^+ cells were astrocytes whose fraction linearly increased with EAE severity. Moreover, disease-dependent *ieAstrocyte* formation was suppressed by astrocyte-specific genetic removal of the sphingosine 1-phosphate (S1P) receptor $S1P_1$, as well as by pharmacological inhibition using fingolimod (FTY720), an FDA-approved MS disease-modifying $S1P_1$ functional antagonist. Our results identify 1) astrocyte c-Fos activation as a key step in EAE development, 2) the requirement for S1P signaling in *ieAstrocyte* formation and 3) the inhibition of *ieAstrocyte* formation as a novel mechanism of action of fingolimod. Disease-modifying therapy reducing *ieAstrocyte* formation may be a new strategy to treat relapsing and possibly progressive forms of MS with reduced risk of immunosuppression.
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Poster

376. Glial Mechanisms in Neurological Disorders

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Title: Vitamin B_{12}ameliorates disease in animal model of multiple sclerosis through astrocytic sphingosine 1-phosphate signaling

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Abstract: Sphingosine 1-phosphate (S1P) is a bioactive lipid with diverse functions acting through its receptors ($S1P_{1-5}$) expressed on various cell types. Fingolimod (FTY720; Gilenya), upon phosphorylation (FTY720-P), is an analogue of S1P, binding to $S1P_{1,3-5}$, and is the first oral, disease-modifying therapy for multiple sclerosis (MS). The widely known mechanism of action of FTY720-P in MS is through sequestration of pathogenic lymphocytes via $S1P_1$ in the secondary lymphoid organs. Additionally, FTY720 can enter the CNS to access $S1P_{1,3,5}$ in several neural cell types as FTY720-P. It is known that FTY720-P causes internalization and functional $S1P_1$ receptor loss on astrocytes, which is largely responsible for its efficacy in experimental autoimmune encephalomyelitis (EAE), an animal model of MS. This work elucidates how $S1P_1$ on astrocytes can mediate the action of FTY720 in EAE.
Astrocytes are the early-responding cell type in the central nervous system (CNS) of EAE mice and these astrocytes were designated as "immediate early astrocytes" (*ieAstrocytes*) in our previous study. Nuclear RNA sequencing of *ieAstrocytes* was instrumental in our pursuit of exploring how FTY720 mitigates the disease through $S1P_1$ on EAE-activated astrocytes. CD320, a vitamin B_{12} transcobalamin receptor and the only B_{12} uptake receptor in the CNS, was upregulated upon $S1P_1$ loss, therefore it is regarded as a neuroprotective factor. Both CD320-null and B_{12}-deficient mice exhibited severe EAE when compared to their controls. Furthermore, FTY720 lost its efficacy in EAE in the absence of CD320 and B_{12}. This work points towards a combination of FTY720 and B_{12} as a promising therapy for MS.
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Poster

376. Glial Mechanisms in Neurological Disorders

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Title: Gene expression in the frontopolar cortex in schizophrenia

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Abstract: The Frontopolar cortex is one of the least studied areas of the human brain. Cytoarchitectonical studies catalogue it as Brodmann area 10, and functional imaging research suggest a role for this area in attentional control. In addition, it might also be involved in dynamically adjusting the contribution of internal and external information to moment–to-moment cognition.
Disturbances in the ability to discern between the information coming from external vs internal sources might be important in the context of disorders involving deficits in reality monitoring, as is the case in schizophrenia. Functional imaging studies have reported that schizophrenic patients show deficits in rostral prefrontal cortex activation, and it has been suggested that this might be related to patients' deficits in reality monitoring. However, the underlining cellular dysfunctions that may be associated with these disturbances have not yet been studied.
In this study, we aim to analyze the differences in gene expression within the frontopolar cortex between postmortem brain tissue obtained from normal controls and schizophrenic subjects.
Materials and methods: Human brain blocks from the frontopolar cortex of 24 Schizophrenic subjects and 27 controls were obtained from the Brain Donor Program at the University of California, Irvine by agreement with the Pritzker Neuropsychiatric Consortium. The subjects included in the schizophrenic group met diagnostic criteria from the Diagnostics and Statistical Manual of Mental Disorders (DSM-V). In the control group there was no evidence of psychiatric or neurological disorders. Variables accounted for include gender, age and postmortem interval. All subjects used in the study had tissue pH above 6.5 and agonal factor scores (AFS) of zero. Fresh frozen blocks of tissue averaging 500 mg were dissected from the frontopolar region of each subject and processed for RNA extraction to be used for gene expression studies.
Results: Initial test RNA extractions from the fresh frozen tissue rendered adequate RIN values (average 8.1) and RNA content (average 590 ng/uL). Based on our previous results we will focus on reporting the effect of schizophrenia on the astrocytic network of the Frontopolar cortex and will describe gene expression patterns associated with glial activity in schizophrenic subjects compared to normal controls.
Keywords: Schizophrenia, Prefrontal Cortex, Glia, Gene Expression.
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Poster

376. Glial Mechanisms in Neurological Disorders

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Title: The microRNA and mRNA profile of Müller glia after light damage

Authors: *S. G. WOHL[1,2], E. BERCAW[1], A. BAEK[1], I. MOUNT[1], T. A. REH[1]
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Abstract: Retinal injury leads to neurodegeneration accompanied by activation of Müller glia (MG), the principal glia of the neural retina. microRNAs (miRNAs) are negative regulators of gene expression and known to change in injured or diseased tissue. For now, there is not much known about the role of miRNAs in the response of MG to neuronal loss, therefore we characterized microRNAs along with mRNAs following light damage.
A MG specific reporter mouse (Rlbp-CreER: $Stop^{fl/fl}$ tdTomato) was crossed onto an albino mouse with a RPE-65 mutation. For the conditional knock out (CKO) of Dicer1 (Dicer-CKO_{MG}), the tdTomato reporter mouse (with and without the RPE-65 mutation) was crossed to a $Dicer^{fl/fl}$ mouse. We light-damaged the mice for 8h. Tamoxifen was administered intraperitoneally for CreER activation. Retinal cross sections at 1, 6, 12, and 18 weeks after light damage and 1, 3, 6, and 12 months after Dicer deletion were analyzed. For miRNA analysis, MG from wild type, light damaged, and Dicer-CKO_{MG} mice were purified by FACS and analyzed using the NanoStrings nCounter® System. Microarrays were used to identify the genes changing after light damage and compared that to RNA-Seq data performed after Dicer-CKO.
One week after light damage, we found a clear thinning in the ONL due to photoreceptor degeneration, concomitant with MG activation, i.e., migration and upregulation of GFAP, one of the hallmarks for gliosis. This phenotype was similar to the phenotype we observed in the Dicer-CKO_{MG} mouse after 6 months, although migrating MG in the Dicer-CKO did not upregulate

GFAP.
By analyzing the microRNA profile, already one week after light damage, there was a drastic decline of the miRNAs highly expressed in the MG by an average of 60%. This was similar to what we found in the Dicer-CKO_{MG} one month after Dicer deletion: a reduction in levels of the same miRNA by an average of 70%. By comparing genes affected in both, light-damaged and Dicer-deleted MG, we found 1502 genes similarly upregulated such as the transcription factors Maff. Gene ontology revealed that most
upregulated genes are involved in apoptosis and the regulation of the immune response. However, in contrast to the Dicer-CKO_{MG}, microglia cell number did not significantly change after light damage.
Our results show neurodegeneration due to light damage leads to a rapid decline of the MG specific miRNAs and to upregulation of injury/disease-associated genes, similar to what occurs after MG specific Dicer-deletion. Therefore, glial miRNAs might be important for the maintenance of the physiological function and might also play a role in degenerative diseases.

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Poster

376. Glial Mechanisms in Neurological Disorders

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Topic: B.11. Glial Mechanisms

Title: Peripheral inflammation alters the morphological and functional properties of medullary dorsal horn astrocytes

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Abstract: Background: Recent evidence suggests that astrocytes play an important role in central sensitization, characterized by the development of allodynia and hyperalgesia. In many disease of CNS, astrocytes acquire reactive phenotypes, reflected by an increase of glial fibrillary acidic protein expression and hypertrophy of processes. However, whether the electrophysiological properties of astrocytes, too, change following peripheral inflammation is still unknown.
Objective: The aim of this study was: i) to characterize the morphological and electrophysiological properties of medullary dorsal horn (MDH) astrocytes in naïve rats and ii) to investigate whether theses parameters vary in persistent inflammatory conditions.
Materials/**Methods**: All experiments, analysis and reporting were ARRIVE-compliant. Experiments were performed according to European Directive 2010/63/EU on the protection of

animals used for scientific purpose. The protocols were authorized by the French Ministry of Higher Education and Research (No 04521.02). Adult male Sprague-Dawley rats (3-5 weeks) received a facial injection of either saline or complete Freund's adjuvant (CFA). Four days after injection, MDH astrocytes were identified using sulforhodamine 101 and recorded using whole-cell patch clamp technics within the *substantia gelatinosa* of *ex vivo* MDH slices. At the end of each recording, epifluorescence was used to assess whether the recorded astrocyte was actually filled with Alexa Fluor 488. Stained astrocytes were then observed with a Zeiss LSM 800 confocal laser scanning microscope. Z-series were scanned at x63 magnification. Reconstruction and morphologic analysis of the soma and arbor projections of Alexa Fluor 488-labeled astrocyte were performed from confocal image stacks with Fiji software.
Results: Four days after injection, compared with astrocytes from saline-injected rats, those from CFA-injected rats displayed i) depolarized resting membrane potentials, ii) larger membrane capacitances without changes in the input resistance, iii) enhanced density of hyperpolarization activated cationic I_H, but, conversely iv) decreased inwardly rectifying potassium K_{IR} and outward K_D currents. Inflammation also induced morphological changes in astrocytes: the arborization area, soma volume as well as total number of branches increased significantly.
Conclusion: These results reveal for the first time that peripheral inflammation alters MDH astrocyte functions and suggest that the reduction in astrocyte K_D and K_{ir} channels might contribute to inflammatory pain.

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Poster

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Title: Astrocytes in the ventromedial hypothalamus mediate anxiety in mice

Authors: Y. LIU[1,2], J. SHAO[1,2], L. ZHANG[1,3], D. GAO[1], X. ZHOU[1,2], Q. XIAO[1,2], N. HU[4], X. ZHANG[4], J. TU[1], *F. YANG[1]
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Abstract: The ventromedial hypothalamus (VMH) is important in regulating metabolism and mental disorders, suchas anxiety and depression. Recent evidence has demonstrated that astrocytes in hippocampus andamygdala play key roles in mediating anxiety behavior through secretingGamma-Aminobutyric Acid(GABA), brain-derived neurotrophic factor (BDNF), fibroblast growth factor 2(FGF2)and other factors.However, the exact role of astrocytes in VMH in regulating anxiety and underlying mechanism is unclear.We have found that, in chronic stressed mice, GABAconcentration in VMH nucleus was significantly elevated, and optogenetic activating GABAergicneural projections in VMH induced significant anxietylikebehavior. In presentstudy,combining optogenetics, pharmacogenetics, brain slice electrophysiology and behavioranalysis, we furtherdemonstrated in S100β-Cremice that selectivemanipulationof VMH astrocytes can bi-directionally regulate anxiety, which shows a gender based difference that male mice showed more perturbable than female after CNO administration. Furthermore, specific optogenetic activation of VMH astrocyte could induce excitatory response in SF-1 neurons, which might imply that astrocytes can mediate anxiety behavior through regulate SF-1 neurons in VMH. Takentogether, our study emphasized VMH astrocytes' role in regulating anxiety-likebehavior in mice.Considering the pivotal role of astrocytes in reuptake and metabolism of neurotransmitters, more study isneeded to dissect the molecular mechanism of VMH astrocytes-mediatedanxiolysis.

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Poster

376. Glial Mechanisms in Neurological Disorders

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 376.11/F26

Topic: B.11. Glial Mechanisms

Support: Human Spare Parts 2 program, The Finnish Funding Agency for Innovation, TEKES.

Title: Production and characterization of human iPSC-derived reactive astrocyte phenotype

Authors: *S. HAGMAN, T. HYVÄRINEN, S. NARKILAHTI, A. PELKONEN
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Abstract: Astrocytes have a central role in supporting nervous system physiology. However, in neurodegenerative diseases, such as multiple sclerosis and stroke, astrocytes undergo activation into reactive inflammatory phenotype. The role of reactive astrocytes in neurodegenerative

diseases is controversial, but they may affect disease progression by inducing axonal damage and limiting neuronal repair and remyelination. The aim of this study was to induce reactive astrocyte phenotype and characterize their inflammatory nature. To establish reactive phenotype, we treated human induced pluripotent stem cell (hiPSC)-derived astrocytes with inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-1β. Treated astrocytes exhibited morphological change from highly filamentous shape to flattened polygonal appearance. Reactive phenotype was characterized by quantifying GFAP staining intensity, glutamate uptake activity and expression of inflammatory molecules in the mRNA and protein levels. Increased gene expression of known inflammatory factors were detected in reactive astrocytes as compared to controls. Moreover, astrocyte activation induced widespread protein secretion profiles of inflammatory mediators. In reactive phenotype, glutamate uptake activity was impaired when compared to controls. Protocol for inducing reactive astrocytes was promising and astrocytes transformed to inflammatory phenotype. These produced reactive astrocytes can be used in co-culture models together with neurons to study their inflammatory mechanism of axonal damage as well as possible strategies for promoting regeneration.

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Poster

376. Glial Mechanisms in Neurological Disorders

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Support: Department of Science and Technology (DST) India
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Title: SRF regulates the generation of neuroprotective reactive astrocytes in the mouse brain

Authors: *M. JAIN[1], P. Y. LU[2], S. C. R. THUMU[1], S. SOMAN[1], V. VERMA[3], D. GUTMANN[4], J. C. CHELLIAH[3], T. V. ARUMUGAM[5], N. RAMANAN[1]
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Abstract: In response to CNS injury infection and in neurodegenerative disorders, astrocytes become reactive in a process called astrogliosis. To understand the underlying mechanisms,

whole brain transcriptome analysis was done following ischemic stroke in mice. This showed significant downregulation of the stimulus-dependent transcription factor, serum response factor (SRF). To study the role of SRF in astrogliosis, SRF was deleted in astrocytes using a GFAP-Cre transgenic mouse line. In the resultant SRF-GFAP-cKO conditional mutant mice, astrogliosis was observed at 3 weeks of age and persisted throughout adulthood. This was also accompanied by persistent microgliosis. Depletion of microglia following PLX5562 treatment, did not affect astrogliosis suggesting that SRF-deficient reactive astrocytes are not dependent on microglia. To confirm that the gliosis observed in SRF-GFAP-cKO mice is not due to developmental effects of embryonic deletion of SRF, we generated a tamoxifen-inducible conditional mutant mouse, SRF-GFAP-ERT-cKO. Tamoxifen (Tam) administration at 6-8 wk old SRF-GFAP-ERT-cKO mice resulted in astrogliosis at 2 months post-injection that also persisted throughout adulthood. Unlike neurotoxic A1 astrocytes, which have been shown to kill neurons and oligodendrocytes *in vitro*, we observed no evidence of cell death in these SRF mutant mice. Furthermore, we observed no deficits in basal synaptic transmission and LTP was seen in GFAP-ERT-cKO mice. Gene expression analysis by qRT-PCR of whole brain RNA of neocortex of SRF mutant mice showed a predominant expression of neuroprotective A2 reactive astrocyte markers as compared to A1 reactive astrocyte markers. These results together indicated that SRF-deficient astrocytes are unlikely to be neurotoxic. We next asked whether SRF-deficient reactive astrocytes can provide neuroprotection in three models of neural injury: ischemic stroke, stab-wound and kainic acid toxicity. SRF-deficient mice showed significantly decreased cell death after stroke and kainate-induced toxicity, and faster wound-healing following stab-wound. Together, our findings suggest that SRF regulates the generation of neuroprotective astrocytes in the mouse brain and SRF-target genes may provide novel therapeutic targets for treatment following stroke, traumatic brain injury and epilepsy.

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Poster

376. Glial Mechanisms in Neurological Disorders

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Topic: B.11. Glial Mechanisms

Support: NSFC (31430036)
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QYZDJ-SSW-SMC002

Title: Identification of astrocytic dopamine D2 receptor signaling pathways that modulate neuroinflammation in a mouse model of multiple sclerosis

Authors: *Y. WU, S. YIN, S. LU, Y. GUO, Y. YIN, Y. LI, J. HOU, J. ZHOU
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Abstract: A growing body of evidence suggests that astrocytes play critical roles in the regulation of brain function under normal conditions as well as in the pathogenesis of various brain diseases. It is, therefore, important and necessary to determine the regulatory network that controls astrocyte functions. Dopamine is well known as a neurotransmitter, playing important roles in multiple brain functions. Previously, we demonstrated that astrocytic dopamine receptor D2 (DRD2) is an important player in the modulation of brain innate immunity (Shao, et al. *Nature*, 2013). However, the molecular mechanism underlying the function of astrocytic DRD2 are largely unknown. Here we show that the signaling pathway of astrocytic DRD2 is distinct from that of neuronal DRD2. Treatment of primary cultured astrocytes isolated from the spinal cord of mouse pups with DRD2 agonist quinpirole markedly increased phosphorylation levels of AKT. In contrast, the same treatment induced profound down-regulation in phosphorylation levels of AKT in the astrocyte cultures. This up-regulation of phosphor-AKT was also seen in DRD2-over-expressing astrocytes. Interestingly, this astrocytic DRD2-AKT signaling pathway is specific to the spinal cord, given that this signaling pathway showed opposite changes in astrocytes from the cerebrocortex and striatum, indicating regional heterogeneity in astrocyte response following DRD2 stimulation. Moreover, we investigated the impact of astrocytic DRD2 in progression in a mouse model of experimental autoimmune encephalomyelitis (EAE), a commonly used animal model for multiple sclerosis. The clinical severity of EAE was significantly ameliorated after glial DRD2 inhibition, which was not observed in global DRD2 knockout mice. Together, our study suggests that astrocytic DRD2-AKT pathway plays important roles in modulating neuroinflammation.

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Poster

376. Glial Mechanisms in Neurological Disorders

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Topic: B.11. Glial Mechanisms

Support: MH-083728
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NS050274

Title: Disc1 knockdown in mature astrocytes suppresses expression of the glutamate transporters, GLAST and GLT-1, and impairs cognitive function in mice

Authors: *A. V. SHEVELKIN[1,2], C. TERRILLION[2], V. MISHENEVA[2], Y. JOUROUKHIN[2], S.-H. KIM[2], D. FUKUDOME[2], A. SAWA[2], A. KAMIYA[2], M. V. PLETNIKOV[2,3]
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Abstract: The role of a rare psychiatric genetic risk factor, Disrupted-In-Schizophrenia 1 (DISC1), in astrocyte physiology remains poorly understood despite the increasing appreciation of the contributions of astrocytes to neurotransmission and synaptic plasticity in a time- and brain region-dependent manner. We evaluated the effects of *Disc1* knockdown in mature astrocytes of the prefrontal cortex or hippocampus on expression of the glutamate transporters, GLAST and GLT-1, the markers of excitatory and inhibitory synapses within astrocyte areas, and associated learning and memory. Male and female 8-week-old C57BL6/j mice were injected with AAV-*Gfa*::GFP-miR30-*Disc1* (*Disc1* KD) or scrambled AAV-*Gfa*::GFP-mir30-Ctrl in the prefrontal cortex (PFC) or the CA1-2 areas of the hippocampus. 3 weeks later, a series of basic and cognitive tests was run followed by quantitative analysis of expression of GLAST and GLT-1 and the markers of excitatory and inhibitory synapses using Imaris software. *Disc1* KD in hippocampal or PFC astrocytes did not alter locomotor activity or anxiety-like behaviors but impaired the performance in Barnes maze. Unlike *Disc1* KD in PFC, *Disc1* KD in the hippocampus also reduced cue-dependent freezing in trace fear conditioning. These behavioral alterations were associated with decreased expression of GLAST and GLT1 in astrocytes and reduced density of excitatory ($Glut1^+$/$PSD95^+$ puncta) but not inhibitory ($VGAT^+$/$Gephyrin^+$ puncta) synapses within the GFP^+ astrocyte coverage areas. Reduced *Disc1* expression in mature brain astrocytes decreased levels of glutamate transporters and density of excitatory synapses within the astrocyte coverage zones and led to impaired learning and memory in a brain region-dependent manner. Our findings suggest that genetic risk factors could contribute to abnormal astrocyte functioning in a brain region-dependent manner, leading to cognitive impairment observed in major psychiatric disorders.

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Poster

376. Glial Mechanisms in Neurological Disorders

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Topic: B.11. Glial Mechanisms

Support: MS society of Canada Grant

Title: The cytokine IL-27 shapes the properties of human astrocytes and neurons in the context of multiple sclerosis

Authors: *F. LEMAITRE[1], A. CARMENA MORATALLA[1], N. FARZAM-KIA[1], E. HADDAD[2], N. ARBOUR[1]
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Abstract: Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) characterized by immune cell infiltration, loss of myelin, and neuronal cell death. Several publications underscore altered gene expression profiles and perturbed functions of astrocytes and neurons associated with MS pathobiology. Notably, enhanced expression of multiple immune molecules by astrocytes and neurons in MS brains is observed. The impact of immune mediators produced by astrocytes and/or neurons on ongoing MS neuroinflammation is still incompletely resolved. The cytokine interleukin-27 (IL-27) triggers both pro and anti-inflammatory responses upon binding to its receptor (IL-27R). IL-27 can reduce disease severity in a mouse model of MS but its role in MS is still unknown. We previously showed that IL-27 expression is elevated in MS brains compared to controls and that human astrocytes both in MS lesions and *in-vitro* express this cytokine and its cognate receptor IL-27R. *We speculate that local CNS IL-27 production alters immune related functions of astrocytes and neurons in MS brains.* To answer this question, we use primary cultures of human astrocytes or neurons generated from fetal material. We demonstrated that IL-27 triggered signaling pathways including STAT1 phosphorylation and the NF-kB pathway in human astrocytes. We also observed that IL-27 enhanced protein expression of the immunoregulatory molecules PDL-1 and IDO-1, which could dampen T cell activation. IL-27 increased the surface expression of ICAM1, an adhesion molecule involved in T cell infiltration and astrocytes/T cell immunological synapse formation. Moreover, in response to IL-27, human astrocytes secreted key immune mediators such as CXCL9, 10 and 11, and IL-18BP. We also demonstrated that human neurons expressed IL-27R and responded to IL-27 *in-vitro* by triggering STAT1 phosphorylation. However, IL-27 induced a different response in neurons compared to astrocytes. Although IL-27 also augmented the expression of PD-L1 in neurons, it did not induce IDO-1 expression by neuronal cells. Our results support the notion that IL-27 distinctly alters immune functions of human astrocytes and neurons. Such alteration could have an impact on the immune responses in the brain of MS patients.

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Poster

376. Glial Mechanisms in Neurological Disorders

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Topic: B.11. Glial Mechanisms

Support: MS Society of Canada
FRQS

Title: Human neurons and astrocytes express NKG2D ligands and are susceptible to NKG2D-mediated immune responses

Authors: *A. CARMENA MORATALLA[1], L. LEGROUX[1], F. LEMAÎTRE[1], N. FARZAM-KIA[1], E. HADDAD[2], A. PRAT[1], N. ARBOUR[1]
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Abstract: Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS). MS pathological hallmarks include focal demyelination, axonal loss and immune cell infiltration. Our group has identified NKG2D as a relevant player in MS pathobiology. NKG2D is an activating co-receptor expressed by several immune effector cells. In humans, it binds to various ligands (NKG2DL), including MICA, MICB and ULBP (1 to 6) which are induced by environmental triggers such as inflammation. The expression of each of these ligands is controlled by different mechanisms, suggesting unique roles played by specific NKG2DL. Binding of NKG2DL to their receptor stimulates immune effector cells and triggers cytotoxic and inflammatory responses. We have shown that human oligodendrocytes express NKG2DL *in vitro* and the blockade of NKG2D reduced their killing by immune effector cells. Moreover, our group has previously shown MICA/B expression in MS lesions; however, other NKG2DLs have not been investigated. MS is characterized not only by myelin/oligodendrocyte destruction but also neuronal death and perturbed astrocyte functions. Therefore, we hypothesize that *human neurons and astrocytes exposed to inflammatory conditions also upregulate NKG2DL and consequently become susceptible to NKG2D-mediated responses by immune effector cells.* Our goal is to characterize the expression of each NKG2DL in MS brains and in primary cultures of human CNS cells and to determine the impact of such expression on the neural-immune cell interactions. We observed that both neurons and astrocytes express different NKG2DL at the mRNA level. Notably, only ULBP4 was detected by multiple techniques (FACS, western blot, immunocytochemistry) at the protein level on astrocytes and such expression was increased in the presence of inflammatory cytokines such as TNF and IFNγ. However, the expression of ULBP4 on neurons was more modest and not modulated by inflammation. ULBP4 was detected in MS brains. We are currently studying the functional impact of ULBP4 expression. Our results

suggest that not only oligodendrocytes, but also human neurons and astrocytes, can express specific NKG2DL and consequently become susceptible to NKG2D-mediated immune responses by effector cells such as CD8 T cells.

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Poster

376. Glial Mechanisms in Neurological Disorders

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Title: α7 nicotinic acetylcholine receptor signaling modulates fetal sheep brain astrocytes transcriptome in response to endotoxin stress: Comparison to microglial transcriptome

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Abstract: Mechanisms of astrocytes' contribution to fetal neuroinflammation are poorly understood. α7 nicotinic acetylcholine receptor (α7nAChR) signaling may polarize glia towards a neuroprotective role under inflammatory conditions. Conversely, prenatal stress (PS) is accompanied by inflammation in the mother and offspring. PS increases expression of the Glu transporter VGluT1 resulting in higher levels of GLT1. We hypothesized (1) under exposure to lipopolysaccharide (LPS), α7nAChR agonist stimulation in fetal astrocytes augments their

neuroprotective profile, while the antagonistic stimulation reduces it; (2) a LPS double-hit (first in vivo, then in vitro) exacerbates these effects similar to microglia (PMID 28878260); (3) LPS exposure induces upregulation of glutamine transporters in glia akin to PS. Using an in vivo - in vitro fetal sheep model (PMID: 27856275), we validate these hypotheses via RNA-Seq analysis in primary fetal astrocyte cultures exposed to LPS in the presence of a selective α7nAChR agonist or antagonist. We compare these findings to the previously published results in identically conducted microglia experiments. Principal component analysis showed two transcriptome clusters: control, LPS single-hit and double-hit astrocytes pre-treated with α7nAChR agonist and LPS single-hit and double-hit astrocytes pre-treated with α7nAChR antagonist. The top down-regulated signaling pathway unique to LPS treatment was Ephrin A signaling (p-value 5.77E-04). α7nAChR agonist reduced this down-regulation (p-value 2.51E-02). α7nAChR antagonist had no significant effect. We used IPA to annotate VGLUT1 gene network with our findings to test for evidence that the network is being perturbed by LPS treatment. Across all treatment comparisons, two differentially expressed genes (DEGs) were identified in astrocytes: JAK1/2 (2.732 (log ratio) up-regulated, FDR 3.91E-05) and SLC1A2 (2.350 up-regulated, FDR 1.80E-04). In comparison, microglia exposed to LPS regardless α7nAChR manipulation showed a single DEG: SLC1A2 up-regulated 3.53 (FDR 5.17E-04). The LPS double-hit scenario did not change the overall pattern; α7nAChR agonist in astrocytes exposed to LPS in vivo also showed a -0.806 down-regulation of SH3GL1 (endophilin-A2, FDR 4.74E-02).Ephrin signalling contributes to a α7nAChR-dependent neuroprotective astrocyte phenotype. Low grade neuroinflammation results in changes similar to PS with regard to reprogramming glial cells to a higher glutamine uptake. PS and exposure to LPS may act synergistically to exacerbate the impairment of neuron-glial glutamatergic interaction.

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Poster

376. Glial Mechanisms in Neurological Disorders

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Paralyzed Veterans America (3080)
Dr. Miriam and Sheldon G. Adelson Medical Foundation

Wings for Life

Title: Transcriptional regulators of astrocyte reactivity

Authors: *J. E. BURDA[1], A. ROGERS[1], T. M. O'SHEA[1], J. KIM[1], Y. AO[1], R. KAWAGUCHI[2,3], G. COPPOLA[2,3,4], M. V. SOFRONIEW[1]
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Abstract: Astrocytes respond to diverse central nervous system (CNS) insults with functional changes that can range broadly from modulating inflammation to impacting on neural tissue remodeling and mediating neuroprotection. Genetic regulation of these responses is poorly understood. Here, using large scale genomic meta-analyses of mouse and human-derived data, we identified transcriptional regulators of astrocyte reactivity in multiple diverse CNS disorders. 25 transcriptional regulators were common across all eight disorders investigated. These regulators included both previously known molecules such as STAT3 and select SMADs, and newly identified molecules, whose critical roles are being validated by astrocyte-specific gene deletion in mouse models of CNS injury and disease. Numerous additional transcriptional regulators were found to be selective to subsets of disorders and some to only a single disorder. Notably, there was significant disorder selective bias of downstream molecules targeted by these regulators. Ontology and pathway analysis of downstream gene targets suggests that conserved transcriptional regulators may drive functionally-distinct gene regulatory programs in astrocytes depending on disease context. Our findings strongly support a model whereby astrocyte reactivity is highly diverse and disorder dependent rather than a stereotypic response constant across disorders. We provide a framework for understanding and potentially modulating the genetic control of astrocyte reactivity in different contexts. Supported by NIH-NINDS NS084030 and K99NS105915, Paralyzed Veterans America, the Dr. Miriam and Sheldon G. Adelson Medical Foundation, and Wings for Life.

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Poster

376. Glial Mechanisms in Neurological Disorders

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NWO Spinoza award . Grant Number: 2008

Amsterdam Neuroscience proof-of-concept . Grant Number: 2014

Title: Astrocyte dysfunction leads to neuronal network hyperexcitability in the leukodystrophy MLC

Authors: *R. MIN[1,2,4], M. DUBEY[2,4], E. BROUWERS[2,4], E. M. C. HAMILTON[2], O. STIEDL[5], M. BUGIANI[3], H. KOCH[6], M. H. KOLE[7], U. BOSCHERT[8], R. C. WYKES[9], H. MANSVELDER[4], M. S. VAN DER KNAAP[2]
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Abstract: Loss of function of the astrocyte specific protein MLC1 leads to the white-matter disease megalencephalic leukoencephalopathy with subcortical cysts (MLC). Patients have a high water content in the brain white matter. At the cellular level, the first occurrence in MLC is astrocyte swelling, followed by progressive myelin vacuolation. Previous studies on patient material and isolated cells from MLC mouse models show a role for MLC1 in astrocyte volume regulation and suggest that disturbed brain ion and water homeostasis is central to the disease. However, how astrocyte dysfunction in MLC affects neuronal networks is unclear.
Analysis of an extensive MLC patient inventory indicated that an early seizure onset is common in MLC. We studied the cellular basis of seizures in two mouse models for MLC (*Mlc1*-null and *Glialcam*-null mice). Although we did not observe spontaneous seizures in MLC mice, these mice exhibited a lowered threshold for kainate-induced seizures. Additionally, wireless local field potential recordings revealed epileptiform brain activity in MLC mice. Intrinsic excitability of pyramidal neurons, assessed with whole-cell patch-clamp recordings in brain slices, was unchanged in MLC mice. However, potassium rises in brain slices upon synaptic stimulation were increased in MLC mice. We are currently investigating whether synaptic transmission is altered in MLC mice, and how astrocyte swelling relates to epileptiform brain activity. In conclusion, our results indicate that astrocyte dysfunction in MLC leads to hyperexcitability of neuronal networks. These findings give important insight into the role of astrocyte volume regulation in neuronal network excitability and epilepsy.

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Poster

376. Glial Mechanisms in Neurological Disorders

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Title: Regulation of the astrocytic response to neural injury by LZK

Authors: *M. CHEN[1], C. G. GEOFFROY[2], J. MEVES[3], A. NARANG[3], Y. LI[3], M. NGUYEN[3], V. KHAI[3], X. KONG[1], C. STEINKE[3], L. ELZIERE[3], M. GOLDBERG[1], Y. JIN[3], B. ZHENG[3]
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Abstract: Reactive astrocytes impact recovery and repair after injuries, and contribute to disease pathogenesis in the central nervous system (CNS). The molecular regulation of astrocyte reactivity remains incompletely understood. Using genetic loss- and gain-of-function analyses *in vivo*, we identify leucine zipper-bearing kinase (LZK) as an activator of astrocyte reactivity after CNS injury. Deletion of LZK in adult astrocytes impairs astrogliosis and increases lesion size after spinal cord injury. Conversely, overexpression of LZK in adult astrocytes enhances astrogliosis and reduces lesion size. Remarkably, LZK overexpression is sufficient to induce widespread astrocyte reactivity and upregulation of known astrogliosis regulators SOX9 and STAT3, in the absence of injury. These findings have broad implications in understanding the multicellular response to injury and promoting CNS repair.

Disclosures: M. Chen: None. **C.G. Geoffroy:** None. **J. Meves:** None. **A. Narang:** None. **Y. Li:** None. **M. Nguyen:** None. **V. Khai:** None. **X. Kong:** None. **C. Steinke:** None. **L. Elziere:** None. **M. Goldberg:** None. **Y. Jin:** None. **B. Zheng:** None.

Poster

376. Glial Mechanisms in Neurological Disorders

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 376.21/G10

Topic: B.11. Glial Mechanisms

Support: Office of Naval Research N000141512016
NIH

Title: Beneficial effects of low alcohol exposure, but adverse effects of high alcohol intake on glymphatic function

Authors: *I. LUNDGAARD[1,2], W. WANG[2], A. EBERHARDT[2], H. VINITSKY[2], B. REEVES[2], S. PENG[2], N. LOU[2], R. HUSSAIN[2], M. NEDERGAARD[2,3]
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Abstract: Prolonged intake of excessive amounts of ethanol is known to have adverse effects on the central nervous system (CNS). Here we investigated the effects of acute and chronic ethanol exposure and withdrawal from chronic ethanol exposure on glymphatic function, which is a brain-wide metabolite clearance system connected to the peripheral lymphatic system. Acute and chronic exposure to 1.5 g/kg (binge level) ethanol dramatically suppressed glymphatic function in awake mice. Chronic exposure to 1.5 g/kg ethanol increased GFAP expression and induced mislocation of the astrocyte-specific water channel aquaporin 4 (AQP4), but decreased the levels of several cytokines. Surprisingly, glymphatic function increased in mice treated with 0.5 g/kg ethanol following acute exposure, as well as after 1 month of chronic exposure. Low doses of chronic ethanol intake were associated with a significant decrease in GFAP, with little changes in the cytokine profile compared with the saline group. These observations suggest that ethanol has a J-shaped effect on glymphatic system whereby low ethanol doses increase glymphatic function.

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Poster

376. Glial Mechanisms in Neurological Disorders

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Program #/Poster #: 376.22/G11

Topic: B.11. Glial Mechanisms

Support: Dr. Miriam and Sheldon G. Adelson Medical Research Foundation
NIH F32-087783
LLHF 2014-A-033-FEL

Title: Reactive astrocyte heterogeneity after stroke: Identifying new targets for neural repair

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Abstract: Stroke is one of the most common forms of death and disability worldwide, yet few treatment options exist. Tissue repair and recovery after injury is a complex process, involving both scar formation around the site of the injury that likely isolates the damage and prevents spread of immune-related damage molecules as well as remodeling of the neighboring and, in some cases, contralateral tissue. Astrocytes are central to these processes and respond differently in these distinct compartments. While it has long been clear that astrocytes respond to damage in a gradated fashion, the details of these responses are unknown. Here, we use morphologic and phenotypic tools to identify distinct zones of reactive astrocytes after stroke, which we used to inform a transcriptomic analysis to comprehensively map zone-specific astrocytic responses to both white matter and cortical stroke. We are using these transcriptomic maps to identify potential intervention points to promote repair and recovery, with a particular focus on astrocytic control of vascular development after white matter stroke.
In order to map the morphologic changes astrocytes undergo after stroke, we developed astrocyte-specific spaghetti monster reporter lentiviruses, injected them sparsely into the cortex or white matter at the time of stroke, and assessed individual astrocyte morphology at 7 days post-stroke. These studies revealed stereotyped changes in the morphology of both cortical and white matter astrocytes within defined regions surrounding the injury. We also assessed phenotypic changes in post-stroke astrocytes, using markers for proliferation, reactivity, and proteins previously identified as differentially regulated by stroke. Together, these studies were used to define zones of reactive astrocytes. To map the transcriptomic changes zone-specific astrocytes undergo after stroke, we used GFAP-Cre/Ribotag mice, in which astrocytic ribosomes are tagged, to selectively isolate astrocyte-enriched mRNA in laser captured zones. Astrocytes undergo dramatic, zone-specific changes in both stroke models; while some changes are

preserved between white matter and cortical stroke, others are not. In particular, cortical astrocytes appear to trigger greater angiogenesis and vessel maturation after stroke than do white matter astrocytes. Angiogenesis has been associated with increased recovery after gray matter strokes; therefore, we are exploring the function of astrocyte-induced angiogenesis in white matter stroke.

Disclosures: A.J. Gleichman: None. **R. Kawaguchi:** None. **M.V. Sofroniew:** None. **G. Coppola:** None. **S. Carmichael:** None.

Poster

376. Glial Mechanisms in Neurological Disorders

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Program #/Poster #: 376.23/G12

Topic: B.11. Glial Mechanisms

Support: R21NS077330
R01NS087033

Title: Role of Orai1 in LPS-mediated cytokine production

Authors: *J. JIANG, H. HU
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Abstract: Ca^{2+} signals in astrocytes mediate a remarkable variety of cellular functions, such as glutamate release and cytokine production. Among the various mechanisms by which cellular Ca^{2+} signals are generated, store-operated calcium channels (SOCs) have recently emerged as a widespread pathway for regulating many Ca^{2+}-dependent functions. We have demonstrated that SOC proteins are expressed in astrocytes and activation of astrocytic SOCs results in cytokine production. However, whether SOCs are involved in cytokine production in a pathological condition is not clear. Lipopolysaccharide (LPS), a component of the Gram-negative bacterial cell wall, is the most well-established inducer for the experimental inflammation. Using the specific siRNAs against STIM2 and Orai1, two major components of SOCs, we observed that knockdown of STIM2 or Orai1 dramatically decreased LPS-induced TNF-α and IL-6 production. While we further confirmed that knockout of Orai1 drastically attenuated LPS-induced cytokine production, it remains unknown how Orai1 is involved in this process. LPS-induced Toll-like receptor4 (TLR4) signaling has been shown to activate a number of kinases including mitogen-activated protein kinases (MAPK). To characterize the signal transduction pathways in astrocytes, we tested effects of several MAPK inhibitors on LPS-induced activation of MARKs and cytokine production. MEK/ERK inhibitor PD 98059, JNK inhibitor SP600125 and p38 inhibitor SB202190 significantly attenuated LPS-induced cytokine production and abolished LPS-induced increases of phosphorylation of ERK, p38 and JNK, respectively. Consistently,

knockout of Orai1 markedly decreased LPS-induced activation of MAPKs. Interestingly, STIM2 and Orai1 were significantly increased in LPS-treated astrocytes compared to vehicle treated astrocytes. LPS-induced-downstream effects were blunted by a TLR4 antagonist. These results indicate TLR4 activation is functionally coupled to the SOC channel Orai1 in astrocytes. Our findings highlight an important role of SOCs in LPS-induced cytokine release and may suggest that SOCs are potential therapeutic targets to treat inflammatory diseases of the central nervous system.

Disclosures: J. Jiang: None. **H. Hu:** None.

Poster

376. Glial Mechanisms in Neurological Disorders

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 376.24/H1

Topic: B.11. Glial Mechanisms

Support: UTSA COS Biology Startup

Title: Modeling neuroinflammatory states associated with astrocyte function

Authors: *Z. S. JORDAN[1], A. M. MAROOF[2], K. THANGAMANI[3], C. HUTCHINSON[2], L. JOHNSON[2], M. C. VARELA[4], K. MEYER[2], A. KHAN[2], S. ALI[2]
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Abstract: Astrocytes are essential for the functional maturation of neuronal circuits. Reactive astrocytes are found in neurodegenerative diseases (NDDs) at specific brain regions relevant to disease pathology. However, it's not clear whether reactive astrocytes are a cause or a consequence of NDD progression. Inducing a reactive state causes mouse astrocytes to change their function from neurotrophic to neurotoxic. We generated astrocytes from human induced pluripotent stem cells (hIPSCs), derived from NDD patients and healthy controls, and compared their quiescent and reactive states to primary human astrocytes to confirm astrocyte fate specificity and potential function. We treated astrocytes with the three cytokines: TNF-α, IL-1α, and IFN-γ, which are known to induce a reactive state in astrocytes *in vitro*. A panel of gene products known to be differentially regulated in the reactive state were assayed via immunocytochemistry and qRT-PCR in order to confirm a robust induction of the reactive state by cytokine treatment. Functional assessment of hIPSC-derived astrocytes will be assessed through glutamate uptake, calcium signal propagation, neuronal fate specification, synaptogenesis, synaptic phagocytosis, and neuronal survival. Therefore, we modeled the reactive astrocyte state *in vitro* with or without mature neurons. We used assays for astrocyte functions which support neuron growth and survival in order to determine the mechanisms in

which neurodegeneration may be initiated, following infection, traumatic brain injury, or other stressors that induces reactive astrogliosis.

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Poster

376. Glial Mechanisms in Neurological Disorders

Location: SDCC Halls B-H

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Program #/Poster #: 376.25/H2

Topic: B.11. Glial Mechanisms

Support: NIH R01 GM105775-05
NIH R01 EY12736
NIH T32-EY13933-16
Simons Society of Fellows

Title: Synaptic refinement and glial engulfment in a mouse model of fragile X syndrome

Authors: *M. A. LEE[1], M. WEISENHAUS[2], P. JURAKHAN[4], M. SHIRASU-HIZA[2], C. MASON[3]
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Abstract: Fragile X Syndrome (FXS) is the leading monogenic cause of both autism and intellectual disability and results from the silencing of the *Fmr1* gene, which codes for the translational regulator, FMRP. FXS is characterized by structural and functional defects at the synapse. In particular, elongated dendritic spines and defects in LTD are hallmarks of the disorder, as well as structural defects in axons. Glia are known regulators of both synapse growth and elimination. Despite FMRP expression in glia and reports of glial defects such as altered astrocytic glutamate signaling in FXS, little is known about glial function and dysfunction in FXS. The Shirasu-Hiza lab has identified a defect in glial phagocytosis in the *Drosophila* model of FXS. This defect is associated with decreased levels of activated glia, marked by expression of Draper, the *Drosophila* homolog of the mammalian astrocyte phagocytic receptor, MEGF10. Whether such a defect occurs in vertebrates is unknown.
The mouse retinogeniculate system is a classic system in which to study synapse refinement. Retinal ganglion cell (RGC) axons extend from each eye to the dorsal lateral geniculate nucleus (dLGN), and their arbors and synapses from opposite side (contralateral) and same side (ipsilateral) eyes overlap. During the first postnatal week, excess branches and synapses are refined so that by postnatal day 10, each dLGN neuron only receives inputs from a single eye—

either contralateral or ipsilateral. Both microglial and astrocytic phagocytosis are known to participate in arbor and synapse refinement (Schafer et al, Neuron, 2012; Chung et al, Nature 2013). We are investigating the role of glial phagocytosis in synapse refinement in the mouse retinogeniculate system in FXS. We first examine gross synaptic refinement in the retinogeniculate system of the *Fmr1* KO mouse by labeling RGCs from each eye with anterograde tracers conjugated to different fluorophores and find developmental segregation of ipsilateral and contralateral RGCs is enhanced in the first postnatal week (P7) of *Fmr1* KO mice when compared to wild-type littermates. Subsequently, segregation in the *Fmr1* KO mutant is comparable to wild-type littermates at P14. By adulthood (P40), however, retinogeniculate inputs are less segregated in the *Fmr1* KO dLGN than in wild-type. In combination with CTB-labeling of RGCs, we are assessing astrocytic engulfment of presynaptic inputs in the *Fmr1* KO::Aldh1l1-eGFP mouse. We are also investigating RGC synapse number and structure. This work will help to advance our understanding of the role of FMRP in glial function and may elucidate the role of glia in development and adult-stage refinement of the visual system.

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Poster

376. Glial Mechanisms in Neurological Disorders

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 376.26/H3

Topic: B.11. Glial Mechanisms

Support: NZ Neurological Foundation
HOPE-Selwyn Foundations of NZ
Maurice and Phyllis Paykel Trust

Title: Acute sensitivity of astrocytes in the Substantia Nigra to oxygen and glucose deprivation compared to the hippocampal CA1 region

Authors: *J. LIPSKI, R. N. KARUNASINGHE
Univ. Auckland, Auckland, New Zealand

Abstract: Astrocytes have a diverse spectrum of functions that are essential for regulating brain homeostasis. However, their role in brain ischemia still remains to be fully understood. The Substantia Nigra is a midbrain nucleus that is critical for movement control. Although its dopamine-producing neurons are implicated in the aetiology of Parkinsons disease, little is known of the acute effects of ischemia in this region. We recently reported that oxygen and glucose deprivation (OGD, 10 min) in brain slices, an *in vitro* ischemia model, generated a profound depolarization and swelling of neurons in the Substantia Nigra pars reticulata (SNr) but

not the dopaminergic neurons in the Substantia Nigra pars compacta (SNc). The SNr response resembled the phenomenon of 'anoxic depolarization,' known to also spread through the CA1 hippocampal region. The current study aimed to characterise the effects of OGD on astrocytes in the Substantia Nigra. Conventional intracellular recordings were made from astrocytes located at the border between SNc and SNr subregions in midbrain slices obtained from P21-23 rats. After fixing, immunoreactivity for the astrocyte-specific protein (GFAP) was also assessed. Nigral findings were compared with the relatively well-established astrocytic responses in the CA1 hippocampal region. OGD evoked a slow, then fast-phase depolarization of nigral astrocytes (n=13 cells from 9 rats), with the fast response developing during the anoxic depolarization and a rapid increase in extracellular K^+ ion concentration. This two-phased response resembled the OGD-evoked depolarization of hippocampal astrocytes (n=8 cells from 7 rats). However, unlike the prompt repolarization seen in hippocampal cells after returning O_2 and glucose, nigral astrocytes remained depolarized near 0 mV during reperfusion. In addition, immunoreactivity for the astrocytic protein GFAP markedly decreased in the Substantia Nigra after OGD (8 OGD *vs* 8 control slices submerged in standard ACSF, from 5 rats), while the hippocampus was unchanged (8 OGD and control slices, from 3 rats). These data indicate an acute post-ischemic withdrawal of astrocytic support in the Substantia Nigra.

Disclosures: J. Lipski: None. **R.N. Karunasinghe:** None.

Poster

376. Glial Mechanisms in Neurological Disorders

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 376.27/H4

Topic: B.11. Glial Mechanisms

Title: Effects of iron and tumor necrosis factor-alpha on astrocyte iron and glutamate homeostasis

Authors: *S. MUKEM[1,2], W. CHAIYANA[3], C. L. CHEEPSUNTHORN[4], P. CHEEPSUNTHORN[3]
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Abstract: Elevated levels of brain iron and pronounced inflammation have been correlated with neuropathological hallmarks of many degenerative brain disorders. Astrocytes are the most abundant cell type in the brain with diverse functions, including regulation of iron and glutamate homeostasis. This study sought to investigate the effects of iron and tumor necrosis factor-alpha (TNF-α) on the aforementioned functions of astrocytes using human 1321N1 astrocytoma cells.

Exposure to 50 ug/ml ferric ammonium citrate (FAC) increased calcein-chelatable iron content in 1321N1 cells, which was further enhanced when combined with 10 ng/ml TNF- α. These results correlated with a significant reduction in ferroportin mRNA levels and elevation of intracellular reactive oxygen species (ROS). TNF-α alone or, even better, in combination with FAC significantly stimulated the secretion of glutamate, as well as interleukin-6 (IL-6), from 1321N1 cells. Our findings suggest that astrocytes in degenerative brain disorders with iron accumulation and augmented inflammation are under stress due to their tendency to accumulate iron. Such astrocytes can release more of glutamate and IL-6, which together can drive neuronal cell death.

Disclosures: S. Mukem: None. **W. Chaiyana:** None. **C.L. Cheepsunthorn:** None. **P. Cheepsunthorn:** None.

Poster

376. Glial Mechanisms in Neurological Disorders

Location: SDCC Halls B-H

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Program #/Poster #: 376.28/H5

Topic: B.11. Glial Mechanisms

Title: Automated detection of markers of astrocyte activation

Authors: *L. G. FRANKLE, D. CARLYN, A. KOOTHAL, C. LU, R. CLEMENTS
Kent State Univ., Kent, OH

Abstract: Astrocyte activation is an important biomarker of multiple sclerosis (MS) and can be used to estimate severity of disease models. There are various ways to detect astrocyte activation including the concentration of proteins such as GFAP as well as morphological changes such as increased cell body size and altered cell body shape, and increased number, thickness, and branching of processes. While these morphological changes can be assessed manually with the aid of image processing software such as FIJI, automated programs could serve to increase the speed and ease of such assessments, potentially aiding in determinations of the extent to which mice being treated with cuprizone to mimic MS are being affected by it or conversely are being rescued by protective treatments. This would be a useful tool for future studies that use a mouse cuprizone model of MS, or any other mouse model that involves morphological changes to astrocytes. This plugin was developed using Java and works by selecting a seed point and using a region growing algorithm to expand throughout the cell. Processes are distinguished from the cell body by identifying whether a cubic radius surrounding each voxel includes only voxels belonging to the cell, and not background, in which case it's a body voxel. These methods segment astrocyte voxels from background voxels and body voxels from branches so determinations about cell body volume and process length and number can be reached. Data obtained by utilizing the plugin was compared to manual data to assess accuracy on metrics of

process counts and cell body volume. Manual data for the nuclear (DAPI) channel is also included to assess patterns in size and chromatin condensation as it correlates to cuprizone treatment and activation.

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Poster

377. Parkinson's Disease: L-DOPA Induced Dyskinesia

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 377.01/H6

Topic: C.03. Parkinson's Disease

Support: NIH Grant UH3NS100553
Michael J Fox Grant

Title: The effects of medication on both non stepping and compensatory stepping responses to progressive external postural disturbances in individuals with Parkinson's disease

Authors: *C. P. HURT[1], D. J. KUHMAN[2], H. C. WALKER[3]
[1]Physical Therapy, Univ. of Alabama at Birmingham, Birmingham, AL; [2]Rehabil. Sci., Univ. of Alabama at Birmingahm, Birmingham, AL; [3]Neurol., UAB, Birmingham, AL

Abstract: Parkinson's Disease (PD) is associated with motor and non-motor symptoms. Among the motor symptoms is poor balance function, which increases fall risk. Medications (e.g., Levadopa) alleviate some, but not all, PD symptoms. For instance, effects of drug therapy on balance function have been equivocal. Our objective was to explore the effects of PD medication on a measure of balance function - the ability to resist stepping while experiencing progressive external disturbances to balance. This preliminary analysis of an ongoing study includes four participants with PD, Hoehn and Yahr 2 or 3. Each participant was tested after at least 12 hours off (OFF) and then on (ON) PD medication on the same day. We delivered progressively larger balance disturbances by increasing treadmill belt accelerations over 400 ms, starting at 0.5 m/s^2 and incrementing by 0.5 m/s^2 until a participant failed 4 times at a given acceleration (i.e., their step threshold). Participants stood quietly on the treadmill before each disturbance and were asked to avoid taking a step. We used motion capture to collect kinematic data for the feet and the body's center of mass (CoM). With these data, we calculated margin of stability (MoS), which considers CoM position and velocity with respect to the body's base of support (computed with kinematic data of the feet). Positive MoS suggests system stability and negative MoS suggests system instability. We found the minimum MoS (MoS_{min}) in the non-stepping trial immediately preceding the first trial where stepping threshold occurred ("sub-threshold trial"). For stepping trials, we found the MoS value at foot strike of the compensatory step (MoS_{fs}) and

the step distance. Step thresholds increased after administration PD medications in 3 individuals (3 to 5 m/s^2, 5 to 5.5m/s^2, 3.5 to 5.5 m/s^2) and did not change in 1 individual (5 m/s^2). MoS_{min} during sub-threshold trials were qualitatively different OFF (0.0 ± 2.0 cm) vs. ON (-3.1 ± 2.0 cm). That individuals were able to withstand a more negative MoS while ON indicates greater capacity for generating non-stepping compensatory responses. For trials in which a step occurred, both MOS_{fs} (OFF: 6.7 ± 8.7 cm; ON: 9.8 ± 11 cm) and step distance (OFF: 7.1 ± 9.0 cm; ON: 12.7 ± 10.0 cm) qualitatively increased with medication. These data indicated that PD medication enhanced compensatory stepping mechanics. In general, this preliminary analysis of an on-going study suggests that medications enhance balance function in individuals with PD. Going forward we will use joint kinetics to characterize improved internal muscular responses with medication.

Disclosures: C.P. Hurt: 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 Funding BRAIN Initiative grant UH3NS100553. **D.J. Kuhman:** None. **H.C. Walker:** 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 Funding BRAIN Initiative grant UH3NS100553.

Poster

377. Parkinson's Disease: L-DOPA Induced Dyskinesia

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 377.02/H7

Topic: C.03. Parkinson’s Disease

Title: Dopamine d_1r or d_3r agonist exposure induces cross-receptor behavioral sensitization: Functional implications of d_1r-d_3r interactions

Authors: *K. E. LANZA, S. M. MEADOWS, K. CHEMAKIN, B. TOPPING, N. E. CHAMBERS, C. SAITO, C. R. BISHOP
Psychology, Binghamton Univ., Binghamton, NY

Abstract: Parkinson’s Disease (PD) results in motor deficits which are symptomatically treated with L-DOPA. However, chronic use of L-DOPA inevitably leads to the development of L-DOPA-induced dyskinesia (LID) characterized by abnormal involuntary movements (AIMs). Though there are many mechanisms that contribute to LID, accumulating data have implicated the involvement of both dopamine (DA) D1 sensitization and D3 receptor upregulation (D1R and D3R, respectively). Importantly, D3R upregulation occurs largely on D1R-bearing cells and interactions between these receptors have been postulated to underlie LID development. Changes to D3R expression appear to depend on chronic stimulation of D1R. However, it is unknown

about whether and how repetitive D3R stimulation induces D1R behavioral sensitivity. The current investigation tested the functionality of putative D1R-D3R interactions by examining the presence of cross-agonism, hypothesizing that D1R or D1R agonist treatment would induce reciprocal behavioral sensitization. To this end, female adult Sprague-Dawley rats were rendered hemi-parkinsonian via a unilateral 6-hydroxydopamine lesion in the medial forebrain bundle. Three weeks later, rats were tested for off-treatment motor deficits with the Forepaw Adjusting Steps (FAS) test and subsequently organized into 4 equally motor impaired groups (n=8-10 per group). Thereafter, each group received 4 injections every 4-5 days of either saline, the D1R agonist SKF38393 (1.0 mg/kg) or the D3R agonist PD128907 (0.1 mg/kg). AIMs were monitored following each injection for 3 hours to track the development of dyskinesia. For the 5^{th} injection, each group received an acute injection of the opposite agonist to test for the possibility of cross-sensitization. AIMs were monitored for the same testing period. Results showed that subchronic administration of both SKF38393 and PD128907 induced the development of dyskinesia. More importantly, cross-agonism tests revealed reciprocal cross-sensitization; chronic treatment with either SKF38393 or PD128907 induced sensitization to a single administration of the opposite agonist. Therefore, the current study provides key behavioral data demonstrating the role of D3R in dyskinesia and provides behavioral evidence of D1R and D3R functional interactions.

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Poster

377. Parkinson's Disease: L-DOPA Induced Dyskinesia

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 377.03/H8

Topic: C.03. Parkinson’s Disease

Title: Cholinergic neurons in the rostral pedunculopontine nucleus contribute to dyskinesia in the hemi-parkinsonian rat

Authors: *N. E. CHAMBERS[1], J. SERGIO[1], M. COYLE[1], B. TOPPING[1], C. SAITO[1], K. E. LANZA[1], S. CLARK[3], C. R. BISHOP[2]
[2]Psychology, [1]Binghamton Univ., Binghamton, NY; [3]Univ. at Buffalo - The State Univ. of New York, Buffalo, NY

Abstract: Parkinson’s disease (PD) is associated with motor deficits and dopamine (DA) cell loss in the substantia nigra pars compacta. L-DOPA, the current standard treatment for PD, produces abnormal involuntary movements known as L-DOPA-induced dyskinesia (LID) within a decade of treatment. Although the causes of LID are multi-faceted, elevated cholinergic tone, to which the pedunculopontine nucleus (PPN) may contribute, has been implicated. Notably, the

PPN innervates basal ganglia structures, is implicated in parkinsonian motor deficits and drug-induced motor stereotypy, and displays upregulation of immediate early genes that contribute to dyskinesia expression in rats. Despite compelling evidence that the PPN is involved in movement and motor stereotypy, the role of cholinergic PPN neurons in dyskinesia is unknown. Thus, the goal of the current study was to examine the role of PPN cholinergic neurons in parkinsonian motor deficits and in LID and DA-agonist-induced dyskinesia in the hemi-parkinsonian rat model. In the current study, we employed a between-subjects design with four surgical groups. Diphtheria-urotensin II fusion toxin (DTX-UII) was used to selectively lesion cholinergic PPN neurons. Each group received a unilateral surgery targeting the PPN with either saline or DTX-UII and the medial forebrain bundle with either saline or the DA neurotoxin 6-OHDA. Gait analyses (via CatWalk), forepaw adjusting steps (FAS), and locomotor chambers were used to assay parkinsonian motor deficits and their reversal. Dyskinesia was measured via the abnormal involuntary movements test. Lesion severity and specificity was verified via PPN ChAT and NeuN and nigral TH expression. We hypothesized that dual lesion (DTX-UII + 6-OHDA) and DA lesion groups would show motor impairments, and that dual lesioned rats would show less dyskinesia compared to DA lesioned rats. The results of this study show that dual and DA lesioned rats displayed parkinsonian motor deficits. Importantly, dual lesioned rats exhibited less LID than DA lesioned rats. In addition, dual lesioned rats displayed less severe D1- and D2-receptor agonist-induced dyskinesia than DA lesioned rats. Taken together, this suggests that the PPN may contribute to dyskinesia through innervation of both the direct and indirect pathways. Interestingly, L-DOPA's motor efficacy was maintained in dual lesioned rats, supporting PPN cholinergic neuron modulation as a promising therapeutic target for reducing LID without interfering with motor benefits of L-DOPA.

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Poster

377. Parkinson's Disease: L-DOPA Induced Dyskinesia

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 377.04/H9

Topic: C.03. Parkinson's Disease

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Title: Optogenetic and chemogenetic induction of unilateral hemiparkinsonism is not associated with the risk of levodopa induced dyskinesias

Authors: *K. M. LE[1], S. CHINNIAH[1], V. IYER[1], N. PATEL[2], C. LIEU[1], M. PENNOCK[1], E. HANDLEY[1], A. ZENEROWITZ[1], E. DICKEY[1], S. SAVALIYA[1], T. SUBRAMANIAN[1], K. VENKITESWARAN[1]
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Abstract: L-dopa provides effective symptomatic relief in Parkinson's disease (PD), but its long-term use causes levodopa-induced dyskinesias (LID). Loss of continuous dopaminergic stimulation (CDS) is an accepted hypothesis for the pathogenesis of LID. The "loss of CDS hypothesis" would predict that hemiparkinsonian (HP) patients and HP animals would develop unilateral LID. However, our studies show that HP rats and monkeys with preserved interhemispheric nigrostriatal fibers not develop LID. This suggests that loss of interhemispheric nigrostriatal connections may be critical for the genesis of LID. We tested the hypothesis that unilateral inhibition of the nigrostriatal pathway will lead to reversible hemiparkinsonism that will not be associated with dyskinesias. We used recombinant viral vectors to conditionally express Natronomonas halorhodopsin (NpHR3.0) and hM4Di, a Designer Receptor Exclusively Activated by Designer Drug (DREADD) to cause unilateral silencing of the nigrostriatal pathway. Experiments were performed in 2 separate model systems. In model #1, Sprague Dawley rats and in model #2 transgenic TH-Cre rats were used. In both models, recombinant viruses AAV5-Ef1a-DIO-eNpHR3.0-EYFP or AAV8-hSyn-DIO-hM4Di-mCherry was injected into the left substantia nigra pars compacta (SNpc). A cre-mediated switch, AAV2- Ef1a-mCherry-IRES-WGA-Cre, was injected unilaterally into left striatum in model #1 animal. Animals were repeatedly tested for onset and reversal of right HP (RHP) state. The eNpHR3.0 treated animals served as controls for the hM4Di treated animals and vice versa. Our results show that both eNpHR3.0 and hM4Di treated animals consistently developed RHP as characterized by mean unilateral reduction of vibrissae-evoked forelimb placement test scores by 75% ($p<0.5$). Striatal microdialysis indicated near complete diminution of dopamine levels that was temporally correlated with DREADD activation. In both models, RHP was completely reversible with no residual parkinsonism or adverse events. Repeatedly inducing RHP did not cause dyskinesias. Further, chronic treatment with L-dopa round the clock along with induction of RHP did not induce dyskinesias. No phototoxicity was noted. These experiments support the notion that unilateral inhibition of the nigrostriatal pathway with intact interhemispheric nigrostriatal connections does not lead to dyskinesias and chronic intermittent fluctuations of endogenous striatal dopamine is not associated with dyskinesias. These findings support the notion that preservation of interhemispheric nigrostriatal fibers and dopaminergic synapses will mitigate/prevent LID in PD.

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Poster

377. Parkinson's Disease: L-DOPA Induced Dyskinesia

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Topic: C.03. Parkinson’s Disease

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Title: Muscarinic but not nicotinic agonists prevent increase in dyskinesias with optogenetic activation of striatal D1 medium spiny neurons in parkinsonian mice

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Abstract: Dyskinesias are a disabling motor complication that arises with prolonged L-dopa treatment. Evidence indicates that direct pathway medium spiny neurons (D1 MSNs) play a primary role in the development of dyskinesias. Our previous work showed that D1 MSN stimulation induced dyskinesia-like abnormal movements in L-dopa naïve mice. L-dopa treatment alone induced dyskinesias (LIDs) to a greater extent than optical stimulation. However, combined L-dopa administration and stimulation resulted in an additive increase indicating that complex adaptive responses extending beyond activation of D1 and/or D2 receptors contribute to dyskinesias. Here, we used optogenetics to investigate whether nicotine treatment affects the direct control of striatal D1 MSN activity on dyskinesias in parkinsonian mice. Mice expressing cre-recombinase under the control of the D1 receptor promoter were unilaterally lesioned with 6-hydroxydopamine. AAV5-ChR2-eYFP or AAV5-control-eYFP was injected into the dorsolateral striatum, and optical fibers implanted. After stable virus expression, mice were optically stimulated and dyskinesias rated. The mice were subsequently treated with saline or L-dopa (until stably dyskinetic) and randomly assigned to saccharin or nicotine treatment. After a month of treatment, the effect of D1 MSN stimulation was assessed in all treatment groups. As previously, D1 MSN stimulation induced dyskinesia-like abnormal movements in L-dopa-naive animals and enhanced LIDs in L-dopa-primed mice. Nicotine decreased LIDs by ~50%, as before, but was unable to prevent the enhancing effect of D1 MSN stimulation. In fact, combined L-dopa and D1 MSN stimulation increased AIMs in the nicotine group to the same level as before nicotine treatment and equivalent to that observed in the saccharin group. These combined data suggest that nicotinic receptors (nAChRs) do not exert a direct control over D1 MSN activity to regulate LIDs. As recent studies have shown that enhancing muscarinic receptor type 4 (M4 mAChR) signaling decreases D1 MSN activity and ultimately reduces AIMs, we next examined the effect of VU0467154 on the effect of D1 stimulation on LIDs with and without nicotine treatment. VU0467154 reduced LIDs in saccharin but not nicotine-treated mice. Interestingly, it prevented the enhancement in LIDs with D1 stimulation in both groups. Overall,

the data indicate that while M4 mAChRs directly modulate D1 MSN activity to decrease LIDs, nAChRs may reduce them by indirectly affecting D1 MSNs and/or via additional mechanisms. Studies will now focus on examining the molecular mechanisms involved.

Disclosures: X.A. Perez: None. **T. Bordia:** None.

Poster

377. Parkinson's Disease: L-DOPA Induced Dyskinesia

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Title: The kappa opioid receptor antagonist nor-BNI accelerates development of L-DOPA-induced dyskinesia in a model of mild Parkinson's disease

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Abstract: Levels of the opioid peptide dynorphin, which is an endogenous ligand selective for kappa-opioid receptors (KORs), and its mRNA and peptide precursors are differentially dysregulated in Parkinson's disease (PD) and following the establishment L-DOPA-induced dyskinesia (LID). In the dopamine (DA)-depleted PD state, levels of dynorphin and its precursors are downregulated. In contrast, established LID is associated with levels of dynorphin and its precursors that are upregulated. It remains unclear whether these alterations contribute to the pathophysiological mechanisms underlying PD motor dysfunction and the subsequent emergence of LID, or whether they are compensatory. The KOR-antagonist nor-BNI has previously been shown to block sensitization in PD rats (Newman et al. 1997). Therefore, we set out to test if nor-BNI can change the development of LID during the priming period. To assess this, we have evaluated the effect of nor-BNI administration (3 mg/kg; *s.c.*) on the dose- and time-dependent development of abnormal voluntary movements (AIMs) in response to chronic escalating doses of L-DOPA treatment in a mild striatal 6-OHDA lesion rat LID model (70-80% DA depletion, compared to >95% in the standard LID model). Within this mild-dyskinesia paradigm, independent nor-BNI and vehicle-treated groups (n=6-7) were tested multiple times (2-4 times) across 5 escalating doses of L-DOPA (6, 12, 24, 48, 72 mg/kg*; i.p.*) and evaluated for

total limb axial and orolingual (LAO), and locomotor AIMs scores under blinded conditions. When cumulative AIMs scores were analyzed separately according to L-DOPA dose, we discovered that nor-BNI increased the expression of LAO AIMs only at the 12 (two-tailed t-test t=2.76 df=50, *p*=0.0081) and 24 mg/kg (two-tailed t-test, t=2.473 df=50, *p*=0.0169) L-DOPA doses. At the end of the experiment, after the 72 mg/kg L-DOPA dose, the AIMs levels were equal in both groups. When cumulative total locomotor AIMs scores were analyzed separately according to L-DOPA dose, at the 12 mg/kg dose a trend of an increased expression of locomotor AIMs after nor-BNI was seen (two-tailed t-test t=2.76 df=50, *p*=0.0733), and again locomotor AIMs were equal after the 72 mg/kg L-DOPA dose. In summary, nor-BNI significantly accelerated the rate of development of LAO but not locomotor LID in a mild striatal-lesion rat PD model, but did not increase the AIMs once LID was established. Given recent data showing that nor-BNI can increase DA release in the nucleus accumbens shell as measured with fast-scan cyclic voltammetry, it might be of interest to test if a similar increase in DA also occurs in the dorsolateral striatum and contributes to the described effect of nor-BNI.

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Poster

377. Parkinson's Disease: L-DOPA Induced Dyskinesia

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Program #/Poster #: 377.07/H12

Topic: C.03. Parkinson’s Disease

Support: NS073994

Title: Striatal ΔFosB gene silencing reduces abnormal involuntary movements induced by L-DOPA in hemiparkinsonian rats

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Abstract: Long-term dopamine replacement therapy in Parkinson's disease (PD) leads to the development of motor complications including L-DOPA-induced dyskinesia (LID). Several studies have shown that the transcription factor ΔFosB, a truncated form of FosB, plays an important role in the development of dyskinesias. Specifically, our experiments in rodent models of PD demonstrated that the transgenic ΔFosB overexpression in the striatum induces rapid development of abnormal involuntary movements (AIMs) in the absence of the typically

required regular L-DOPA treatment. To confirm the key role of this transcription factor, here we investigated whether inhibiting ΔFosB expression reduces and/or delays AIMs development. rAAV-ΔFosB shRNA-GFP or the control virus rAAV-scrambled shRNA-GFP was injected into the left striatum of rats with 6-OHDA lesions of the left medial forebrain bundle (n = 24). After apomorphine test screening, animals were evaluated for their baseline motor responses to L-DOPA, and then started on L-DOPA daily administration for 2 weeks. The whole L-DOPA motor response (rotation, cylinder and stepping test performances) and AIMs were assessed every four days, using standardized rating scales for rodents.
AIMs scores were significantly reduced in the rats injected with rAAV-ΔFosB shRNA compared to animals injected with the control vector over the course of chronic L-dopa treatment. Rotation counts, and scores from cylinder and stepping tests were no different between the two groups, indicating that silencing the striatal ΔFosB gene had no impact on the antiparkinsonian action of L-DOPA. These results confirm the crucial role of ΔFosB in the underlying mechanisms of AIMs, and suggest that ΔFosB gene silencing could be a useful therapeutic approach for LID.
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Poster

377. Parkinson's Disease: L-DOPA Induced Dyskinesia

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Topic: C.03. Parkinson’s Disease

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Title: Effect of sodium nitroprusside and 7-nitroindazole on cyclic nucleotides in a rat model of L-DOPA-induced dysinesia

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Abstract: The nitric oxide (NO) system has been proven to be a valuable modulator of L-DOPA-induced dyskinesia (LID) in parkinsonian rodents. Recent studies suggest that, under

certain circumstances, drugs with opposing pharmacological profiles may exhibit similar effects on LID. Our group has previously shown the anti-dyskinetic properties of NO synthase (NOS) inhibitor 7-Nitroindazole (7-NI) and of NO donor sodium nitroprusside (SNP) on reducing LID in hemiparkinsonian rats. This paradox effect is yet to be comprehended. Our aim was to investigate whether these drugs could have a common mechanism of action, perhaps by acting on different cyclic nucleotide systems in the lesioned striatum. For that, unilaterally 6-OHDA-lesioned rats with similar lesion intensity (as evaluated by the stepping test) were separated into groups and treated for 14 days with SAL (saline 0,9%, gavage, n=6), 7-NI/SAL (7NI 30 mg/kg i.p., n=6), SNP/SAL (NPS 4 mg/kg i.p., n=6), Veh/L-DOPA (L-DOPA 20 mg/kg, gavage, n=6), 7-NI/L-DOPA (7NI 30 mg/kg i.p., n=6) or SNP/L-DOPA (SNP 4 mg/kg i.p., n=6). We measured cAMP and cGMP levels in the dorsal striatum ipsilateral to the lesion, 10-15 min after last drug administration. We confirmed that 7-NI inhibited the manifestation of LID (P=0.0003). Our results also revealed the prolonged administration of SNP attenuated LID manifestation (U=12.08, P=0.0003). Chronic treatment with L-DOPA led to an increase in cAMP ($F_{1,30}$=15.98, P=0.0004) and cGMP ($F_{1,30}$=17.72, P=0.0002) levels in the lesioned dorsal striatum when compared to animals treated with SAL. Cyclic nucleotides levels were differentially regulated in animals that received 7-NI or NPS: 7-NI prevented the increase of both cAMP ($F_{2,30}$=21.00, P=0.0001) and cGMP levels ($F_{2,30}$=36.84, P=0.0001) whereas NPS prevented the increase of the cAMP levels ($F_{2,30}$=21.00, P=0.0001) and increased cGMP levels ($F_{2,30}$=36.84, P=0.0001) in L-DOPA-treated groups. In this way, our results revealed the reduction of cAMP levels might be a common mechanism in LID modulation by NOS inhibitor and NO donor. cAMP levels decrease might be an important mechanism to the antidyskinetic ability of the NO donor and the NOS inhibitor.

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Poster

377. Parkinson's Disease: L-DOPA Induced Dyskinesia

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Program #/Poster #: 377.09/H14

Topic: C.03. Parkinson’s Disease

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Title: The lack of Manf in the central nervous system causes activation of unfolded protein response without an effect on the dopamine system in mice

Authors: *E. PAKARINEN[1], V. VÕIKAR[2], P. PIEPPONEN[3], M. SAARMA[1], M. LINDAHL[1]
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Abstract: Neurodegenerative diseases, such as Parkinson's disease, share pathological features of protein aggregation and protein misfolding in cells. Accumulation of protein aggregates causes endoplasmic reticulum (ER) stress, which activates unfolded protein response (UPR) to reduce stress. Mesencephalic astrocyte-derived neurotrophic factor (MANF) is an ER-located protein secreted in response to ER stress. Moreover, MANF protects cells from ER stress both in vitro and in vivo. The deficiency of MANF in mice leads to pancreatic beta cell loss and diabetes caused by ER stress in beta cells. In the rodent model of Parkinson's disease MANF has shown both neuroprotective and neurorestorative actions. However, the role of endogenous MANF in the dopamine system in mice is still not understood. To examine whether MANF acts as a physiological trophic factor for dopamine neurons, we created MANF conditional knockout (cKO) mice with the deletion of Manf in the central nervous system by using transgenic Nestin Cre mice. In this study we aimed to characterize the morphology and function of the midbrain dopamine system of MANF cKO mice and examine the effect of MANF absence on ER stress levels in the brain. Results reveal chronic activation of one of the UPR pathways, IRE1, in the brains of MANF cKO mice. This activation, however, might provide protection, since no cell loss is observed in the dopamine system. Aged MANF cKO mice have normal number of midbrain dopamine neurons and unchanged dopamine levels in the striatum. Moreover, motor behavior stays similar to control mice during aging. Also, the functionality of dopamine neurons tested with amphetamine administration is unaffected in aged MANF cKO mice. In conclusion, mice with the deletion of MANF in the central nervous system have prolonged activation of UPR in their brains. Furthermore, MANF deficiency in mice is dispensable for the maintenance of dopamine neurons during aging.

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Poster

377. Parkinson's Disease: L-DOPA Induced Dyskinesia

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Topic: C.03. Parkinson's Disease

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Science for Life Laboratory

Title: Neurotransmitter alterations in Parkinson's disease and L-DOPA-induced dyskinesia analyzed by molecular-specific mass spectrometry imaging

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Abstract: There is a great need to define the molecular changes associated with Parkinson's disease (PD) patients that develop L-Dopa-induced dyskinesia (LID). In the present research we have studied neurochemical processes in PD, and specifically LID, using MALDI-mass spectrometry imaging (MSI). We introduce a novel molecular-specific approach to image and quantify a large number of different neurotransmitters, their precursors and metabolites simultaneously directly in tissue sections using mass spectrometry (MS). Samples were obtained from a biobank of brain tissue (*Macaca mulatta*). Regional analysis (coronal sections) of neurotransmitters produced by the basal ganglia neurons in the PD and LID states were investigated by MALDI-FTICR MS imaging (Solarix 7T). Non-parametric test (Mann-Whitney U) as well as orthogonal correction partial least squares discriminant analysis (OPLS-DA) were performed in 21 selected brain regions. Using the novel MSI approach we were able to map almost the complete catecholamine neurotransmitter system, the precursors and most of their metabolites, directly in brain tissue sectionsin the PD and LID experimental model. L-Dopa and dopamine was significantly increased in most measured brain structures of the dyskinetic animals and the dopamine metabolites 3-MT and HVA were significantly increased in several cortex regions as well as the hippocampus. In summary, our methodology enables *in situ* mapping of patterns within and between neurotransmitter systems and showed novel neurotransmitter alterations in PD and LID. Quantitation of functional neurotransmitter balances may be a useful approach in studies of neurodegenerative disorders but also in drug development as a biomarker-based rationale for targeted modulation of neurotransmitter networks.

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Poster

378. Dystonia, Tremor, and Other Movement Disorders

Location: SDCC Halls B-H

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Topic: C.04. Movement Disorders other than Parkinson's Disease

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Title: Microexon inclusion is associated with distinct expression patterns and subcellular localization of the disease gene TAF1

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Abstract: Mutations in *TAF1*, the TATA-box binding protein associated factor that is central to RNA polymerase II function, can cause two different diseases. One, X-linked dystonia parkinsonism (XDP/DYT3), is characterized by neurodegeneration of the striatum with generalized dystonia and parkinsonism beginning in adulthood. XDP is linked to mutations near the 3'-end of *TAF1*, including an insertion of the retrotransposon SVA (SINE-VNTR-Alu) upstream of a neuron-specific *TAF1* exon. A second disease, X-linked intellectual disability syndrome (MRXS33), is linked to mutations in constitutive exons of *TAF1* that are located 5' to the SVA insertion. MRXS33 causes perinatal growth retardation, facial dysmorphology, psychomotor symptoms, and intellectual disability. To better understand the differences between XDP and MRXS33, we directly compared mRNA and protein expression patterns of the neuron-specific *TAF1* isoform (*N-TAF1*) and the constitutive isoform (*C-TAF1*). This is technically challenging because *N-TAF1* is distinguished from *C-TAF1* only by the inclusion of a six base-pair micro-exon (exon 34'), encoding alanine and lysine.
We used BaseScope™ probes against *C-* and *N-TAF1* for *in situ* hybridization in adult mouse brain, liver, and heart. *C-TAF1* was detected in all of these tissues, whereas *N-TAF1* was restricted to brain. Within the brain, *C-TAF1* was detected in mature neurons, glia, and dividing cells. By contrast, *N-TAF1* was enriched in post-mitotic neurons. We found similar differences in expression with antibodies that we generated against TAF1 peptides that included or excluded the microexon 34' sequence. Both C- and N-TAF1 were widely expressed in the brain including within nuclei of cortical neurons, striatal neurons, and dopamine-containing nigral neurons. However, double immunofluorescence for C- and N-TAF1 showed differential subnuclear localization within post-mitotic neurons.
Splicing factor nSR100/SRRM4 regulates inclusion of microexons in many neuronal genes, including *N-TAF1*. We compared the expression pattern of *nSR100* to *C-* and *N-TAF1* by *in situ* hybridization and immunolabeling. Like N-TAF1, nSR100 was widely expressed in post-mitotic neurons, but differentially enriched among them.
Our findings, together with previous work, suggest that the developmental symptoms of MRXS33 might be absent in XDP because of the restriction of N-TAF1 expression to post-mitotic neurons. However, the striking difference in subnuclear localization of C- and N-TAF1 indicates that other symptom differences are related to separate functions for these two TAF1 isoforms and shows that microexons can have a major impact on subcellular localization.

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Poster

378. Dystonia, Tremor, and Other Movement Disorders

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Program #/Poster #: 378.02/H17

Topic: C.04. Movement Disorders other than Parkinson's Disease

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Title: Validation of a torsinA cerebellar knockdown model of DYT1 dystonia

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Abstract: Dystonia is a movement disorder characterized by sustained or intermittent muscle contractions causing twisting, repetitive movements or abnormal postures. DYT1 dystonia is the most common genetic dystonia. About 60-90% of DYT1 patients carry heterozygous *DYT1 GAG* mutation causing a loss of a glutamic acid of the protein torsinA. Many studies suggest that the mutation results in a loss of function. But how the mutation causes dystonia remains largely unknown. The research in this area is hampered by the lack of overt dystonia-like phenotypes in most of the genetically modified mouse models developed. Recently, acute torsinA knockdown in the cerebellum using small hairpin RNAs (shRNAs) against torsinA mRNA has been demonstrated to induce overt dystonia-like phenotype in adult mice. However, shRNAs or small interfering RNAs (siRNAs) are known to have significant off-target effects that lead to altered expression of unintended genes. To validate the shRNA model of DYT1 dystonia, we generated an acute torsinA knockdown mouse model by bilateral stereotaxic injections of AAV5-CMV-Cre-GFP into the cerebellum of $Dyt1^{loxP/loxP}$ mouse. Expression of Cre led to cre-loxP-mediated recombination and eliminated the expression of torsinA in AAV-infected cells. The motor behavior of these mice was assessed by the dystonia scale at 3 weeks, 7 weeks and 13 weeks after injection, respectively. Thirteen weeks after AAV injection, mice were euthanized, and the brains were harvested. Green fluorescence could be seen only in the cerebellum and the torsinA level was quantified by western blot. Electrophysiological properties of the Purkinje cells were characterized using the cell-attached patch-clamp recording in the brain slices. Overt dystonia-like phenotypes were found in 5 out of 7 mice. However, an unexpected bidirectional circling was found in 3 of the most severely affected mice when the mice were lifted to examine hindlimb clasping. This resembles the vestibular deficits seen in other mutant mice such as vertigo 2 Jackson mice. Vertigo is not a typical symptom of DYT1 dystonia patients. Our findings are consistent with the previous results that acute cerebellar knockdown of torsinA expression can produce the overt dystonia phenotype, however, the vertigo phenotype we

observed questions the validity of the shRNA model as a phenotypic DYT1 dystonia mouse model.

Disclosures: Y. Li: None. **Y. Liu:** None. **E. Rodriguez-Lebron:** None.

Poster

378. Dystonia, Tremor, and Other Movement Disorders

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 378.03/H18

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: A grant from Restless Legs Syndrome Foundation
NIH grant NS65273
NIH grant NS82244

Title: The role of BTBD9 in the pathogenesis of restless legs syndrome (RLS): Cortical and dopaminergic neurons

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Abstract: RLS is a prevalent sensorimotor disorder characterized by an urge to move due to uncomfortable sensations in the legs, often at night or at rest, which is generally relieved by movement. Genome-wide association studies have identified mutations in *BTBD9* to confer a higher risk of RLS. We developed *Btbd9* knockout (KO) mice as an animal model for the disease. RLS patients have been reported to show functional alterations in the cortex, especially sensorimotor cortex. Furthermore, the primary prescription for RLS patients are D2/D3 dopaminergic agonists and alterations in dopaminergic system have been widely found in both RLS patients and RLS animal models. Reduced expression of fly BTBD9 homolog in a subset of dopaminergic neurons can lead to sleep disruptions. Therefore, both cerebral cortex and dopaminergic system appear to be critical for the pathogenesis of RLS, with the exact functions of BTBD9 in these brain regions largely unknown. Here, we conducted manganese-enhanced MRI study in *Btbd9* KO mice and found increased neural activity in primary somatosensory cortex (S1) and rostral piriform cortex (PirR). Next, we performed behavioral studies with cerebral cortex-specific *Btbd9* conditional knockout mice (BCKO) and dopaminergic neuron-specific conditional knockout mice (BDATKO). We did not find motor restlessness and increased thermal sensation in BCKO or BDATKO mice. Instead, the BCKO mice showed a significant decrease of thermal sensation. Finally, we made use of the simple nervous system of *Caenorhabditis elegans* and studied egg-laying and locomotor behaviors to define the genetic

interactions between the worm BTBD9 homolog and the dopaminergic system. We found loss of *hpo-9*, the *C. elegans* homolog of *BTBD9*, resulted in hyperactive egg-laying behavior and altered responses to exogenous dopamine. Additionally, dopamine receptor mutation *dop-1* partially, but *dop-3* completely, suppressed the effect of *hpo-9* in egg-laying behavior. The function of HPO-9 in locomotion was modulated by *dop-1* and *dop-3*, with *hpo-9* and *dop-1* working in a similar way. Our finding is consistent with the previous study of increased D1 receptor (D1R) protein expression in the lumbar spinal cord of RLS animal models. Our results therefore indicate that systematic BTBD9 deficiency increases the neural activity of S1 and PirR. However, loss of BTBD9 only in cortical neurons or dopaminergic neurons is not sufficient to cause RLS-like phenotypes in mice. In *C. elegans*, HPO-9 deficiency altered the dopaminergic system by its interaction with dopamine receptors, especially *dop-1*. Taken together, our results revealed a complex mechanism by which mutation of BTBD9 can lead to RLS.

Disclosures: A. Doroodchi: None. **Y. Sheng:** None. **K. Dexter:** None. **B. Salvato:** None. **M. DeAndrade:** None. **M. Miller:** None. **M. Febo:** None. **R. Xiao:** None. **Y. Li:** None.

Poster

378. Dystonia, Tremor, and Other Movement Disorders

Location: SDCC Halls B-H

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Program #/Poster #: 378.04/I1

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH 1R21NS103098-01

Title: Changes in motor cortical electrophysiological and two-photon Ca^{2+} responses during episodic dystonia in the tottering (tg/tg) mouse

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Abstract: Episodic ataxia type 2 (EA2) is a rare neurological disorder caused by a mutation in the *CACNA1A* gene, encoding the P/Q-type, voltage-gated Ca^{2+} channel important for initiating neurotransmitter release. Patients with EA2 exhibit both cerebral and cerebellar pathologies, including migraines, absence seizures, and episodic ataxia, which can be triggered by caffeine, stress, or alcohol. Several clinical studies have also reported dystonia occurring in EA2 patients. The *tottering* (*tg/tg*) mouse is a commonly used model for EA2 due an orthologous mutation in the *Cacna1a* gene, resulting in a phenotype similar to EA2, including absence seizures, ataxia, and transient episodes of dystonia. Previous research has implicated the cerebellum in the generation of the dystonic attacks, and it is well known that cerebellar output can help refine and coordinate motor commands. As a profound motor phenotype occurs during the dystonic attack,

we hypothesized there would also be changes occurring in the local cellular networks in the primary motor cortex (M1) of *tg/tg* mice. To investigate this, we recorded extracellular activity with a 16 channel multi-electrode array and two-photon (2P) Ca^{2+} imaging from awake, head-fixed *tg/tg* and wildtype mice on custom treadmills. Mice were either implanted with a chamber to accommodate the electrophysiology headstage, or injected with an AAV construct containing GCaMP6f in M1 and implanted with a 3D-printed, clear brain window for 2P imaging. Following a baseline recording period, the dystonic attacks were triggered by caffeine injections, and electrophysiology and imaging recorded pre-, during, and post-attack. Local field potential (LFP) analysis found generalized spike-and-wave discharges (GSWDs), a hallmark of absence seizures that *tg/tg* mice exhibit, were completely abolished during the dystonic attack and reappeared following an attack. This decrease in GSWDs was also paralleled with a decrease in gamma coherence during the attack, suggesting a decrease in thalamocortical activity. Alpha and beta coherence also decreased, which may be associated with a reduction in inhibitory synchrony that contributes to the dystonic movements. Preliminary results from the 2P imaging revealed an increased number of Ca^{2+} transients per neuron during the attack, however, the single unit recordings of basic firing properties (firing rate, CV, CV2) did not change during the attack. Overall, these results suggest that changes in local M1 coherence may contribute to the episodic dystonia in *tg/tg* mice. Further, the complete dissociation between GSWDs and the dystonic attacks shows that these two instabilities represent unique network states.

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Poster

378. Dystonia, Tremor, and Other Movement Disorders

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Program #/Poster #: 378.05/I2

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Tyler's Hope for a Dystonia Cure. Inc.
NIH Grant NS054246

Title: Abnormal firing of Purkinje cells contributes to the pathogenesis of DYT1 dystonia

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Abstract: DYT1 dystonia is an inherited movement disorder caused by a heterozygous trinucleotide deletion (GAG) mutation in DYT1/TOR1A, coding for torsinA. Growing evidence suggests that the cerebellum plays a role in the pathogenesis of dystonia. Brain imaging of DYT1

dystonia patients showed abnormal activity in the cerebellum. Cerebellum-specific knockdown of torsinA expression in adult mice leads to dystonia-like behavior. We and others reported previously altered dendritic structures in Purkinje cells in *Dyt1 Δ*GAG heterozygous knock-in (*Dyt1* KI) mouse models of DYT1 dystonia. To examine whether there are functional alterations of the Purkinje cells, electrophysiological properties of the Purkinje cell were characterized in the *Dyt1* KI mice using cell-attached patch-clamp recording in brain slices. Both the firing rate and the coefficient of variation (CV) were unchanged in the tonic type of Purkinje cells. However, in the non-tonic type of Purkinje cells in the *Dyt1* KI mice, although the firing rate was unchanged, the CV was significantly increased, suggesting abnormal firing of the non-tonic type of Purkinje cells in the *Dyt1* KI mice. To investigate how the altered Purkinje cell firing patterns contribute to DYT1 dystonia pathogenesis, we used Cre-dependent DREADD (designer receptors exclusively activated by designer drugs) to manipulate the Purkinje cell activity *in vitro* and *in vivo*. *Pcp2-cre*/DREADD double heterozygous mice were generated by crossing DREADD mice with L7/Pcp-2:Cre transgenic mice. Brain fluorescent imaging showed that DREADD was selectively expressed in Purkinje cells in *Pcp2-cre*/DREADD mice. Bath application of CNO (clozapine N-oxide), the DREADD agonist, could selectively excite or inhibit Purkinje cell firing in brain slice preparations. Behavioral tests were performed after intraperitoneal injections of either CNO or saline in *Pcp2-cre*/DREADD mice. Activation of Purkinje cells led to significant deficits in rotarod and beam-walking tests, but not when the Purkinje cells were silenced. None of these DREADD mice showed overt dystonia. The results demonstrated that altered Purkinje cell firing pattern, as seen in *Dyt1* KI mice, could potentially lead to motor coordination and balance deficits that have been reported in multiple genetic DYT1 dystonia mouse models. Taken together, our results support a role of cerebellum in the pathogenesis of DYT1 dystonia.

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Poster

378. Dystonia, Tremor, and Other Movement Disorders

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 378.06/I3

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: CONACYT a JFR N° 220871
DGAPA-PAPIIT IN214716 JFR

Title: Findings of myotonic dystrophy type I by diffusion tensor imaging

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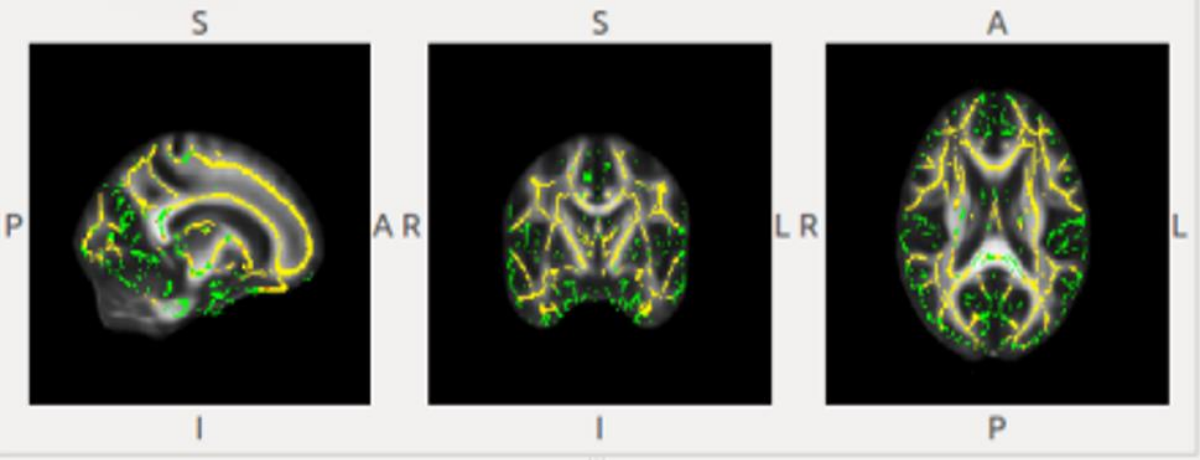
Abstract: Myotonic Dystrophy Type I (DM1) is a degenerative and hereditary disorder, its more typical symptoms are muscle weakness and hypotonia, which may lead to several complications like respiratory failure and cardiac arrest. DM1 has proven difficult to diagnose. It affects the quality of life of the patients and its symptoms could lead to different diagnostics that could be confused even with normal aging. For this reason it is important to find biomarkers that help in the assessment and in the follow up of treatment for this disease.The aim of this project is to find biomarkers that help us to characterize the evolution of this disorder. In the present work 37 DM1 patients and 35 healthy controls volunteer participants were matched by age, sex and level of education, then underwent an MRI session in a 3T Philips Ingenia scanner with a 32-channel head sense coil. Diffusion Tensor Images (DTI) were acquired with the following parameters: 33 diffusion directions, b=800 s/mm^2, isometric spatial resolution (2mmx2mmx2mm), TR=7103 ms, TE=60 ms, Gap=0, 70 slices. For the analyisis of data a t-test was applied for unpaired samples, and the FSL5.0.8 software was used. For the post processing DTI images were corrected by movement and eddy currents and TBSS script was applied. Clusters with statistical significance (P<0.05) were found in the regions of orbitofrontal white matter paths, anterior cingulate cortex white matter paths and corpus callosum. Atrophy in white matter paths is significant in frontal brain areas, which may imply that different control processes could be altered.



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Poster

378. Dystonia, Tremor, and Other Movement Disorders

Location: SDCC Halls B-H

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Topic: C.04. Movement Disorders other than Parkinson's Disease

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Bachmann Strauss Dystonia & Parkinson Foundation

Title: Phenotyping cervical dystonia: using clinical video recordings and computer vision to capture triaxial head posture

Authors: Z. ZHANG[1], J. P. VU[1], Q. CHEN[1], E. CISNEROS[1], C. N. BENADOF[1], T. J. SEJNOWSKI[2], J. S. PERLMUTTER[3], G. T. STEBBINS[4], C. COMELLA[4], *D. A. PETERSON[5]
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Abstract: The objective of this study was to test the hypothesis that computer-vision based video analysis software can capture abnormal head postures in cervical dystonia. Cervical dystonia (CD) is one of the most common forms of the adult onset focal dystonias. The most prominent phenotypic feature of cervical dystonia is abnormal head postures. These are usually quantified subjectively with the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS), an ordinal clinical rating scale. Advances in computer vision have produced algorithms to infer head posture from conventional digital video recordings. But they have not been evaluated for measuring pathological head postures in CD. We analyzed digital videos from 21 patients recruited at Rush University Medical Center with isolated CD and a heterogeneous mix of abnormal head postures in each of the 3 axes of rotation: pitch (antero/retrocollis), roll (laterocollis, or "tilt"), and yaw (torticollis). We used OpenFace to estimate head orientation on each video frame, quantified as degrees of rotation in each axis. Mean orientation was calculated while patients were instructed to let their head drift to its most comfortable (dystonic) position with eyes closed. Of the 21 patients, 3 could not be evaluated because of extreme CD or facial hair which confounded face detection in OpenFace. For the remaining 18 patients, mean head orientation calculated from the videos was positively correlated with the ordinal severity ratings from the TWSTRS in each of the three axes of rotation (pitch $R = 0.85$, roll $R = 0.88$, and yaw $R = 0.69$, all $p < 0.005$). Our results demonstrate that computer-vision based video analysis software can capture abnormal head rotations in CD. This approach provides a measure of CD motor features that is inherently objective, critical to improving outcome measures for clinical trials of new treatments and regressors in human research on CD pathophysiology. In future

work, we plan to investigate heuristics for mitigating cases of extreme severity and excessive facial hair. We also plan to extend our analyses to a cohort of over 200 CD patients recruited from 10 sites in North America through the Dystonia Coalition's project to validate the Comprehensive Cervical Dystonia Rating Scale.

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Poster

378. Dystonia, Tremor, and Other Movement Disorders

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Program #/Poster #: 378.08/I5

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: DFG Grant LA 3830/1-1
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DAAD

Title: A dystonia mouse model with dysregulated pontine plasticity

Authors: *D. LAM[1], R. H. WILLIAMS[1], E. LUJAN[4], K. TANABE[4], N. L. SAW[5], G. HUBER[2], J. MERL-PHAM[3], M. J. PLASTINI[6], M. ZECH[1], A. GEERLOF[2], M. SHAMLOO[5], S. HAUCK[3], M. WERNIG[4], J. WINKELMANN[1]
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Abstract: The movement disorder dystonia can be caused by variants in the C-terminal domain (CTD) of the α3 subunit of collagen VI (COL6A3). Variants in other domains cause mechanistically unrelated muscular dystrophy phenotypes. To dissect this domain specificity, we generated mice expressing a truncated COL6A3, lacking only the CTD (*Col6a3*^{CTT} allele). Homozygous *Col6a3*^{CTT} mice had a dyskinetic phenotype, with impaired beam walk and rotarod performance, reminiscent of dystonia observed in patients with *COL6A3* CTD variants. Unlike a reported functional null allele, we observed no nonsense-mediated decay of the truncated transcript and no signs of muscular dystrophy. Within the brain, *Col6a3* is exclusively expressed in the septum and basal pontine nuclei (BPN). Given the critical role of the BPN in relaying corticocerebellar motor control and learning signals, we used whole cell patch clamp electrophysiology to investigate synaptic function in BPN neurons. BPN neurons of *Col6a3*^{CTT} homozygous mice have increased frequency of spontaneous and mini excitatory postsynaptic current (sEPSC and mEPSC) compared to age-matched wildtype controls, indicating enhanced

presynaptic excitatory tone. Neuroanatomical tracing indicated that excitatory input is primarily of cortical origin. We hypothesize that COL6A3 mediates corticopontine synaptic plasticity via an intrasynaptic interaction. Overactivation of the corticocerebellar pathway, consistent with the hyperkinetic nature of dystonia, represents a novel pathophysiological substrate for dystonia as well as a potential therapeutic target.

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Poster

378. Dystonia, Tremor, and Other Movement Disorders

Location: SDCC Halls B-H

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Program #/Poster #: 378.09/I6

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant 5R01HD081346-04

Title: The effect of EMG-based sensory feedback training of a trajectory-constrained self-feeding task on motor control for adolescents with secondary dystonia

Authors: *S. AMANO[1], E. AMBROSINI[2], E. BIFFI[3], A. GALBIATI[2], C. CASELLATO[4], A. PEDROCCHI[2], T. D. SANGER[1]
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Abstract: While brain injury in early childhood or at birth not only immediately result in motor dysfunction, but also potentially cause a long-term consequence in motor learning, as sensory deficits, often observed in secondary dystonia, could result in motor learning deficit. Therefore, developing a novel intervention that can provide an augmented sensory feedback to reverse abnormalities in motor learning is imperative for children with secondary dystonia to have a better quality of life. The purpose of the study was to determine the effect of a 5-day motor training of a trajectory-constrained self-feeding task with our EMG-based sensory feedback in persons with secondary dystonia. Three subjects with secondary dystonia, whose ages ranged from 12 to 20, underwent two of a 5-day training of a self-feeding task; 1) with (BF+) and 2) without (BF-) a vibrotactile biofeedback device. On each day, each subject was instructed to transport a marble in a spoon between two targets, positioned 20cm apart along the sagittal plane, and to complete 10 repetitions without dropping a marble. Under BF+ condition, we attached a battery-powered wearable EMG feedback device on subject’s anterior or lateral

deltoid muscle. This device indicates the level of muscle activity at the site of our targeted muscle, by proportionate changes in the speed of a silent vibration motor. At baseline and following the training period, averaged movement time (MT) of 10 repetitions were recorded. To quantify the quality of movement, spoon trajectory was also collected at 100Hz to compute movement jerk (i.e., time derivative of acceleration normalized by duration and distance) during the task. The change scores after the training period in MT and normalized jerk were compared between BF+ and BF- condition. Improvement in MT after the training period appears to be negligible regardless of intervention (BF+: +.14±.70s, BF-: -.07±.23s). On the other hand, training with sensory feedback was beneficial to improve the quality of movement. In particular, after a 5-day training with the biofeedback device, all of three subjects reduced movement jerk (mean change: -267.4± 215.4). While average jerk across subjects also decreased (-67.6±450.0) under BF- condition, two of three subjects increased movement jerk. These results are consistent with the hypothesis that distorted or reduced sensory feedback or abnormal attentional mechanisms may perpetuate motor symptoms in children with dystonia. Most importantly, they provide evidence that correction of sensory deficits or task inattention can have direct beneficial effects. We are actively recruiting more children with secondary dystonia to confirm the hypothesis.

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Poster

378. Dystonia, Tremor, and Other Movement Disorders

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 378.10/I7

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Characterization of the stress response in the P/Q-type calcium channel knockout mouse model quirky

Authors: *M. GRÖMMKE[1], S. HERLITZE[2], M. D. MARK[3]
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Abstract: The cerebellum is an important component of the motor system due to its involvement in coordination, optimization and execution of movements. Malfunctions within the cerebellar network cause several disorders with an immense impairment of motor behavior. Especially, mutations of the P/Q-type calcium channel result in motor related diseases like episodic ataxia type 2 (EA2) which is associated with ataxia and episodes of dyskinesia. A previous study demonstrated that conditional knockout of the P/Q-type calcium channel in cerebellar granule cells leads to cerebellar ataxia and dyskinesia in mice (*quirky*). Interestingly, stress is a common

trigger of dystonic events in EA2 patients as well as in *quirky* mice. In the cerebellum stress is predominantly associated with the release of the transmitter norepinephrine (NE) from neurons of the *Locus coeruleus* (LC). The activity of the LC, however, is to a great extent mediated by the corticotropin-releasing factor (CRF) and the LC is also known to release CRF as a co-transmitter. Since *quirky* mice display ataxia and dyskinesia triggered by stress it is of great interest to determine how NE and CRF have impact on stress-induced dystonic events and how the cerebellar network is affected in these mice. The short restraint stress-test which reliably induces dystonic events in *quirky* but not in C57BL/6 control mice was performed and the effect of intraperitoneally injected α_1-adrenergic receptor antagonist prazosin and CRF-receptor type 1 antagonist NBI-35965 was determined. The administration of prazosin reduced the occurrence of dystonic attacks in *quirky* mice whereas NBI-35965 had no beneficial effect indicating that the stress-response is mediated by NE and not CRF in these mice. Furthermore, *in vivo* electrophysiological recordings of cerebellar Purkinje cells (PCs) in anaesthetized mice were performed and the impact of NE on the spontaneous activity of PCs was investigated. Exogenous application of NE evoked a strong inhibition of the PC activity in *quirky* and C57BL/6 control mice. The NE-induced inhibition was stronger in *quirky* mice than in control mice and the additional application of prazosin led to a decrease of the NE-induced inhibition in *quirky* mice. These results demonstrate that the release of NE in the cerebellum leads to a greater inhibition of PC activity in *quirky* mice which in turn enhances the cerebellar output signal onto premotor areas of the brain leading to dyskinesia. It can be suggested that the NE-induced effect is primarily mediated by G_q-coupled α_1-adrenergic receptors located on cerebellar molecular layer interneurons and thus by a stronger inhibitory drive onto PCs.

Disclosures: M. Grömmke: None. **S. Herlitze:** None. **M.D. Mark:** None.

Poster

378. Dystonia, Tremor, and Other Movement Disorders

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 378.11/I8

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Chronic multi-electrode recordings of the basal ganglia-thalamocortical circuitry in children with generalized dystonia reveal functional mechanisms of deep brain stimulation

Authors: *E. ARGUELLES[1], M. ABOLFATH-BEYGI[2], R. DESHPANDE[2], D. FERMAN[1], T. D. SANGER[2]
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Abstract: Dystonia is a movement disorder characterized by involuntary muscle contractions and abnormal postures. Dystonic symptoms have been associated with basal ganglia (BG) dysfunction, where abnormal neural activity causes a complex set of physiological changes

causing abnormal neural organization. Studies of the BG and thalamus in dystonia have focused on single electrode recordings under vary constrained conditions, making it difficult to study multi-neuronal function under natural behavior. Here we present single unit and local field potential data from 14 pediatric patients with generalized dystonia implanted with up to 100 microelectrodes and 60 macroelectrodes (Ad-Tech MM16C) distributed in multiple brain regions, including STN, GPi, Vo, VIM, and VPL. EMG was recorded from arm and leg muscles. Data were collected during one week, in which the patients performed different reaching tasks. Mutual information between single units and dystonic EMG was quantified and compared across the different brain areas. 9Hz stimulation was applied systematically to all implanted nuclei. Deep brain and cortical evoked potentials were recorded and analyzed to infer connectivity between the implanted regions. Permanent DBS targets were selected based on clinical improvement in motor symptoms with no negative side effects during high frequency stimulation. Our results show low firing rate neurons correlating with dystonic EMG. Mean firing rates of 5Hz or lower were found in all recording sites. Fast firing rate neurons >20Hz were poorly correlated with EMG. Deep brain evoked potentials were not present in all projected areas from the stimulation site, showing an inconsistent connectivity pattern with respect to the expected anatomical connections between the implanted areas, likely due to differences in the etiology and anatomical distribution of injury in each patient. Finally, the permanent implantation target varied between children, and in general, stimulation that ameliorate dystonic symptoms corresponded to nuclei in which neuronal activity was highly correlated with dystonic EMG and those that were able to propagate evoked potentials through the BG-thalamocortical loop. We hypothesize that nuclei with the highest relationship with EMG are those with dominant effect on the abnormal dynamic in the BG-thalamocortical circuit, given that dystonic EMG is associated with abnormal posture, unwanted movement, and overflow. Moreover, DBS is most effective when it can propagate through the circuit.

Disclosures: E. Arguelles: None. **M. Abolfath-Beygi:** None. **R. Deshpande:** None. **D. Ferman:** None. **T.D. Sanger:** None.

Poster

378. Dystonia, Tremor, and Other Movement Disorders

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 378.12/I9

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Intracranial microelectrode recordings during deep brain stimulation correlate with sensory nerve evoked potentials to facilitate targeting

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Abstract: Deep brain stimulation (DBS) of the ventral intermediate nucleus (VIM) has been an effective treatment of the motor symptoms in movement disorders such as dystonia and essential tremor (ET). Recently, chronic peripheral nerve stimulation (PNS) has been demonstrated to be a non-invasive alternative for a subpopulation of patients with ET. In this study, we explore the effects of low frequency PNS in basal ganglia and thalamic nuclei. We present sensory evoked potential (SEP) data from 14 pediatric patients with generalized dystonia recorded from depth electrodes implanted in VIM, subthalamic nucleus (STN), ventral oral (Vo) and ventral posterolateral (VPL) nuclei. Patients were implanted temporarily with 8 or 10 Ad-Tech MM16C depth electrodes to simultaneously record single neuron activity and Local Field Potentials (LFP) from 80 or 100 micro contacts respectively. PNS at 9Hz was delivered using a standard clinical system (Xltek) to the median nerve. Artifact trigger average responses were computed and further analyzed. The results were contrasted with postoperative CT scans to confirm the location of the electrodes. The patients underwent the final DBS implants after this procedure, after a clinical decision was made taking into account this analysis and observed clinical benefits in the patients.
Our results show consistent SEPs in afferent sensory thalamic nuclei VIM and VPL, with smaller amplitudes in Vo. SEPs in STN were inconsistent or absent. These results are in consensus with studies in human SEPs done in the past using macro electrode recordings, and have helped to establish a relation between macroscopic SEP and cellular micro electrode recordings.High frequency LFPs overlapped in time with low frequency SEPs, and distinct peaks in frequency were observed at 1 kHz and 60Hz, after finding the Power Spectral Density (PSD) of the observed evoked response.
This method has low demands in terms of the necessary equipment. It can help in identifying the VIM as a target for DBS tremor suppression. It is also capable of distinguishing electrode locations in VIM, STN and Vo and can thus be used as an easy and reliable tool to assess the actual location of the depth electrodes with respect to the intended targets. The electrode locations can be corrected during the final implant surgery.

Disclosures: R. Deshpande: None. **E. Arguelles:** None. **D. Ferman:** None. **T.D. Sanger:** None.

Poster

378. Dystonia, Tremor, and Other Movement Disorders

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 378.13/I10

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant R01NS073872
NIH Grant R21NS098930

Title: Functional studies of the candidate gene, CACNA1G identified in essential tremor families

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Abstract: Essential tremor (ET) is one of the most common neurological disorders. The primary motor feature of ET is a 4 -12 Hz kinetic tremor (i.e., tremor that is seen during voluntary movements such as eating or writing), which occurs in the arms and hands. Non-motor features can also include cognitive deficits, dementia and psychiatric manifestations. Emerging evidence suggests that ET is a disease or family of diseases of the cerebellum. The etiology of ET remains largely unexplained; however, genetic factors are thought to strongly contribute to the etiology of ET. Using a whole genome sequencing (WGS) approach, we identified mutations in *CACNA1G*, which encodes the T-type low-voltage activated calcium channel, $Ca_V3.1$, in three early-onset ET families (FASET; R01NS073872). $Ca_V3.1$ is expressed in various motor pathways and has been previously implicated in neuronal autorhythmicity and ET. High expression of $Ca_V3.1$ in Purkinje neurons and its active involvement in producing tremors characteristic of ET, make it a promising candidate gene. Electrophysiology studies by whole cell patch clamp recordings were performed in HEK293 cells expressing the $Ca_V3.1$ mutant channels. Significant differences in the gating of mutant $Ca_V3.1$ channels were observed compared to the wildtype channel. Although the effects on activation and inactivation kinetics were subtle small changes, a shift of steady state inactivation towards more positive voltages (+3.4mV) was observed for one of the mutations analyzed (p.Arg1235Gln) ($p<0.05$) . A shift of activation toward positive voltages has also been reported for the $Ca_V3.1$ p.Arg1715His mutation identified in a family with SCA42 (Coutelier et al., 2015). Computer simulation modeling for the $Ca_V3.1$ p.Arg1235Gln mutant channel or $Ca_V3.1$ p.Arg1715His mutation suggests that both mutations result in decreased neuronal excitability. To complement these studies, immunocytochemical studies are currently being performed in order to examine the possible changes in subcellular localization caused by each mutation. In summary, functional studies of the *CACNA1G* mutations identified in ET families suggest that *CACNA1G* is an ET gene and that ET may represent a family of disorders of neurological channelopathies. Further evaluation of ion channels as candidate genes for ET is warranted.

Disclosures: S. Sonti: None. **Z. Odgerel:** None. **E. Louis:** None. **L. Clark:** None.

Poster

378. Dystonia, Tremor, and Other Movement Disorders

Location: SDCC Halls B-H

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Program #/Poster #: 378.14/I11

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: MnDrive Graduate Fellowship in Neuromodulation
NIH Grant 5R01NS081118-03

Title: Behavioral and electrophysiological characterization of harmaline-tremor in non-human primates

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Abstract: Many of the recent advancements in deep brain stimulation (DBS) technology have leveraged the preclinical, MPTP-treated non-human primate model of Parkinson's disease. Preclinical animal models of Essential Tremor (ET) that display action and postural tremors have received much less attention. Studies from the early 1970s in non-human primates have indicated that administration of the MAO-inhibitor drug, harmaline, can induce transient 6-12 Hz action and postural tremors, which are broadly comparable to those in individuals with ET. What remains unclear for this preclinical animal model are: (1) the behavioral time-course of harmaline tremors, (2) whether the effects of harmaline habituate over time as reported for multi-dose harmaline-exposure in rodents, and (3) the electrophysiological responses to harmaline treatment in non-human primates. In this study, two non-human primates were treated with systemic, intramuscular harmaline in dosages ranging from 8 mg/kg to 12 mg/kg, while measuring limb tremor with a tri-axial accelerometer and while recording local field potentials (LFPs) from a chronically implanted DBS lead in the cerebellar-receiving area of motor thalamus. In comparison to the pre-drug state, administration of harmaline caused a broad increase in kinematic tremor power between 1-25 Hz and more focal spectral peaks centered at 13Hz in one subject. Thalamic LFPs also exhibited an increase in oscillatory activity between 5-10Hz, which was associated with the onset and duration of the harmaline-induced tremors. Consistent with previous reports, harmaline-induced tremor is a transient phenomenon with tremors subsiding approximately 2 hours after dosing. Additionally, these effects were found to be repeatable across multiple sessions over several weeks. The non-human primate model of action and postural tremor using systemic harmaline is likely to be a useful preclinical animal model for further development of novel neuromodulation technologies.

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Poster

378. Dystonia, Tremor, and Other Movement Disorders

Location: SDCC Halls B-H

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Program #/Poster #: 378.15/I12

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Measuring tremor continuously and in real-world contexts using a wearable device during an essential tremor clinical trial

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Abstract: Essential tremor is a common movement disorder characterized by tremor, most commonly in the hand, during a voluntary movement or maintenance of a posture. The current standard for measuring tremor severity in clinical trials is clinical assessments, which has its inherent limitations of requiring clinic visits and infrequent sampling. Wearable devices, on the other hand, could provide significant additional value by measuring tremor continuously, both inside and outside of the clinic. Here, we present data from an open-label Phase 2a clinical trial evaluating the effects of SAGE-217, a GABA-A positive allosteric modulator, in patients with essential tremor. The objective of this analysis is to compare tremor measurements from a wearable wristband device (E4 wristband, Empatica, Milano) against clinical scales, and to determine whether the device can detect pharmacological modulation of tremor. At regular time intervals prior to and following SAGE-217 administration, subjects were assessed in the clinic with the TETRAS clinical scale and with a finger-worn accelerometer (Kinesia ONE, Great Lakes NeuroTechnologies, Cleveland) worn during defined tasks. The Empatica wristband device was continuously worn by patients during the trial and measurements from the device were captured both inside and outside of the clinic. In the majority of subjects, an oscillating signal within the expected tremor-frequency range (3-8 Hz) was clearly measurable from the wristband device. Custom algorithms were developed to derive a continuous, time-varying tremor score from the device. Across subjects, the mean wristband-derived tremor score over the trial correlated with both the TETRAS score ($r=0.68$, $p<0.01$) and the Kinesia score ($r=0.86$, $p<0.0001$). Treatment with SAGE-217 resulted in a reduction of tremor that was measurable based on the TETRAS, Kinesia, and from the Empatica wristband device. Additionally, treatment effects of SAGE-217 and their time course were also measurable from patients wearing the device outside of the clinic. These results support the notion that a wearable-device may be able to accurately and continuously measure tremor in clinical trial settings and could provide additional value by measuring tremor in real-world situations outside of clinical assessment windows.

Disclosures: **B.J. Farley:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **A.E. Bullock:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **I. Kaul:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **D.P. Nguyen:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **S.J. Kanes:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **J.J. Doherty:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **M.C. Quirk:** A. Employment/Salary (full or part-time):; Sage Therapeutics.

Poster

378. Dystonia, Tremor, and Other Movement Disorders

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 378.16/I13

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH R21 NS096258

Title: Deep brain stimulation in essential tremor: Tremor and dysmetria in the upper and lower limb

Authors: *A. CASAMENTO MORAN, S. DELMAS, S. L. BRACKSIECK, B. YACOUBI, M. S. OKUN, A. W. SHUKLA, D. E. VAILLANCOURT, E. A. CHRISTOU
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Abstract: Essential tremor (ET) is one of the most common movement disorders in humans. The hallmark symptom of ET is a 4 to 8 Hz action tremor of the upper limbs. Deep brain stimulation (DBS) can reduce tremor amplitude and improve quality of life. However, it remains unknown whether DBS improves tremor and accuracy of goal-directed movements with the upper and lower limb. Here, we test whether DBS reduces upper and lower limb tremor and dysmetria (movement error) in ET patients. Nineteen ET patients treated with DBS (70.1 ± 8.1, 7 women) and 10 healthy (HC) age-matched controls (68.0 ± 6.2, 8 women) performed ballistic goal-directed movements with the wrist and ankle joint. ET performed the session twice, once with DBS on and once with DBS off (counterbalanced order). We quantified the tremor and dysmetria of the ballistic goal-directed movements. When DBS was switched off, ET exhibited greater tremor and dysmetria than healthy controls for both the wrist and ankle joints (all $P<0.05$). Turning on the DBS, reduced the tremor amplitude of the wrist ($P=0.04$) but not the tremor of the ankle *($P = 0.66$)* during ballistic goal-directed movements. In contrast, DBS did not reduce dysmetria for neither the wrist *($P = 0.3$)* nor ankle *($P = 0.08$)*. Our findings suggest that although DBS can reduce the tremor of the upper limb, it has no significant effect on lower limb tremor or upper and lower limb dysmetria.

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Poster

378. Dystonia, Tremor, and Other Movement Disorders

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 378.17/I14

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Towards a wearable sensor driven closed-loop deep brain stimulation paradigm for Tourette syndrome

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Abstract: Introduction Tourette syndrome (TS) is a neuropsychiatric disorder characterized by a combination of involuntary motor and vocal tics with symptoms potentially worsening into adulthood. Deep brain stimulation (DBS) has emerged as a potential treatment option for medically refractory TS. We hypothesize that DBS delivered in response to pathology can ameliorate symptoms and provide additional benefits, such as a longer battery life of the device and a reduction in side effects of prolonged stimulation. Herein, we demonstrate that external wearable sensors can provide a reliable control signal for closed-loop DBS. Additionally, we show that there are underlying differences within the muscle signal between voluntary and pathological movements. **Method** Data were collected from 3 TS subjects during routine monthly programming visits for DBS at the University of Florida Health Shands Hospital. During the visits, external wearable sensors recorded electromyography (EMG) and acceleration from muscles most afflicted by the subjects' tics. The subjects were instructed to perform a series of tasks that included mimicking their tics, volitional movements, or freely ticing. Each recording lasted between 3-4 minutes with intermittent periods of rest. Videos were concurrently recorded with wearable sensor data and labeled for movement onset by a movement disorder neurologist. Data were collected when DBS was off to minimize tic suppression. To align the data with movement onset, a trigger was sent and picked up by both the video and the wearable sensors. **Results** Frequency analysis demonstrated statistically significant differences in several EMG power bands, which were noted by computing the p-values (false-discovery rate corrected) between each type of movement for each feature band within each sensor. The top five significant features for both the left and right side of the body were corroborated using Receiving Operating Characteristic curves and inputted into a linear discriminant analysis classifier. Specificity (65-95%) and sensitivity (80-95%) were above chance level for each respective subject and side, suggesting that EMG characteristics can successfully distinguish between a tic and a volitional movement. Other analyses, such as cross-correlation, display significant differences in the delay of muscles during the two types of movements. This work has vast implications on the future of closed-loop DBS in not only TS, but also other movement disorders, such as Essential tremor. Overall, this research will lead to both a deeper

understanding of the effects of DBS on muscular physiology and a broader understanding of brain-muscle connectivity.

Disclosures: J. Cagle: None. **W. Deeb:** None. **M. Okun:** None. **A. Gunduz:** None.

Poster

378. Dystonia, Tremor, and Other Movement Disorders

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 378.18/I15

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH R01 NS096008
NSF CAREER 1553482

Title: Electrophsiology-guided deep brain stimulation targeting in tourette syndrome complements anatomical targets for improved outcomes

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Abstract: Tourette syndrome (TS) is a continuous lifelong condition that is highly prevalent and socially embarrassing. Although motor and vocal tics usually wane by the late teenage years, some individuals require medical or behavioral interventions. However, these may be ineffective and TS may persist into adulthood. Deep brain stimulation (DBS) has emerged as a promising treatment option for these tic conditions. In our patient cohort, two 4-contact macroelectrodes are placed during DBS surgery in the centromedian-parafascicular thalamic (CM-Pf) region bilaterally, which is known to suppress tic activity in TS patients. However, due to different imaging techniques and clinician preferences, the final target locations vary from patient to patient. In a previous study we identified neural correlates associate with tic onset in patients chronically implanted with Medtronic Activa PC+S neurostimulator, a highly novel next generation technology that can record brain activity while stimulation. Herein, we identify the anatomical correlates of Tourette syndrome using both imaging techniques and neurophysiological recordings from DBS electrodes for the better understanding of TS and improved targeting in future DBS interventions. To date, 6 TS patients are enrolled in the study, each having received bilateral Activa PC+S implants for chronic neural recordings. Tic features, a low frequency power in the theta-alpha ranges, is computed with respect to tic onset. Pre-operative T1 magnetic resonance imaging (MRI), inverted-T1 MRI, and post-operative computed tomography (CT) were obtained for each patient. The post-operative CT and pre-operative inverted-T1 MRI were co-registered to T1 MRI and the lead locations were identified.

Our results show that standard AC-PC coordinates do not reveal accurate lead placement due to the difference in brain size and neural anatomy from patient to patient. Both nonlinear MNI transformation and reverse linear atlas transformation shows similar lead locations with target location in anterior CM-Pf. In addition, for functional recordings, we identified one patient with an absence of tic features when initially implanted. After 4 months of treatment, the patient continued to experience less than 10% improvement in his tic severity. A review of his anatomical location showed deviated lead location. After replacement of this lead, we were able to identify the tic features and his tic improvement is on par with other patients. Therefore, with the identification of tic correlates functional recording may confirm target location after anatomical localization and intraoperative recordings with the DBS leads may improve DBS outcomes.

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Poster

378. Dystonia, Tremor, and Other Movement Disorders

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Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Creative-Pioneering Researchers Program through Seoul National University (SNU) Ministry of Education of the Republic of Korea and the National Research Foundation of Korea (NRF-2016S1A5A8020309)

Title: Changes in joint coordination during gait in individuals with cerebral palsy

Authors: *J. PARK, P. PATHAK, K. KIM, D. XU
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Abstract: Motor dysfunction present in cerebral palsy (CP) patients has deemed to be the origin of instability in gait mechanics thereby increasing the risk of fall. Abnormal coordination of multiple joints with CP is often observed. Thus, proper organization of a redundant set of joints is critical to ensure the gait stability by stabilizing salient performance variables such as whole body center-of-mass (COM), head position, etc. Hence, the purpose of the current study was to examine the difference in COM and head stabilizing synergy during a gait cycle between CP patients and control subjects. 7 diplegic CP (GMFCS level II) patients and 10 age-& gender-matched controls were recruited. The subjects were asked to perform 3 trials with 4 strides per trial (12 cycles in total) walking at self-paced moderate speed and each cycle was normalized to 100%. The geometrical models included 11 & 5 sagittal plane segmental angles set as elemental variables for COM and head, respectively, whereas the sagittal plane coordinates of COM and

head as performance variables. The framework of uncontrolled manifold (UCM) analysis was utilized to quantify the indices of synergy (ΔV) for stabilization of the COM and head. The synergy indices in time-series were then divided into four phases and averaged across subjects: 1) double stance to left foot mid-swing, 2) left foot mid-swing to double stance, 3) double stance to the right foot mid swing & 4) right foot mid-swing to double stance. For the control group, there was a rise (i.e., stabilization) and drop (i.e., destabilization) in the ΔV trajectory with the transition from stance to swing phase. However, CP patients showed no systematic changes of ΔV in the sagittal plane for both COM and head stabilization. For phases divided dataset, a two-way ANOVA supported these findings setting factors as Group (2 levels: Control & CP) and PV (2 levels: COM & Head) for each phase separately. For phases 1 & 3, ΔV for control subject was larger than that for CP ($p<0.05$), and there was no difference between ΔV of COM and head for both groups. For phase 2, ΔV for control subject was larger than for CP ($p<0.05$), and ΔV of COM was larger than ΔV of the head ($p<0.05$). For phase 3, there were no significant differences between groups & PVs. Finally, there was no significant interaction of factors for all phases. These results imply that the ability to modulate co-variation patterns (e.g., destabilization for swing phase to stabilization for stance phase) among the segmental angles to stabilize the COM and head position during moderate speed walking were impaired in the individuals with CP, which may be caused by spastic contraction of a group of muscles within lower extremities.

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Poster

378. Dystonia, Tremor, and Other Movement Disorders

Location: SDCC Halls B-H

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Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH NS054124
NIH NS97484
Swartz Foundation

Title: EEG study on chronic tic disorder patients

Authors: *M. MIYAKOSHI[1], K. TUNG[2], E. LLOYD[2], S. MAKEIG[1], S. LOO[2]
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Abstract: Measuring ongoing brain activity underlying chronic tic disorder (CTD) is difficult. Here, we applied the mobile brain-body imaging (MoBI) concept to collect data from 45 (8-12 yr) children (26 CTD, 19 Healthy Controls). EEG data for five children were unusable, leaving data for 40 children (23 CTD, 17 HC) in three conditions: Tic Freely, Suppress Tics without

reward, and Suppress Tics with reward in 5-min blocks embedded in 40-50 min recording sessions. EEG signals were recorded from 128 EGI hydrocel electrode channels. During Suppress Tics with and without reward, patients used a custom joystick to express their current intensity of tic urge. The EEG data for each subject in all three conditions were concatenated and processed using custom EEGLAB scripts. For the CTD subgroup, data cleaning retained 47.2 (SD 29.5, range 13-117) trials. Independent component analysis (ICA) decomposition was performed on data from each subject. Resulting independent components (ICs) were selected for group-level analysis if their equivalent dipole model was within the MNI template brain volume and the residual variance of their scalp map from the equivalent dipole projection was <=20%. The resulting 631 ICs from 20 CDT subjects were clustered based on equivalent dipole location into 15 clusters. IC tic event-related spectral perturbations (IC ERSPs) were computed, normalized, and collapsed into standard frequency bands. Non-zero ERSP band deviations were detected using one-sample t-tests with FDR. More non-zero time points were pre-tic than post-tic. Two frontal IC clusters exhibited pre-tic theta/alpha decreases, while several centro-parietal clusters had pre-tic beta/gamma decreases.were clustered based on equivalent dipole location into 15 clusters. IC tic event-related spectral perturbations (IC ERSPs) were computed, normalized, and collapsed into standard frequency bands. Non-zero ERSP band deviations were detected using one-sample t-tests with FDR. More non-zero time points were pre-tic than post-tic. Two frontal IC clusters exhibited pre-tic theta/alpha decreases, while several centro-parietal clusters had pre-tic beta/gamma decreases.

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Poster

378. Dystonia, Tremor, and Other Movement Disorders

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Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: National Natural Science Foundation of China 81130021
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Title: PRRT2 mutations lead to loss of function in the pathogenesis of paroxysmal kinesigenic dyskinesia

Authors: *Y. PAN[1], Q. LIU[2], Y. YANG[3], X. LI[3], S.-H. LI[4]
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Abstract: PKD (Paroxysmal Kinesigenic Dyskinesia) is a rare autosomal dominant inherited paroxysmal movement disorder, which is characterized by recurrent, brief attacks of abnormal involuntary movements induced by sudden voluntary movements. Our previously study had identified that mutations in the PRRT2 (proline-rich transmembrane protein 2) gene are associated with PKD and that the c.649dupC missense mutation is the major causative mutation. Since heterozygous mutations of PRRT2 in patients cause symptoms and may create truncated PRRT2, it remains unknown whether PRRT2 pathogenesis involves the gain of toxicity of mutant PRRT2. By generating PRRT2 KO and KI mice that express the hotspot c.649dupC mutation and by comparing WT and truncated PRRT2 resulted from the c.649dupC mutation, we have found that this mutation generates a truncated PRRT2 that is unstable and degraded rapidly. Furthermore, PRRT2 KO and KI mice are indistinguishable for their behaviors. Our studies demonstrate that PRRT2 mutations lead to the loss of function in the disease and suggest that its treatment requires the recovery of the normal function of PRRT2.

Disclosures: Y. Pan: None. **Q. Liu:** None. **Y. Yang:** None. **X. Li:** None. **S. Li:** None.

Poster

378. Dystonia, Tremor, and Other Movement Disorders

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Program #/Poster #: 378.22/J2

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Leblang Charitable Foundation

Title: Tracking ABCD1 expression using HA-tagged ABCD1 following intrathecal AAV9 delivery

Authors: *Y. GONG[1], A. BERENSON[1], F. LAHEJI[1], A. VOLAK[2], C. MAGUIRE[2], F. EICHLER[3]
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Abstract: Mutations in the gene encoding the peroxisomal ATP binding cassette transporter (ABCD1) cause elevations in very long chain fatty acids (VLCFA) and the neurodegenerative disease adrenoleukodystrophy. Previously we showed that gene correction in the Abcd1-/- mouse after intrathecal delivery of recombinant AAV9 encoding hABCD1 under the broadly active CBA promoter led to a 30% decrease of VLCFA levels in spinal cord. However, this gene and lipid correction was not achieved in dorsal root ganglia (DRG). To optimize detection of ABCD1 and better understand its biodistribution, we developed a method using hemagglutinin (HA)-tagged ABCD1 to track ABCD1 following AAV9 delivery. A C-terminal HA-tagged human ABCD1 cDNA was cloned into pAAV-CBA vector and packaged into rAAV9 in 293T cells.

When we treated 293T cells with AAV9-ABCD1-HA ($1X10^4$gc/cell and $1X10^5$gc/cell), there was a dose-dependent increase of ABCD1 protein expression using an anti-ABCD1 antibody; meanwhile, a strong signal was also detected using the HA-tag antibody, suggesting successful tagging of the ABCD1 protein. To confirm the localization of ABCD1 with HA-tag *in vitro*, we assessed mixed brain glial cells from postnatal mice treated with AAV9-ABCD1-HA at $5X10^5$gc/cell for 5 days. Immunostaining demonstrated exact co-localization of ABCD1 with the HA-tag. To corroborate the feasibility of using AAV9-ABCD1-HA to track ABCD1 *in vivo*, we carried out intrathecal delivery of AAV9-ABCD1-HA via an osmotic pump (IT pump). Tissues were harvested after 15 days of injection and then fixed for immunostaining using an HA-tag antibody. The results showed that delivery led to extensive expression of ABCD1-HA across different cell types in the spinal cord but was largely limited to neurons in DRGs.. To assess for functionality after AAV9-hABCD1 delivery, we measured VLCFA across different cell types *in vitro*. To our surprise, we could correct VLCFA levels in mixed brain glial cells but not in isolated DRG satellite cells. Cells treated with HA-tagged-ABCD1 were also collected for VLCFA analysis. In conclusion, AAV9-ABCD1-HA provides a more reliable way for tracking ABCD1 distribution after *in vivo* delivery. Compared with the less reliable ABCD1 antibody staining, HA-tag staining provides higher specificity and demonstrates that AAV9-ABCD1-HA localizes mainly to neurons in DRG. This result was corroborated with an AAV9 vector encoding GFP. Studies are pending to assess whether the HA tag still allows ABCD1 activity. These results may provide insight into our observation that we did not achieve VLCFA correction in DRG after AAV9-ABCD1 delivery, as the proper cell type was not transduced.

Disclosures: Y. Gong: None. **A. Berenson:** None. **F. Laheji:** None. **A. Volak:** None. **C. Maguire:** None. **F. Eichler:** None.

Poster

378. Dystonia, Tremor, and Other Movement Disorders

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 378.23/DP04/J3

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Gordon and Betty Moore Foundation
Alfred P. Sloan Foundation

Title: Multidimensional analysis and detection of informative features in diffusion MRI measurements of human white matter

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Abstract: The white matter contains long-range connections between different brain regions. Tractometry uses diffusion-weighted magnetic resonance imaging (dMRI) data to quantify tissue properties along the trajectories of these connections in the human brain in vivo (Yeatman et al. 2012). In previous work, the results of tractometry were usually analyzed using mass univariate approaches: group comparisons or regression models computed separately for each point along every one of the tracts. Alternatively, tissue properties such as fractional anisotropy (FA) and mean diffusivity (MD) were computed for a specific tract of interest based on an a priori hypothesis. In the present work, we developed a method based on the sparse group lasso (Simon et al., 2013) that takes into account all of the tissue properties measured in all of the tracts, and selects informative features by enforcing sparsity, both at the level of individual tracts and tissue properties, but also across the entire set of tracts and all of the measured tissue properties. The sparsity penalties for each of these constraints is identified using a nested cross-validation scheme. Using data from a previous study that measured dMRI in patients with amyotrophic lateral sclerosis (ALS) and matched controls (Sarica et al. 2017), we demonstrate that this method is accurate, exceeding the previous results, that were based on a priori feature selection, in classifying patients and controls, with ~84% accuracy, an area under the receiver operating characteristic curve of 0.93, and an average precision of 0.95. Moreover, our method automatically identifies as important for this classification the parts of the white matter known to be affected by ALS within the corticospinal tract. In a regression setting, data from another previous study (Yeatman et al. 2014) can be used to accurately predict “brain age”. Thus, this multivariate analysis approach both (a) achieves high cross-validated accuracy for precision medicine applications of dMRI data and (b) identifies relevant features of brain anatomy to further our neuroscientific understanding of clinical disorders.

Disclosures: A. Richie-Halford: None. **J.D. Yeatman:** None. **N. Simon:** None. **A.S. Rokem:** None.

Poster

378. Dystonia, Tremor, and Other Movement Disorders

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 378.24/J4

Topic: E.07. Rhythmic Motor Pattern Generation

Support: ALS reseach grant from JALSA(Japan ALS Association)

Title: Time-varying phase synchronization network within sensorimotor area in a patient with amyotrophic lateral sclerosis

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Abstract: [Object/Background] Phase synchronization in oscillatory brain signals have been considered as a possible mechanism of functional integration and information transmission between different brain regions. However, local functional network within sensorimotor cortex have not been fully elucidated. The purpose of this study is to reveal phase synchronization network within sensorimotor area in a patient with amyotrophic lateral sclerosis. **[Methods]** In this study, we retrospectively analyzed the ECoG data obtained in our past clinical research carried out at Osaka University Hospital in 2013. One male patient with advanced-stage amyotrophic lateral sclerosis (ALS) participated in the research and clear written informed consent was obtained from the participant. The clinical research was approved by the Ethics Committee in Osaka University Hospital and the experimental protocol was carried out in accordance with the Declaration of Helsinki. ECoG data was recorded using custom-made 94ch high density ECoG electrodes which were designed to be located on the four anatomical regions within sensorimotor area on the left hemisphere: hand motor area (M1 hand), primary motor area other than hand motor area (M1), premotor area (PM), primary sensory area (S1). Phase synchronization network was assessed by two commonly used phase synchronization measures, inter-site phase clustering ISPC (which have originally termed as phase locking value PLV) and phase lag index PLI between all possible inter-electrode pairs (${}_{94}C_2 = 4371$) using morlet wavelet analysis with the center frequencies in alpha, beta, low and high gamma band range at every time-points around the onset of motor imagery task. **[Results]** Phase synchronization based all to all inter-electrode connectivity matrixes and time course of phase synchronization in subnetworks were examined. Grouping of electrodes based on the anatomical positions and the results of phase synchronization based connectivity between electrodes are highly corresponding to each other. Among the networks, some inter-regional synchronization showed characteristic increase and decrease around the timing of motor intention, whereas most of the intra-regional synchronization was relatively constant throughout the period. These findings are mostly apparent in beta frequency range and not obvious in other frequency band. **[Conclusion]** The present high-density micro ECoG study revealed time-varying transitions of phase synchronization network within sensorimotor area in a patient with ALS. Characteristic change around the timing of motor intention was obsearved in some inter-regional synchronization.

Disclosures: Y. Fujimoto: None. **M. Hirata:** None.

Poster

379. ALS Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 379.01/J5

Topic: C.06. Neuromuscular Diseases

Support: VA Merit grant BX003625 to SD

Title: Mapping neuron- and glia-specific non-coding gene regulatory elements in motor cortex of ALS patients

Authors: *M. K. JAISWAL[1,2], P. APONTES[2], A. KOZLENKOV[1,2], W. BYNE[1,2], R. SATTLER[3], V. BELZIL[4], S. DRACHEVA[1,2]
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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by degeneration of neurons in the motor cortex (MCX), brain stem and spinal cord. About 90% of ALS cases occur sporadically with unknown genetic etiology, while the rest of cases are linked to genetic mutations mostly associated with familial ALS. We posit that vulnerability to ALS is rooted in a complex interaction between genetic mutations, genetic risk factors, and environmental insults. The latter have the potential to modify the genetic risk to ALS by affecting epigenetic modifications, thus causing dysregulation of gene expression networks and ultimately resulting in ALS-specific cellular pathophysiology. The goal of our work is to examine epigenetic signatures in MCX of ALS decedents in order to elucidate epigenetic correlates that may represent risk factors for ALS.
Epigenetic modulation occurs primarily at the level of the non-coding gene regulatory elements (GREs), such as gene promoters and enhancers, the latter being the most numerous GREs in the human genome. Although the position of enhancers cannot be easily inferred, both active promoters and enhancers are characterized by Histone 3 Lysine 27 acetylation (H3K27ac) modifications. Whereas considerable information exists for the functional epigenome in homogenate (i.e., cellularly heterogeneous) brain tissues from ALS decedents, the multicellular complexity of the brain precludes reliable annotation of cell-type-specific epigenomes from existing data sets. Therefore, cell-type-specific variations are likely to be missed in preparations of mixed cell types. Here we examined the epigenetic signatures of two major populations of brain cells (neurons and glia) in the MCX of ALS and control subjects, thereby significantly increasing the likelihood of detecting epigenetic correlates specific for ALS.
We applied fluorescence-activated nuclear sorting (FANS) with Alexa488-conjugated anti-NeuN antibodies and DNA-binding dye 7-AAD to separate neuronal and glia nuclei from diseased cases and controls. We then mapped neuron- and glia-specific GREs employing low-input native ChIP-seq assay for H3K27ac. The specificity of ChIP was confirmed using qPCR for sequences with known enrichment for H3K27ac and negative controls. Libraries were generated using NEBNext Ultra DNA Library Prep, KAPA-quantified, and sequenced using PE50 protocol and HiSeq 2500 instrument**.** ChIP-seq data analysis is currently in progress. This study will, for the first time, provide information on ALS-related epigenetic alterations unique to individual cell types.

Disclosures: M.K. Jaiswal: None. **P. Apontes:** None. **A. Kozlenkov:** None. **W. Byne:** None. **R. Sattler:** None. **V. Belzil:** None. **S. Dracheva:** None.

Poster

379. ALS Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 379.02/J6

Topic: C.06. Neuromuscular Diseases

Support: MRC/MNDA CSF to PF
4 Year studentship funded by the Leonard Wolfson Experimental Neurology Centre (Wolfson Foundation)

Title: The stress granule response of wild-type and ALS FUS-mutant neurons to somatic and axonal stressors

Authors: *M. P. BENTHAM, N. BIRSA, C. BODO, C. MADURO, A. DEVOY, E. M. FISHER, G. SCHIAVO, P. FRATTA
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Abstract: Stress granules (SGs) are cytoplasmic ribonucleoprotein aggregates which form in response to cellular stress and disassemble following the cessation of stress. Mutations in human SG proteins cause the neurodegenerative disease amyotrophic lateral sclerosis (ALS) and have been demonstrated to alter granule dynamics. Further, SGs have a compositional protein overlap with the post-mortem cytoplasmic inclusions that characterise ALS, suggesting that SGs may act as a precursors to, or seed, these inclusions.
We cultured primary neurons in compartmentalised devices, applying the oxidative stressor sodium arsenite to either the somal or axonal compartment, to investigate the kinetics of SG formation. We observed a delayed SG assembly response in neuronal somas when arsenite was applied axonally,
Further, we investigated the effect of ALS-causing mutations on the SG response, using a novel FUS-mutant mouse model. This model expresses a humanised C-terminal disease-causing mutation that results in a frameshifted amino acid sequence downstream of the mutation, eradicating the nuclear localisation signal of the FUS protein. This frameshift sequence allowed for the generation of antibodies able to distinguish between wild-type and mutant FUS. Using these antibodies, we observed that the normally predominately nuclear FUS protein significantly mis-localised to the cytoplasm in neurons heterozygous and homozygous for the mutation. Additionally, we demonstrated that the mutant protein is present in SGs at a significantly higher level than the wild-type protein.
As FUS binds a number of the other RNA-binding proteins present within SGs, we investigated the effect of this mutation on other granule components in wild-type, heterozygous and homozygous neurons and fibroblasts.

Our results demonstrate the ability of neuronal axons to respond to exogeneous oxidative stressors and highlight the importance of the SG response in ALS-mutant cells.

Disclosures: M.P. Bentham: None. **N. Birsa:** None. **C. Bodo:** None. **C. Maduro:** None. **A. Devoy:** None. **E.M. Fisher:** None. **G. Schiavo:** None. **P. Fratta:** None.

Poster

379. ALS Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 379.03/J7

Topic: C.06. Neuromuscular Diseases

Support: Cariplo grant (2012-0513)
JPND (DAMNPATHS)

Title: Key role of SMN/SYNCRIP and RNA-motif 7 in spinal muscular atrophy (SMA): RNASeq and motif analysis of human motor neurons

Authors: F. RIZZO[1], M. NIZZARDO[1], S. VASHISHT[2], E. MOLTENI[2], V. MELZI[1], I. FARAVELLI[1], S. SALANI[1], M. BUCCHIA[1], M. TAIANA[1], A. BORDONI[1], N. BRESOLIN[1], *G. P. COMI[3], U. POZZOLI[2], S. CORTI[1]
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Abstract: Spinal muscular atrophy (SMA) is a motor neuron (MN) disorder and remains the most common genetic cause of infant mortality. SMA is caused by mutations in the survival motor neuron 1 gene (SMN1), which impairs the function and survival of MNs in the spinal cord. The reasons for the selective vulnerability of MNs linked to SMN reduction remain unclear. Therefore, we performed deep RNA sequencing on human SMA MNs derived from patients' induced pluripotent stem cells (iPSCs) to detect specific altered gene splicing/expression and to identify the presence of a common sequence motif in these genes. Many deregulated genes, such as the neurexin and synaptotagmin families, are implicated in critical MN functions. The overexpression of *NRXN2* was shown to improve human SMA-MN survival and increase motor axon length, suggesting a role as a modifier gene. Motif-enrichment analyses of differentially expressed/spliced genes, including neurexin2 (*NRXN2*), revealed a common motif, motif 7, which is a target of SYNCRIP. Interestingly, SYNCRIP interacts only with full-length SMN, binding and modulating several MN transcripts, including SMN itself. SYNCRIP overexpression rescued SMA-MNs due to the subsequent increase in SMN and their downstream target *NRXN2* through a positive loop mechanism. SMN/SYNCRIP complex through motif 7 may account for selective MN degeneration and represent a potential therapeutic

target. This study produced novel insights into the role of SMN loss in mRNA processing and its selective effects on human SMA-MNs, highlighting novel targets for therapeutic strategies.

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Poster

379. ALS Mechanisms

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Program #/Poster #: 379.04/J8

Topic: C.06. Neuromuscular Diseases

Support: JPND networks SOPHIA (01ED1202A)
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the EU (NADINE 246513, FAIR-PARK II 633190)
the German Research Foundation/DFG (SFB1279)
the foundation of the state Baden-Württemberg (D.3830)

Title: Proteomic analysis of cerebrospinal fluid from patients with C9orf72 hexanucleotide repeat expansion

Authors: *P. BARSCHKE, P. OECKL, P. STEINACKER, A. LUDOLPH, M. OTTO
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Abstract: Aim: The hexanucleotide (GGGGCC) repeat expansion in the non-coding region of the *C9orf72* gene is the most common mutation associated with genetic amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Until now, it is unknown which factors define whether *C9orf72* mutation carriers establish ALS or FTD. Our aim is to identify protein biomarker candidates in the cerebrospinal fluid (CSF) from ALS and FTD patients with *C9orf72* mutations which differentiate between FTD and ALS and might be indicative for the outcome of the *C9orf72* mutation.
Methods: We analyzed the CSF proteome of symptomatic ALS (n=17) and symptomatic FTD (n=8) patients with *C9orf72* mutations and asymptomatic mutation carriers (CAR, n=11) by isobaric tags for relative and absolute quantitation (iTRAQ). CSF samples were digested with trypsin/LysC. After iTRAQ labeling the samples were pooled, fractionated by strong cation exchange (SCX) into six fractions and analyzed by LC-MS/MS. The MaxQuant and Perseus software were used for protein identification, quantification and data evaluation.

Results: In total, 2099 proteins were identified. By comparing the CSF proteome of ALS vs. FTD, ALS vs. CAR and FTD vs. CAR 468, 800 and 120 proteins were shown to be differentially regulated with a p-value lower than 0.05, respectively. Of these proteins 7, 17 and 2 proteins were at least two-fold up or downregulated.
Discussion and outlook: We identified an upregulation of neurofilament medium polypeptide and chitotriosidase-1 in the CSF of ALS patients compared to FTD and CAR, reflecting the neurodegeneration and neuroinflammation. However, we aim to identify new early biomarker candidates. We chose 17 promising protein candidates from our pool of differentially regulated proteins for further validation experiments. Currently targeted mass spectrometric approaches were established.

Disclosures: P. Barschke: None. **P. Oeckl:** None. **P. Steinacker:** None. **A. Ludolph:** None. **M. Otto:** None.

Poster

379. ALS Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 379.05/J9

Topic: C.06. Neuromuscular Diseases

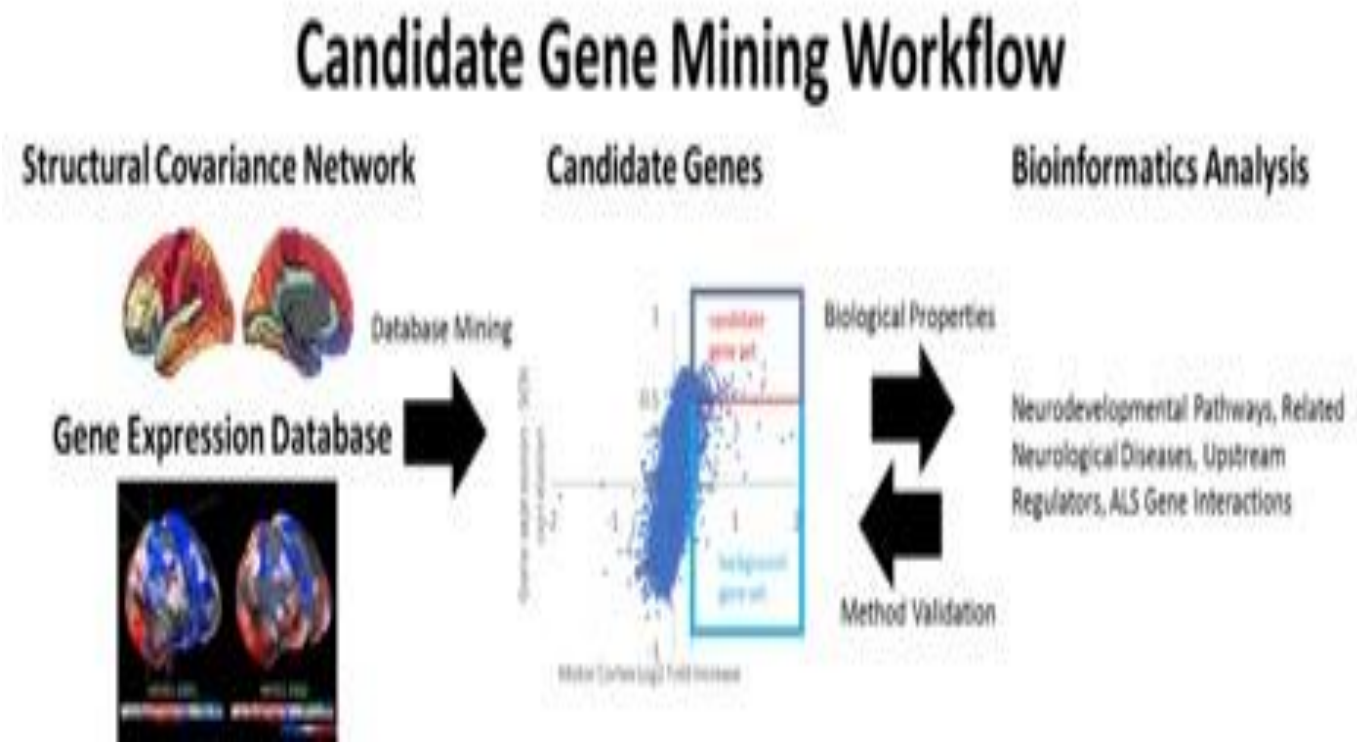
Support: Z01 NS002976

Title: An MRI-based structural covariance network approach to candidate gene discovery in motor neuron disorders

Authors: C. J. HUANG[1], *M. FLOETER[2]
[1]NINDS, NIH, Bethesda, MD; [2]NIH, Bethesda, MD

Abstract: Degeneration occurs differentially across cortical regions in amyotrophic lateral sclerosis (ALS) and primary lateral sclerosis (PLS). Atrophy and cortical thinning is greatest in the precentral gyrus, but typically affects other cortical regions. Correlated thinning between cortical regions could result from expression of common genes that render different regions more susceptible to triggers for degeneration. To examine this hypothesis, we constructed a precentral seed-based structural covariance network derived from MRIs in a cohort of healthy men and women using the Desikan-Killiany parcellations in FreeSurfer. Networks of patients with ALS and PLS were also constructed and were similar to healthy adults. The precentral network of healthy adults was used to query the gene expression database in the Allen Human Brain Atlas. A candidate set of 109 “risk factor” genes was identified that had log2 expression fold > 0.3 in the motor cortex and whose expression pattern across cortical regions was correlated with the precentral network, r>0.5. The biological functions included genes associated with excitability and synaptic function and gene expression regulation. There was no overlap of the candidate

gene set with a database of genes previously known to be associated with ALS (ALSoD). Ingenuity Pathway Analysis bioinformatic software showed that the candidate gene set was enriched in genes involved in neurodevelopmental pathways and in genes associated with neurodegenerative diseases compared to a control set of genes expressed in the motor cortex that were not correlated with the precentral network. There were numerous pathway interactions among the candidate gene set. Our MRI-based exploratory method for mining the Allen Human Brain Atlas has identified a set of genes that are candidates for further study of risk factors for neuronal vulnerability in motor neuron diseases. (supported by Intramural Program NINDS NIH)



Disclosures: C.J. Huang: None. **M. Floeter:** None.

Poster

379. ALS Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 379.06/J10

Topic: C.06. Neuromuscular Diseases

Support: The Pathological Society
British Neuropathological Society

Title: DNA methylation in amyotrophic lateral sclerosis

Authors: *C. APPLEBY-MALLINDER[1], P. R. HEATH[2], R. HIGHLEY[2]
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Abstract: Introduction Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease causing degeneration and death of motor neurones (MNs). ALS can be sporadic (sALS) or familial, with a number genes having being implicated as being pathogenic including C9orf72 (C9ALS), which causes an intronic GGGGCC repeat expansion, and TDP43. DNA methylation is an epigenetic mechanism whereby a methyl (CH3) group is attached to a cytosine, usually resulting in gene expression repression. DNA methylation has been implicated in other neurodegenerative diseases, including Fragile X syndrome, but little work has been conducted in ALS. **Research objective and rationale** Initial studies conducted to investigate the role DNA methylation plays in ALS have been conducted on heterogeneous tissue types, but no studies have been conducted on the specific interaction between MNs from native tissue and DNA methylation. By conducting experiments on MNs extracted from native post-mortem human spinal cord tissue, we aim to elucidate the role of DNA methylation in MN decline, without the interactions from other cell types, which may mask MN-specific DNA methylation changes. **Methods** 5 control, 5 sALS and 6 C9ALS samples were used (n=16). Cases were age and sex matched, with sex differences being assessed. No significant differences were observed. MNs were extracted from the anterior horn of FFPE human cervical spinal cord tissue using laser capture microdissection (LCM). DNA was then extracted, and underwent bisulphite conversion. DNA methylation analysis was conducted using the Infinium HD Methylation assay, and analysed using RnBeads (Assenov *et al.* 2014). **Results** A positive correlation between TDP pathology and global methylation was observed, with C9ALS displaying the highest global methylation and TDP pathology levels. 61 genes and 74 promoters were found to be significantly differentially methylated in control versus disease. 43 of the gene hits, and 42 of the promoter hits displayed hypermethylation when compared to controls. When comparing the gene and promoter hits, 16 were found to be significantly differentially expressed in both genes and promoters, with 10 being hypermethylated. **Conclusions** Our studies suggest DNA methylation is a contributory factor in ALS.

Disclosures: C. Appleby-Mallinder: None. **P.R. Heath:** None. **R. Highley:** None.

Poster

379. ALS Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 379.07/J11

Topic: C.06. Neuromuscular Diseases

Support: NSF Graduate Fellowship

Title: Transforming growth factor beta signaling in SOD1^{G93A} ALS

Authors: *C. BRAINE[1], D. CASTRO[2], T. MANIATIS[3], H. PHATNANI[4]
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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 this study, we use *in vitro* pharmacological and genetic approaches to manipulate different pathways within the TGF-β superfamily in co-cultured ES-derived motor neurons and astrocytes. Inhibition of the Classical and Activin arms of the pathway have differing effects on motor neuron survival. Furthermore, these effects vary depending on the ALS mutation carried by the motor neurons, suggesting that these signaling pathways play different roles in different models of ALS. To fully understand these phenotypes we put forward two objectives: 1. Characterize drug dependent changes in astrocytes and motor neurons independently; and 2. Determine how these changes are affected by the ALS mutations carried by these cells.
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 hypothesize that disease related disruption of TGF-β signaling in motor neurons contributes to their death. We will test this using cre lines or viral therapies to target two different TGF-β receptors in motor neurons or glia in the SOD1^{G93A} ALS animal model followed by gene expression profiling. These studies will help to define the role of TGF-β signaling in diseased and healthy motor neurons and begin to unravel how this pathway changes the motor neuron astrocyte relationship.

Disclosures: C. Braine: None. **D. Castro:** None. **T. Maniatis:** None. **H. Phatnani:** None.

Poster

379. ALS Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 379.08/J12

Topic: C.06. Neuromuscular Diseases

Support: NIH/NINDS 1R01NS062055

Title: Altered oxidative signaling impairs intracellular calcium dynamics in human ALS astrocytes

Authors: *V. GRANATIERO, C. KONRAD, K. BREDVIK, G. MANFREDI, H. KAWAMATA
BMRI, Weill Cornell Med. Col., New York, NY

Abstract: Amyotrophic lateral sclerosis (ALS) is an untreatable, fatal disease leading to motor neuron degeneration and muscle paralysis. ALS astrocytes play a non-cell autonomous role in motor neuron death by secreting factors that modify the neuronal environment. In human induced pluripotent stem cell-derived astrocytes carrying the G93A SOD1 familial ALS mutation, but not in isogenic controls, we demonstrate an unprecedented connection between endoplasmic reticulum (ER) oxidative protein folding, H_2O_2 signaling, calcium dynamics, and cell secretion. In mutant astrocytes, slower ER oxidative protein folding and H_2O_2 production decrease Nrf2 signaling, and downregulate ER antioxidant enzyme expression. The decrease of these enzymes enhances ER calcium pump activity, resulting in excessive ER calcium accumulation and signaling. In turn, excessive ER calcium signaling triggers activation of mitochondrial energy metabolism and enhanced secretion of small molecules, such as ATP. These findings suggest that restoring hormetic H_2O_2 signaling in astrocytes could be a target for ALS therapy.

Disclosures: V. Granatiero: None. **C. Konrad:** None. **K. Bredvik:** None. **G. Manfredi:** None. **H. Kawamata:** None.

Poster

379. ALS Mechanisms

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Program #/Poster #: 379.09/J13

Topic: C.06. Neuromuscular Diseases

Support: NINDS Funding: HHSN271200800033C

Title: The NINDS repository: Publicly available DNA and cell lines sampled from individuals diagnosed with neurological disorders

Authors: *L. SCHEINFELDT[1], S. HEIL[1], J. SANTANA[1], A. GREEN[1], A. AMBERSON[1], A. RESCH[1], R. ZHANG[2]
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Abstract: Neurological disorders present serious health concerns and challenges to health and health-related quality of life in the United States. Most neurological disorders are complex diseases involving genetic and environmental risk factors, many of which are not yet clearly

understood. The identification of reliable genetic risk markers will, therefore, contribute to preventive efforts, earlier diagnoses, disease management options, and new targeted therapeutics. To this end, The National Institute of Neurological Disorders and Stroke (NINDS) Repository offers a public resource containing DNA and lymphoblastoid cell lines with corresponding de-identified clinical and demographic data representing a diverse set of neurological disorders and neurologically normal controls. To date, NINDS Repository samples have been used in over 400 peer-reviewed scientific publications, including genome-wide association studies, case-control studies, whole-genome and whole-exome sequencing studies, structural variation studies, and candidate gene studies. The genetic and genomic data collected by these studies have been deposited in the Database of Genotypes and Phenotypes (dbGaP), a NIH/NLM sponsored repository for restricted-access data from studies investigating the contributions of genetic variation to phenotypic variation and disease (http://www.ncbi.nlm.nih.gov/gap). Since its establishment, specimens from over 39,000 individuals have been successfully banked and can be accessed through an online catalog (http://catalog.coriell.org/NINDS): individuals diagnosed with cerebrovascular diseases (N>12,100), Parkinsonism (N>5,600), motor neuron diseases (N>2,500), epilepsy (N>6,000), Tourette syndrome (N>4,100), Dystonia (N>2,600), and neurologically normal controls (N>7,400). More recently, over 2300 African American cerebrovascular disease and population control DNA samples from the REasons for Geographic and Racial Differences in Stroke (REGARDS) project were added to the NINDS Repository and are now available through the catalog to the scientific community.

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Poster

379. ALS Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 379.10/J14

Topic: C.06. Neuromuscular Diseases

Support: ALSA Grant 18-IIA-418
ALSA Grant 15-PDF-211
NIH F32 NS090831
Doctorate School of Molecular Medicine, Università degli Studi di Milano

Title: The role of the cytoskeleton in modulating nucleocytoplasmic transport in ALS

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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease of unknown etiology specifically affecting upper and lower motor neurons. Recent evidence from multiple cellular and animal models of ALS points at defects in nucleocytoplasmic transport (NCT) as central to the disease pathogenesis. NCT is a tightly regulated process that controls the cellular distribution of, among others, RNA-binding proteins (RBPs) involved in the regulation of mRNA processing. Notably, nuclear depletion of RBPs such as TDP-43 is a hallmark of ALS, suggesting a link between NCT, mRNA regulation, and ALS pathology. Here we investigate the possibility that mutations in PFN1, an actin binding protein associated with familial ALS, affect motor neuron survival via the disruption of NCT. Our data suggest that disruption of actin homeostasis due to mutant PFN1 expression alters the stability and function of the nuclear pore, leading to nuclear depletion and cytoplasmic accumulation of RBPs. Furthermore, we investigate the functional consequences of these defects in the regulation of RNA metabolism and the relevance of theses defects to ALS pathology. Collectively, our data support the hypothesis that cytoskeletal dynamics and mRNA regulation are two pathways linked through the function of NCT, and that disruption to NCT itself may play a central role in multiple forms of ALS.

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Poster

379. ALS Mechanisms

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Topic: C.06. Neuromuscular Diseases

Support: Packard Center for ALS Research
ALS 16-IIP-278
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RO1 NS085207
Barrow Neurological Institute

Title: ADAR2 mislocalization and widespread RNA editing aberrations in C9orf72 mediated ALS

Authors: *S. P. MOORE[1,2], A. STARR[1], I. LORENZINI[1], E. MENDEZ[1], J. LEVY[1], C. BURCIU[1], J. CHEW[3], V. BELZIL[3], J. ROBERTSON[4], E. ALSOP[5], L. PETRUCELLI[3], K. JENSEN[5], R. G. SATTLER[1]
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Abstract: The hexanucleotide repeat expansion GGGGCC (G4C2)n in the *C9orf72* gene is the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Recent findings indicate that dysfunction of nuclear-cytoplasmic transport of RNA binding proteins is disrupted in C9orf72 (C9). Similarly, we have identified that the RNA editing enzyme Adenosine Deaminase Acting on RNA 2 (ADAR2) is significantly mislocalized in C9orf72 repeat expansion mediated ALS. ADAR2 is responsible for adenosine (A) to Inosine (I) editing of double stranded RNA and its function has been shown to be essential for survival in ADAR2 knock out mice, which die shortly after birth. Additionally, altered ADAR2 function due to downregulation has been associated with sporadic forms of the disease. We provide evidence to suggest the mislocalization of ADAR2 in human induced pluripotent stem cell derived neurons (hiPSNs) of C9 patients, a C9 mouse model and C9 ALS post mortem tissue. To begin to understand the mislocalization specific to C9orf72 ALS, we have preliminary evidence that ADAR2 is mislocalized in the presence of the Arginine containing RAN translated dipeptides GR and PR. Surprisingly, we do not see any editing deficits at the GluA2 Q/R site in C9 cells and patient tissue. Current efforts are focused on discovering the overall impact of global A to I RNA editing deficits in C9 disease pathogenesis. Analysis of editing at over 400,000 known RNA editing sites indicates that there are vast RNA A to I editing deficits in Sporadic and C9orf72 mediated ALS. We have identified RNA editing aberrations in many pathways including the crucial EIF2 signaling pathway and the protein ubiquitination pathway. Our findings suggest that the mislocalization of ADAR2 which predominantly occurs in C9orf72 mediated ALS is responsible for the alteration of RNA processing events that may impact vast cellular functions.

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Poster

379. ALS Mechanisms

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Program #/Poster #: 379.12/K2

Topic: C.06. Neuromuscular Diseases

Support: R01NS069601
R01NS102451
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GR.10235006

Title: Dysregulation of Mdm2 and Mdm4 alternative splicing underlies motor neuron death in spinal muscular atrophy

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Abstract: Spinal muscular atrophy (SMA) is a neurodegenerative disorder caused by ubiquitous deficiency in the Survival Motor Neuron (SMN) protein. Reflecting the emerging multiple roles of SMN in RNP assembly and post-transcriptional gene regulation, disruption of several distinct SMN-dependent RNA pathways has been proposed to contribute to SMA pathogenesis. However, proving clear mechanistic links between select SMN-dependent RNA processing events and specific, clinically relevant features of SMA remains an outstanding challenge. We sought to address this issue by focusing on the molecular mechanisms of motor neuron death - the classical hallmark of SMA in both patients and mouse models. We recently demonstrated that p53 is a key driver of motor neuron degeneration in SMA mice, but the molecular mechanisms linking SMN deficiency to p53 activation and motor neuron death are unknown. Here, we demonstrate that the function of SMN in the assembly of spliceosomal snRNPs regulates alternative splicing of Mdm2 and Mdm4, two non-redundant repressors of p53. We show that decreased inclusion of critical regulatory exons of Mdm2 and Mdm4 - which is most prominent in vulnerable SMA motor neurons - acts as a key biological switch governing initiation of the p53 response in SMA mice. Furthermore, we directly link snRNP dysfunction to the dysregulation of Mdm2 and Mdm4 alternative splicing and upregulation of p53. Importantly, increased skipping of Mdm2 and Mdm4 exons regulated by SMN is necessary and sufficient to synergistically elicit robust p53 activation in wild type mice. Conversely, correction of either deficit by restoration of full-length Mdm2 or Mdm4 is sufficient to suppress p53 induction and motor neuron degeneration in SMA mice. These findings reveal that loss of SMN-dependent regulation of Mdm2 and Mdm4 alternative splicing underlies p53-mediated death of motor neurons in SMA, establishing a causal link between snRNP dysfunction and neurodegeneration.

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Poster

379. ALS Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 379.13/K3

Topic: C.06. Neuromuscular Diseases

Title: Short interference peptides as blockers for TDP-43 phosphorylation prevent TDP-43 aggregation and cell death

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Abstract: Background: Detergent insoluble inclusions of TDP-43 protein are the signature neuropathology in over 90% of amyotrophic lateral sclerosis (ALS) cases and approximately half of frontotemporal dementia (FTLD-TDP) cases. In TDP-43 proteinopathy disorders, neurons contain aggregates of TDP-43 protein. TDP-43 in the aggregates is extensively post-translationally modified, with phosphorylated TDP-43 (pTDP) being the most consistent and robust marker of pathological TDP-43 deposition. Since phosphorylated TDP-43 is not observed in the absence of neurodegeneration, abnormally phosphorylated TDP-43 has been hypothesized to mediate TDP-43 toxicity in these neurodegenerative disease states. Casein kinase I delta (CK-1δ) has been reported to phosphorylate TDP-43. TDP-43 phosphorylation sites targeted by CK-1δ correspond to the sites identified in Sarkosyl-insoluble fractions of brains from ALS patients.
Objective: The overall objective is to understand the role of TDP-43 phosphorylation in forming aggregates and how it mediates neuronal toxicity.
Methods: In our study, high density peptide array analysis was used to identify the interaction regions between TDP-43 and CK-1δ. We designed potential peptide-based kinases blockers to effectively interfere with TDP-43 and CK-1δ association. Immunocytochemistry and Western blots were used to quantify the effects of peptides. LDH and MTT assays were used to confirm cell death rate in an Ethacrynic acid (in vitro) model which also caused TDP-43 aggregation.
Results: we found that the endogenous TDP-43 could be phosphorylated and tended toward aggregation following CK-1δ overexpression but did not translocate from nucleus to cytoplasm. We found that the peptides we designed to block CK-1δ -TDP-43 interactions prevented TDP-43 phosphorylation and aggregation caused by either CK-1δ overexpression or Ethacrynic acid treatment. In addition, the peptides reduced the cell death induced by Ethacrynic acid in both SY5Y cells and primary cultured cortical neurons.
Conclusions: The above results indicate that TDP-43 phosphorylation may play an important role in the progress of these neurodegenerative diseases. Our interference peptides which block TDP-43 phosphorylation could be a useful strategy for developing potential new therapeutics.

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Poster

379. ALS Mechanisms

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Program #/Poster #: 379.14/K4

Topic: C.06. Neuromuscular Diseases

Support: Packard Center
Arizona Alzheimer's Consortium
MDA
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Title: Synaptic deficits in c9orf72-als/ftd patient-derived human stem cell differentiated neurons and *in vivo* models of c9orf72

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Abstract: The hexanucleotide repeat expansion GGGGCC (G_4C_2) found in the non-coding region of the *C9orf72* (C9) gene represents the most common genetic abnormality in amyotrophic lateral sclerosis (ALS) (40-50%) and frontotemporal dementia (FTD) (10-30%). ALS and FTD patients have genetic, pathologic and symptomatic overlap. Therefore, understanding the molecular mechanisms of disease pathogenesis in this ALS/FTD disease spectrum could lead to the development of novel therapeutic strategies. Cognitive decline as seen in normal ageing or Alzheimer's disease (AD) is characterized by changes in neuronal morphology, spine density and progressive synapse loss. We hypothesize that similar mechanisms are responsible for the dementia symptoms caused by the C9 mutation and that these events arise early during disease progression before any neurodegeneration has occurred. Furthermore, based on recent findings in AD and FTD, we hypothesize that this synaptic dysfunction may involve the neural-immune complement pathway. Here we present preliminary data supporting this hypothesis using C9-ALS/FTD patient-derived human induced pluripotent stem cells differentiated into motor neurons (hiPSC-MNs) and cortical neurons (hiPSC-CNs), in addition to C9 mouse models. We found significant changes in dendritic branching, dendritic length, spine density and detected alterations in the expression pattern of synaptic proteins in hiPSC neurons. We also observed changes in neuronal excitability using longitudinal micro-electrode array analysis. Similar changes in dendritic arborization and dendritic length were observed in homozygous C9orf72 $^{-/-}$ knockout mice. In addition, increased complement pathway activation and decreased expression of pre-synaptic markers were observed in this C9 mouse model. Our data suggest that synaptic deficits are present in C9 ALS/FTD which might be triggered by aberrant neural-immune interactions. These synaptic dysfunctions are hypothesized to contribute to cognitive impairment and neuronal cell death found in C9orf72 patients and therefore provide first mechanistic insights into the dementia symptoms caused by this mutation.

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Poster

379. ALS Mechanisms

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Program #/Poster #: 379.16/K6

Topic: C.06. Neuromuscular Diseases

Support: Aage og Johanne Louis-Hansens Fond

Title: Distinct autoimmune antibody responses towards TDP-43 in amyotrophic lateral sclerosis

Authors: *T. BRUDEK[1,2], A. KALLEHAUGE NIELSEN[2], J. FOLKE[2], S. OWCZAREK[2], K. SVENSTRUP[3], K. WINGE[4], B. PAKKENBERG[2,5], S. AZNAR[2]
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a progressive heterogeneous neurodegenerative disorder with fatal outcome and with no curative treatment available. ALS is characterized by the degeneration of motor neurons, with 97 % of ALS patients displaying a common histopathological phenotype in disease-affected tissues, namely aggregation of the TAR-DNA binding protein (TDP)-43 in the cytoplasm of motor neurons. Accumulating evidence from unrelated studies suggest that lower levels of naturally occurring autoantibodies (NAbs) towards pathological proteins are implicated in the impaired clearing mechanism in neurodegenerative diseases. The aim of the study was therefore to investigate the affinity of anti-TDP-43 NAbs in plasma and cerebrospinal fluid (CSF) from 41 ALS patients in comparison with 41 healthy individuals.Using ELISA competition assay we explore the affinity of the binding between antibodies and TDP-43 in plasma and CSF. We have utilized the ability of high binding antibodies to form more stable bonds with their antigen compared with lower binding antibody. By incubating plasma samples with increasing concentrations of free TDP-43 monomer and a subsequent measurement of free antibodies on plates with immobilized TDP-43 protein, we were able to separate anti-TDP-43 NAbs into low and high affinity fractions that probably present distinct epitope specificities.The results show significantly reduced levels of high-affinity anti-TDP-43 NAbs in plasma samples from ALS patients. Further, we measured global antibody levels and relative levels of anti-TDP-43 NAbs within the immunoglobulin (Ig)G-subclasses and IgMs in plasma and CSF samples. We found a significant decrease in relative plasma levels of anti-TDP-43- IgG3- and IgM Nabs, and a significant increase of anti-TDP-43 IgG4 Nabs in ALS patients compared to healthy subjects. A significant increase of anti-TDP-43 IgG2 Nabs and global levels of IgG1s, IgG3s, IgG4s and IgMs in CSF samples from ALS patients were also

observed. Additionally, we observed significant increase in IgG Nabs-TDP-43 complexes in ALS plasma samples compared to healthy subjects. These results indicate that a TDP-43 immune aberrancy is apparent in ALS patients and could explain the toxic aggregation of TDP-43. We hypothesize that an immune deterioration may be an important reason for low affinity anti-TDP-43 antibody binding properties in ALS patients resulting in ineffective clearance of TDP-43-antibody complexes containing low affinity antibodies. Data generated in this project can contribute to further development of immune-based therapeutic strategies in ALS.

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Poster

379. ALS Mechanisms

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Program #/Poster #: 379.17/K7

Topic: C.06. Neuromuscular Diseases

Support: Brain Korea 21 PLUS Project for Medical Science, Yonsei University

Title: Strategy for personalized medicine based on ALS patient-derived stem cells using CRISPR/Cas9-mediated genome editing

Authors: *Y. YUN[1], D. BAEK[1], J. KIM[2], S. BAE[3], Y. HA[1]
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Abstract: Purpose Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that causes dysfunction of the motor neuron. Motor neuron which is responsible for controlling voluntary muscles movement in the spinal cord, brainstem and brain progressively deteriorate and die. It leads to muscle weakness, paralysis and eventually respiratory failure to death. The precise cause of ALS is not known, only 5% to 10% of cases are known as associated with genetic problem. Considering various novel genetic mutations have been reported and poorly understood pathophysiology of ALS, the development of personalized therapy that target the patient's own genetic mutation and restore motor neuron function would provide the most promising treatment. Material and methods From a case of male patient presenting with ALS, Next generation sequencing (NGS) discovered a point mutation in a gene named ATP7A which encodes a transmembrane copper-transporting ATPase. In order to assess the genotype-phenotype, impaired ATP7A trafficking, patient-derived fibroblast was directly reprogrammed to generate induced neural stem cell (iNSC). Gene correction of point mutation in ATP7A using CRISPR/Cas9 system was performed in patient-derived induced pluripotent stem cells (iPS). To target the precise mutated sequence, we designed specific sgRNA and donor DNA as well as

proper transfection methods. Results Whole genome sequencing of ALS patient and his parents revealed maternally inherited variant in ATP7A gene. We investigate the pathophysiology in patient-derived cells and impaired ATP7A trafficking phenotype caused by a point mutation. iNSC revealed the cellular dysfunction such as proliferation and apoptosis compared to normal cell line. Similarly, patient-derived iNSC showed less responsiveness to copper compare to normal cell line. We obtained isogenic cell line with ATP7A corrected iPS. Investigation of pathophysiology between patient-derived cells and gene corrected cells revealed that ATP7A function is partly rescued and cellular function such as proliferation and neural differentiation is improved. Conclusion Reprogrammed stem cells from patient somatic cell are suitable to investigate the mechanism, pathology and gene-editing. NGS can give the genetic evidence and candidates of disease. The CRISPR/Cas9 system can target the sequence precisely and homology directed repair (HDR)-mediated gene editing allows a correction of point mutation. As a result, these new technologies are capable of precise genomic analysis in patient genetic background and this strategy would be the most promising approach to personalized precision medicine.

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Poster

379. ALS Mechanisms

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Topic: C.06. Neuromuscular Diseases

Support: ERC 294745 to MA
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Title: Role of neurodegeneration causative genes SMN and C9orf72 in genome instability

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Abstract: The most common motor neuron diseases (MNDs) are amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). While SMA, a childhood MND, is caused by mutations in a single gene (*survival motor neuron* or *SMN1*), ALS has been linked with several causative genes. Intronic GGGGCC repeat expansions in C9orf72 are considered the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), though the mechanisms by which such expansions cause neurodegeneration are poorly understood. Two major pathologies stemming from the RNA expansions have been identified in postmortem tissue: intracellular RNA foci and repeat-associated non-ATG dependent (RAN)

dipeptides, though it is unclear how these and other hallmarks of disease contribute to the pathophysiology of neuronal injury. Adeno-associated virus (AAV) was used to model C9orf72 pathology in neuronal cells and rodent. We report that AAV viral vectors encoding 10 pure, 102 interrupted GGGGCC repeats or 69 V5-tagged DPRs delivered into the cerebrospinal fluid via cisterna magna of wild type mice led to transgene expression in multiple areas of the CNS and cause neurodegenerative phenotype[1.2]. Virus-mediated expression of C9orf72-related RNA and dipeptide repeats in the mouse central nervous system increases double strand breaks and ATM defects and is associated with neurodegeneration. We also report elevated levels of DNA-RNA hybrids (R-loops) and double strand breaks in rat primary neurons, human cells and C9orf72 ALS patient spinal cord tissues.
We also report significant increase in DNA breaks in Smn-depleted motor neurons as well as postmortem spinal cord sections derived from SMA patients. We show that the DNA damage is transcription-dependent as we detect significant decrease in DNA breaks after RNA polymerase II inhibition. These findings suggest that SMN and C9orf72-mediated neurodegeneration is driven, at least partly, by genomic instability.

References:

1. Walker et al., Nat Neurosci. 2017; 20(9):1225-1235. doi: 10.1038/nn.4604.
2. Herranz-Martin et al., Dis Model Mech. 2017; 10(7):859-868. doi: 10.1242/dmm.029892

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Poster

379. ALS Mechanisms

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Topic: C.06. Neuromuscular Diseases

Support: Olle Engkvist Byggmästare Foundation
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Title: ALS Cell Atlas: An online resource to infer gene activity in nine major CNS cell types in ALS patients and mouse models

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Abstract: We still know very little about how various central nervous system (CNS) cell types contribute to ALS onset or progression. Our goal is to infer the timecourse of activity for nine major CNS cell types in spinal cord tissue from end-stage ALS patients and during disease progression in *SOD1*^{G93A} mouse model with the use of single-cell transcriptomes applied to interpret whole tissue expression data. The resulting dataset will be released as an open, interactive online resource.
Our resource will include gene expression specificity rankings for nine major cell types (pyramidal neurons, interneurons, astrocytes, microglia, oligodendrocytes, endothelium, pericytes, vascular smooth muscle and vascular leptomeningeal cells). In order to observe the dynamics of cell activity we analyzed respective cells at pre-symptomatic (28, 42, 56, 70 days) and symptomatic stages (98, 112, 128 days) in *SOD1*^{G93A} mice together with post mortem ALS patient tissue.
We would like to present a demonstration version of the online resource features for genes associated with microglial cells which allowed us to identify novel genes activated during ALS in these cells. Our resource will help to stimulate interest in specific cell type contribution in ALS. It will also help to discover novel drug targets specific for given cell type population in symptomatic and pre-symptomatic stages of ALS progression. In the long run we hope our resource will stimulate drug discovery research and benefit ALS patients.

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Poster

379. ALS Mechanisms

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Program #/Poster #: 379.20/K10

Topic: C.06. Neuromuscular Diseases

Support: ALSA Investigator Initiated Grant 18-IIA-406

Title: Determining the potential roles of G4 Resolvase1 in ALS

Authors: *A. E. CHAMBERS[1], A. E. RICHARDSON[2], S. N. SANDWITH[2], D. W. SAUNDERS[1], H. M. RAIMER[3], J. P. VAUGHN[4], Y.-H. WANG[3], M. A. SMALDINO[1], P. J. SMALDINO[1]
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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. This disease is relentlessly progressive, leading to the inability to eat, speak, move, and breathe. There is no cure, and 80% of all people with ALS will succumb to the disease within 5 years of diagnosis. A

better understanding of the molecular underpinnings of ALS is necessary for the development of effective future therapies. A dramatic increase in the number of GGGGCC-repeat units in the first intron of the *C9orf72* (*C9*) gene causes the most common subset of familial ALS. Healthy individuals harbor between 2 and 30 repeats, while *C9* ALS patients typically have >30 and often hundreds to thousands of the repeat unit. The guanine (G)-rich nature of the *C9*-repeat sequence allows it to fold into secondary DNA structures, termed G-quadruplexes. Expansion of *C9*-repeat DNA leads to transcription of truncated *C9* RNAs that aggregate into toxic foci and sequester essential RNA-binding proteins. *C9*-repeat RNAs are translated into toxic dipeptide repeats, and the net effect is neuronal death. Therefore, *C9* ALS is foundationally a DNA G-quadruplex disease, and identifying the proteins that modulate these structures is essential for understanding and treating *C9* ALS. The enzyme that accounts for the majority of all G-quadruplex helicase activity is G4 Resolvase 1 (G4R1) (aliases: RHAU, DHX36). We hypothesize that G4R1, as the major G-quadruplex helicase, is dysregulated in *C9* ALS. We are testing this hypothesis by comparing G4R1 expression in ALS patient cell lines compared to age-sex matched controls. Our results indicate a significant increase in G4R1 expression in *C9* ALS cell lines compared to matched controls. These findings demonstrate a potential role for G4R1 in *C9* ALS and further investigation is being conducted.

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Poster

379. ALS Mechanisms

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Support: NIH NS061867
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ARCS
ARCS Foundation, Inc. (Achievement Rewards for College Scientists, Inc.)

Title: ALS associated mutations in Matrin 3 alter protein-protein interactions and impede mRNA nuclear export

Authors: *A. BOEHRINGER[1], D. MEDINA[1], K. GARCIA-MANSFIELD[2], N. BAKKAR[1], P. PIRROTTE[2], R. BOWSER[1]
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Abstract: Mutations in Matrin 3 have recently been linked to ALS, though the mechanism that induces disease in these patients is unknown. To define the protein interactome of wild-type and ALS-linked MATR3 mutations, we performed immunoprecipitation followed by mass spectrometry using NSC-34 cells expressing human wild-type or mutant Matrin 3. Gene ontology analysis identified a novel role for Matrin 3 in mRNA transport centered on proteins in the TRanscription and EXport (TREX) complex, known to function in mRNA biogenesis and nuclear export. ALS-linked mutations in Matrin 3 led to its re-distribution within the nucleus, decreased co-localization with endogenous Matrin 3 and increased co-localization with specific TREX components. Expression of disease-causing Matrin 3 mutations led to nuclear mRNA export defects of both global mRNA and more specifically the mRNA of TDP-43 and FUS. Our findings identify a potential pathogenic mechanism attributable to MATR3 mutations and further link cellular transport defects to ALS. Our current work investigates the role of specific protein domains in the interactions between Matrin 3 and TREX proteins, and will explore mRNA export defects in motor neurons derived from patient induced pluripotent stem cells (iPSC).

Disclosures: A. Boehringer: None. **D. Medina:** None. **K. Garcia-Mansfield:** None. **N. Bakkar:** None. **P. Pirrotte:** None. **R. Bowser:** None.

Poster

379. ALS Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 379.22/K12

Topic: C.06. Neuromuscular Diseases

Support: NRF-2016R1D1A1B01009186

Title: Identification of a genetic mutation in microvilli protein EBP50, a new cause of hereditary peripheral neuropathy

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Abstract: We have identified a novel EBP50 gene mutation in a family with peripheral neuropathy. EBP50 is highly expressed at the tip of Schwann cells and at nodes of Ranvier. The expression of EBP50 in sciatic nerve significantly increased during Schwann cell maturation and myelination, while its expression was significantly downregulated in animal models of peripheral neuropathy, suggesting its important role in peripheral nerve system. In addition, heterozygous knockout mice (EBP50 +/-) exhibited distinctive features of peripheral neuropathy such as abnormal myelin formation and decreased motor function. Knocking-down the expression of

EBP50 in Schwann cells reduced the length of cell protrusion and cell motility. Mutations in the EBP50 gene reduced NRG-induced AKT phosphorylation and Schwann cell mobility. In conclusion, we have established a loss-of-function mutation in the EBP50 gene responsible for hereditary peripheral neuropathy in this study. This new discovery will be the basis for the development of genetic peripheral neuropathy therapies.

Disclosures: G. Song: None. **D.P. Gupta:** None. **M. Rahman:** None. **H. Park:** None. **M. Lee:** None. **K. Suk:** None.

Poster

379. ALS Mechanisms

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SynaNet which has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 692340.

Title: Dysfunction of VEGF-mediated mechanisms at different disease stages of $SOD1^{G93A}$ mice model

Authors: *N. REI[1,2], J. A. RIBEIRO[1,2], S. H. VAZ[1,2], C. VALENTE[1,2], A. M. SEBASTIAO[1,2]
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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the progressive degeneration of motor neurons in corticospinal tract, leading to muscle weakness, atrophy, paralysis and death. Recent studies support that vascular endothelial growth factor (VEGF) has a neuroprotective function, protecting motor neurons from degeneration in ALS models. VEGFA binds both VEGF-R1 and VEGF-R2, but with more affinity to VEGF-R1, which may negatively regulate VEGF function, preventing its binding to VEGF-R2. VEGFB binds only to VEGF-R1. We now evaluated if there are any changes in the levels of VEGF proteins (VEGFA and VEGFB), and VEGF receptors (VEGF-R1 and VEGF-R2) at the cerebral cortex (CTX) and spinal cord (SC) of a model of ALS, SOD1(G93A) mice.A qReal-time PCR was used to evaluate mRNA levels (n=3-7) and an ELISA assay (n=4-6) was performed to evaluate the expression of VEGF and VEGF receptors protein levels. Pre-symptomatic (4-6 weeks old) and symptomatic (12-14 weeks old) mice were analysed and data compared with age-matched wild type mice. Significant differences were considered at $p<0.05$. Concerning mRNA expression of VEGF and its receptors, at the CTX of pre-symptomatic mice, there was an

increase in the expression of mRNA levels for VEGFA and VEGFB with no significant change in the expression of mRNA for VEGF receptors. Interestingly, in CTX of symptomatic mice there was a decrease of the mRNA expression for VEGFA and VEGF-R2 and an increase of VEGFB. At the SC of pre-symptomatic mice there was an increase in mRNA levels of VEGF-R1 and VEGFB and a decrease of mRNA levels of VEGFA and VEGF-R2 in the SC of symptomatic mice. Regarding the protein levels, there are no significant changes for VEGFA expression at both, CTX and SC and there is a significant increase of VEGFB at SC of pre-symptomatic mice. Concerning the VEGF receptors, there is an increase at SC of pre-symptomatic mice of both receptors (VEGF-R1 and VEGF-R2) and a decrease at CTX of symptomatic mice.Overall, the data suggest an early dysfunction of VEGF-mediated mechanisms, with an enhanced expression at pre-symptomatic stage, which reverses to a decrease at the symptomatic stage.

Disclosures: N. Rei: None. **J.A. Ribeiro:** None. **S.H. Vaz:** None. **C. Valente:** None. **A.M. Sebastiao:** None.

Poster

379. ALS Mechanisms

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Program #/Poster #: 379.24/K14

Topic: C.06. Neuromuscular Diseases

Support: SERIKA Fund
KAKENHI(16K07045)

Title: Optogenetic enhancement of TDP-43 intermolecular interaction triggers its cytoplasmic mislocalization and inhibits axon outgrowth of spinal motor neurons

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Abstract: Cytoplasmic aggregation of TDP-43 characterizes degenerating motor neurons in most cases of Amyotrophic lateral sclerosis (ALS). However, exact mechanisms of TDP-43 neurotoxicity that lead to neuronal dysfunction and degeneration remain elusive. Here, we show that enhancement of TDP-43 intermolecular interaction is sufficient to trigger its cytoplasmic mislocalization and toxic to spinal motor neurons. We develop an optogenetic approach by which intermolecular interaction of a TDP-43 variant can be enhanced by light illumination in zebrafish larvae. The photo-responsive TDP-43 (optoTDP-43) can function as TDP-43 since it rescues TDP-43 knockout phenotypes under an ambient light condition. In contrast, upon illumination of blue light, optoTDP-43 gradually loses its nuclear-specific localization and

becomes dispersed throughout the nucleus and cytoplasm. Crucially, transient light illumination that causes cytoplasmic mislocalization of optoTDP-43, but not its cytoplasmic aggregate formation, is sufficient to diminish subsequent axon outgrowth of spinal motor neurons. Our results suggest that excessive intermolecular interaction is a fundamental basis of TDP-43 neurotoxicity in spinal motor neurons. Therefore, optoTDP-43 provides a potential avenue for recapitulating pathologies of ALS and other TDP-43 proteinopathies in a spatiotemporally regulated fashion by light illumination.

Disclosures: K. Asakawa: None. **K. Kawakami:** None.

Poster

379. ALS Mechanisms

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Topic: C.06. Neuromuscular Diseases

Support: NIH Grant NS099320

Title: Oligodendrocyte dysfunction and c9orf72 als

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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal, progressive neurodegenerative disease characterized by the degeneration of cortical and spinal motor neurons. An intronic G_4C_2 hexanucleotide repeat expansion (HRE) in the *C9orf72* gene is the most common cause of familial ALS, as well as frontotemporal dementia (FTD). Increasingly, glial cell dysfunction is recognized as a pathogenic mechanism that contributes to ALS pathology and motor neuron death. Furthermore, *C9orf72* promoter activity is enriched in C9-ALS-affected cell types: motor neurons and oligodendrocytes, suggesting that intrinsic changes in these cells may influence disease progression. Oligodendrocytes, the glial cell type responsible for producing myelin and for providing metabolic support to neurons, degenerate early in the gray matter of the mutant SOD1 ALS mouse model as well as in human ALS patients. Subsequently, a downstream proliferative cascade of oligodendrocyte progenitor cells (OPCs) takes place; however, these OPCs are unable to fully differentiate and recapitulate the functions of lost oligodendrocytes. Importantly, the mechanisms underlying these changes, and whether these same events take place in C9-ALS, is unknown. Our group and others have recently demonstrated that

nucleocytoplasmic transport, the critical bidirectional process occurring at the nuclear pore complex (NPC), is disrupted in *C9orf72* animal models as well as in C9-ALS patients. Given that nuclear transposition of transcription factors is critical to oligodendrocyte development, disruption of nucleocytoplasmic transport in these cells may impair their ability to differentiate and support neurons in C9-ALS conditions. Here, we assess whether oligodendrocyte degeneration and impaired OPC differentiation occur in C9-ALS patient tissue and in C9-BAC transgenic mouse models expressing the HRE. We also examine whether NPC pathology and nucleocytoplasmic transport deficits are found in oligodendrocytes and OPCs in these models. Together, these novel experiments shed light on disease-relevant pathways that may be therapeutically amenable, as well as expand on the complex biology of the NPC in specific CNS cell types.

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Poster

379. ALS Mechanisms

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Program #/Poster #: 379.26/K16

Topic: C.06. Neuromuscular Diseases

Support: WFSM Tab A Williams Endowment
The Blazeman Foundation for ALS

Title: Macrophages accumulate at neuromuscular junctions coincident with denervation in the $SOD1^{G93A}$ mouse model of amyotrophic lateral sclerosis

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Abstract: Macrophages are cells associated with tissue homeostasis and the innate immune system and provide a primary, phagocytic response to infectious agents or injured cells. This response is often isolated or directed toward a specific site in a tissue to prevent the spread of infection of injury. Macrophages can exhibit phagocytic, neuroprotective, proinflammatory characteristics or a combination of these, with examples of these activities coming from classic studies of perihperal nerve injury, where myeloid cells have been shown to mediate myelin clearance (phagocytic) and promote axonal regeneration (neuroprotective). Amyotrophic lateral sclerosis (ALS), a disease characterized by the progressive denervation of neuromuscular junctions (NMJs) and the death of motoneurons, provides an intriguing disease model to study macrophage response in, as motoneuron pathology in the disease is present in both the central and peripheral environments. For example, the earliest pathological hallmarks in the $SOD1^{G93A}$

mouse model, those seen in the first postnatal month, include synaptic changes in the spinal cord, vacuolated mitochondria in the dendrites, cell body, axons, and presynaptic terminals. Initial denervation of NMJs and differences in motor behavior are also observed in the first 30 postnatal days. Evidence demonstrating a phagocytic response of tissue macrophages to injured neurons has been shown peripherally in the disease with macrophages accumulating on the sciatic nerve, and centrally with microglia responding to late-stage degeneration of motoneuron cell bodies in the spinal cord, however, potential macrophage responses coincident with early NMJ denervation in muscles have not been explored. Here we show accumulation of macrophages targeting presynaptic terminals and intramuscular axons as early as postnatal day 30 in the tibialis anterior of $SOD1^{G93A}$ mice that undergoes denervation compared to $SOD1^{G93A}$ soleus (that does not undergo denervation) and wildtype, age-matched controls. The macrophages are associated with these structures prior to denervation, but numbers increase with NMJ denervation and axon retraction. These findings suggest that macrophages in the peripheral muscle play a role in disease progression. Potential protective and degenerative roles will be explored and discussed.

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Poster

379. ALS Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 379.27/K17

Topic: C.06. Neuromuscular Diseases

Title: Network aberrations in iTDP-43^{A315T} mouse model of ALS/FTD

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Abstract: Amyotrophic lateral sclerosis (ALS) is a debilitating and fatal disease characterised by progressive loss of motor function, which is now recognised to exist on a disease continuum with frontotemporal dementia (FTD), the second most common form of dementia. Growing evidence suggests that excitotoxity is a major disease mechanism in ALS contributing to the degeneration of motor neurons. A common pathological feature of both ALS and FTD is the cytoplasmic mislocalisation and aggregation of TAR DNA-binding protein 43 (TDP-43) in neurons, occurring in over 90 percent of ALS patients and approximately 50 percent of FTD patients. Furthermore, pathological mutations in *TARDBP* gene encoding TDP-43 have been identified in

ALS/FTD families. As well as progressive motor deficits reminiscent of ALS, we have previously reported cognitive decline and hippocampal atrophy in our inducible mutant TDP-43 mouse line (iTDP-43^{A315T}). To understand the mechanisms underlying this cognitive decline, we first performed RNASeq analysis on hippocampal brain tissue of pre-symptomatic iTDP-43^{A315T} and non-transgenic littermate controls and found up-regulation of several genes involved in synaptic transmission. To see if these differences in mRNA profile translated into functional changes in synapses, we performed electrophysiological recordings on brain slices of iTDP-43^{A315T} mice and their littermate controls and found differences in the amplitude and decay time constant of excitatory postsynaptic current, suggesting alterations in glutamate uptake in iTDP-43^{A315T} mice. Since excitotoxicity is a common feature of ALS patients, we induced excitotoxic seizures with pentylenetetrazole (PTZ) and found that iTDP-43^{A315T} mice had differences in latency to seizure and seizure severity compared to non-transgenic littermates. Taken together, these results suggest novel network aberrations in iTDP-43^{A315T} mice which are consistent with our previously published phenotypic characterisation of these mice, and which may lead to an understanding of important mechanisms underlying the onset and progression of ALS/FTD.

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Poster

379. ALS Mechanisms

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Topic: C.06. Neuromuscular Diseases

Support: ALS Association Grant ID 15-IIP-203
ALS Finding a Cure

Title: Mechanisms underlying neuronal dysfunction in *C. elegans* single-copy/knock-in models for FUS ALS

Authors: *S. N. BASKOYLU[1], N. CHAPKIS[1], K. SCHUCH[2], B. UNSAL[1], J. SIMON[1], D. SAVAS[1], A. C. HART[1]
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Abstract: Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disorder marked by the progressive loss of glutamatergic and cholinergic motor neurons, muscle atrophy and paralysis. It is the most common motor neuron disorder affecting adults, and often leads to death within 3-5 years after disease onset. A number of genes with diverse roles in RNA and protein homeostasis have been associated with ALS, including fused in sarcoma (FUS). FUS encodes a ribonucleoprotein involved in mRNA transcription, splicing, transportation and stress

granule formation. However, it is unclear how mutations in FUS lead to ALS. In patient motor neurons, mutant FUS forms cytoplasmic aggregates with several other proteins implicated in ALS. This, and other evidence, suggest that mutant FUS may function in a common neurodegenerative pathway with other ALS-linked genes. To examine the mechanism of ALS FUS dysfunction, we generated the first single-copy/knock-in models of ALS FUS in *C. elegans* using CRISPR/Cas9-mediated genome editing. Introduction of patient amino acid changes R524S and P525L into the *C. elegans* orthologous gene *fust-1* lead to stress-induced locomotion defects, neurodegeneration and aggregation. Furthermore, single-copy ALS FUS models had defective neuromuscular signaling. Investigation of ALS FUS neuromuscular dysfunction lead to our discovery of several modifier ALS genes in *C. elegans* single-copy/knock-in models of ALS FUS. Notably, loss in an autophagy-related gene fully rescued neuromuscular signaling defects in *C. elegans* ALS FUS models. Currently, we are examining autophagy in *C. elegans* motor neurons. Combined, our findings in single-copy/knock-in models, and other published work on ALS FUS, implicate defective proteolysis pathways as a significant contributing factor in ALS pathogenesis.

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Poster

379. ALS Mechanisms

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Topic: C.06. Neuromuscular Diseases

Support: MIUR - PRIN Grant 2015MJBEM2_006

Title: Sonic hedgehog signalling pathway during regenerative processes in a mouse model of spinal motoneuronal loss

Authors: *M. GULISANO[1], N. VICARIO[2], A. COSTANTINO[3], M. A. S. GIUNTA[2], F. M. SPITALE[2], R. PARENTI[2], R. GULINO[3]
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Abstract: Background - Neuronal loss represents the consequence of direct or indirect insults to neurons, as well as one of the major factors mediating persistent disability. Gliosis, neuroinflammation and neurodegeneration are processes in which different cell populations have an interplay mediating both hostile microenvironment and self-repairing mechanisms. Sonic hedgehog (Shh) signaling, which has been indicated as an important pathway in central nervous

system development and neural stem cells (NSCs) function, may have a role in prompting the repairing and modulating actions of endogenous and/or exogenous NSCs in neurodegenerative conditions. **Methodology** - Somatic NSCs were obtained from the subventricular zone (SVZ) of 8 week old female 129/Sv mice. We studied the Shh pathway on NSCs both *in vitro* and in a mouse model of spinal motorneuronal degeneration induced by cholera toxin B conjugated to saporin (CTB-Sap) injection into the gastrocnemius muscle. **Results** - NSCs were derived, expanded and characterized *in vitro*. We analyzed the effects of Shh signaling pathway modulation on NSCs *in vitro*, finding a significant increase of the NSCs growth rate (2.98±0.58 *vs.* 5.26±0.57, $p<0.05$) and neurospheres diameters (109.9±2.4μm *vs.* 129.6±3.7μm, $p<0.01$) upon Shh pathway activation. We then characterized Shh signaling activation in CTB-Sap mice analyzing neuronal loss, gliosis, inflammation and compensatory self-repairing mechanisms, compared to intact control mice. **Conclusions** - Our results suggest a crucial role of Shh signaling during regenerative processes and NSCs as a potential strategy to support recovery after spinal motoneuronal degeneration, thus representing a promising approach for neurodegenerative disorders.

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Poster

379. ALS Mechanisms

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Topic: C.06. Neuromuscular Diseases

Support: R01-NS094239-02
NS100401-02
Packard Center for ALS
ALSA

Title: Autophagolysosome disruption in *Drosophila* models of ALS/FTD caused by C9orf72 expansion mutations

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Abstract: A GGGGCC hexanucleotide repeat expansion (G4C2 HRE) in the C9orf72 gene is the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia

(FTD). In an RNAi screen for modifiers of HRE-mediated degeneration, our lab previously identified a key role for nucleocytoplasmic transport (NCT) disruption in C9-ALS/FTD (Zhang et al., Nature, 2015). We are investigating pathways that mediate neurodegeneration downstream of nucleocytoplasmic transport disruption. In our screen, we identify two key regulators of autophagy, p62/SQSTM1 and Mitf/TFEB as modifiers of neurodegeneration. In a *Drosophila* model of C9-ALS expressing $(G4C2)_{30}$, we find that p62 is upregulated and forms large aggregates in motor neurons. p62/SQSTM1 plays a key role in autophagy by binding ubiquitinated proteins and delivering them to the autophagosome for degradation via the lysosome. Surprisingly, we find that knockdown of Ref(2)p, the fly homologue of p62/SQSTM1, rescues degeneration in the fly eye and in motor neurons, while knockdown of Mitf enhances neurodegeneration. Immunofluorescence and western blot analysis of autophagy and lysosome markers demonstrates an impairment in autophagosome biogenesis but expansion of dysfunctional lysosomes. Using electron microscopy of the *Drosophila* eye, we observe a remarkable accumulation of expanded multilamellar bodies and autolysosomes that precedes neurodegeneration. Due to these lysosomal defects, we hypothesize that mislocalization of Mitf from the nucleus downstream of NCT defects may cause dysregulation of lysosomes and autophagy. Indeed, we find that Mitf is mislocalized to the cytoplasm in G4C2-expressing cells and in iPS neurons derived from C9-ALS/FTD patients. We also find that genetic and pharmacological induction of lysosomal genes downstream of Mitf rescues neurodegeneration. We propose that C9orf72-HRE expression causes Mitf/TFEB mislocalization from the nucleus and contributes to a feed-forward disruption between NCT disruption and protein aggregation. This study suggests that drugs targeting lysosomal proteostasis pathways may have therapeutic potential for C9orf72-mediated ALS and FTD.

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Poster

380. Neuromuscular Diseases: Other Neuromuscular Diseases

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 380.01/L3

Topic: C.06. Neuromuscular Diseases

Title: Herpes zoster induced brachial plexopathy affecting whole branches, a case report

Authors: *S. KANG
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Abstract: The most common neurologic complication of herpes zoster is chronic pain, and motor paralysis is a less common complication. Until now, a few cases have been reported about

motor paralysis as brachial plexopathy (BPI) after herpes zoster infection. In this case report we report a patient with brachial plexopathy involving whole branch confirmed by needle electromyography. Case A 88 year old female patient visited the hospital with bullous skin lesion in neck, right whole arm. She complained tingling sense, pain, and swelling on the involved area. Under diagnosis of Herpes zoster, she took the anti-viral agents (famciclovir, loxoprofen, epinastine, levosulpiride), and 3 days later, the bullous skin lesion in hands is proceeded to the proximal part of body. Her symptoms were improved with proper medication and two months later, pain was much improved and the skin lesion was changed to chronic scar. However, she was referred for electrodiagnostic study through the orthopedics because she reported that weakness on this right upper extremity had started. On physical examination, muscle power on elbow flexion was grade 4, elbow extension was grade 4, wrist extension was grade 4, finger flexion was grade 3, finger abduction was grade 3, finger extension was grade 3, and finger DIP was grade 3. She showed difficulties in fine motor control and during performing fine motor task she showed mild tremor. In motor nerve conduction study, conduction velocity of median, ulnar and radial nerve was decreased. Amplitude of sensory responses was decreased in median, ulnar, superficial radial, lateral antebrachial cutaneous, and medial antebrachial cutaneous nerve. Needle electromyography showed abnormal spontaneous activities in the muscles innovated from axillary, musculocutaneous, median, ulnar, and radial nerves.
Synthesizing the clinical symptoms, physical examination and eletromyography, she was diagnosed as the brachial plexopathy, whole branch involved, after herpes zoster infection. We performed magnetic resonance imaging (MRI) study, and it showed diffuse swelling of entire right brachial plexus. The patient was enrolled to the occupational therapy and continued medication (steroid). After one month of rehabilitation, weakness and sensory symptoms were much improved. Whole branch involving BPI after herpes zoster infection is a rare case, and electrodiagnostic study is helpful for accurate diagnosis. Proper rehabilitation program would be needed also to improve the motor weakness.

Disclosures: S. Kang: None.

Poster

380. Neuromuscular Diseases: Other Neuromuscular Diseases

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 380.02/L4

Topic: C.06. Neuromuscular Diseases

Support: DFG SPP 1710

Title: Mitochondrial hyperfusion connects GDAP1 pathophysiology in Charcot-Marie-Tooth disease with MNF2

Authors: *A. METHNER[1], C. WOLF[1], A. POUYA[1], A. PFEIFFER[1], D. BUENO[1], H. VON PEIN[1], J. SCHWIRZ[2], K. REHBACH[3], M. PEITZ[3], O. BRÜSTLE[3]
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Abstract: Charcot-Marie-Tooth (CMT) disease is a hereditary polyneuropathy caused by mutation of GDAP1 or MFN2, two mitochondrial proteins that exert opposite effects on mitochondrial shape; GDAP1 is a glutathione-S-transferase which causes mitochondrial fission while MFN2 causes fusion. Both proteins regulate mitochondria-endoplasmic reticulum (ER) contact sites, important cellular signaling hubs, and MFN2 controls mitochondrial hyperfusion, a cellular stress response. No common pathway underlying the disease has yet been identified. We found that neuronal cells from patients with autosomal-recessive CMT4A caused by GDAP1 mutation and GDAP1 knockdown cells have elongated mitochondria and an increased mitochondrial membrane potential. Knockdown uncouples mitochondrial respiration from ATP production, increases mitochondrial turnover, and reduces mitochondria-ER contact sites and mitochondrial [Ca2+]. Interestingly, GDAP1 and MFN2 reside in the same multi-protein complex in the presence of oxidized glutathione and over-expression of wildtype, but not enzyme-dead or mutated GDAP1, inhibits redox-mediated mitochondrial hyperfusion. Our data suggest that GDAP1 regulates mitochondrial hyperfusion via its enzymatic activity, which links CMT4A pathophysiology with MFN2.

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Poster

380. Neuromuscular Diseases: Other Neuromuscular Diseases

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Topic: C.06. Neuromuscular Diseases

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Title: *In vivo* translational profiling of motor neurons in mouse models of Charcot-Marie-Tooth type 2D

Authors: *E. L. SPAULDING[1], R. W. BURGESS[1,2]
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Abstract: Charcot-Marie-Tooth disease (CMT) is a collection of debilitating peripheral neuropathies caused by mutations in over 80 genes. The heterogeneity of the disease, as well as the technical challenge of studying the mammalian peripheral axon *in vivo*, both contribute to the lack of a cure. Dominant mutations in glycyl-tRNA synthetase (*GARS*), the enzyme responsible for both cytosolic and mitochondrial charging of tRNAs with the amino acid glycine, cause Charcot-Marie-Tooth Type 2D (CMT2D). How mutations in *GARS* cause neurodegeneration is unclear, but impaired translation has emerged as a potential toxic gain-of-function mechanism based on work with *Drosophila* models of CMT2D. To test this mechanism in mice, we are profiling translation in motor neuron cell bodies of the spinal cord and axons of the sciatic nerve in mice with mutations in *Gars* that are validated as CMT2D models. *In vivo*, cell type-specific, fluorescent non-canonical amino acid-tagging (FUNCAT) has revealed reduced global translation in mutant motor neuron cell bodies of $Gars^{P278KY/+}$ and $Gars^{C201R/+}$ mice. In contrast, Puromycin labeling of newly synthesized protein in an unaffected tissue, the liver, reveals normal translation in 3 *Gars* mouse models. Surprisingly, an increase in newly synthesized protein is seen in $Gars^{P278KY/+}$ motor axons with FUNCAT, a signature that resembles regenerating motor axons in wild type mice following a sciatic nerve crush. To complement the protein analysis, i*n vivo* ribosome-tagging from $Gars^{P278KY/+}$ and $Gars^{C201R/+}$ motor neuron cell bodies was used to identify mRNAs undergoing translation. This revealed an upregulation of transcripts associated with the integrated stress response, including Activating transcription factor 4 (Atf4) and several of its gene targets. This gene expression signature has been previously associated with metabolic stress in tissues like liver, but has not been described in neurons. Using RNAScope in situ hybridization, we show that activation of the stress response genes occurs in approximately 70% of mutant motor neurons and does not occur in any other cell types of the spinal cord. Thus, a cell autonomous, gain-of-function impairment in translation may contribute to motor neuron degeneration in mouse models of CMT2D.

Disclosures: R.W. Burgess: None.

Poster

380. Neuromuscular Diseases: Other Neuromuscular Diseases

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 380.04/L6

Topic: C.06. Neuromuscular Diseases

Support: CCDA

Title: Investigating the role of a mitochondrial deacetylase in spinal and bulbar muscular atrophy

Authors: J. MAZUK, E. HEINE, K. HAN, D. SIMPSON, D. EXLER, D. GARCIA-CASTRO, *H. L. MONTIE
Bio-Medical Sci., Philadelphia Col. of Osteo. Med., Philadelphia, PA

Abstract: Spinal and bulbar muscular atrophy (SBMA, Kennedy's Disease) is an X-linked neuromuscular disease affecting men. This late onset disease is caused by an expansion of a CAG tract in the androgen receptor (AR) gene, which encodes a glutamine (Q) tract in the protein. Disease is critically dependent upon AR ligands. As mitochondrial dysfunction is a component of SBMA pathogenesis, we have been studying the role of a mitochondrial deacetylase in SBMA. We have modulated this protein genetically and pharmacologically in a number of SBMA cell models. Up-regulating its activity protects cells from the toxic effects of polyQ-expanded AR. We are currently investigating the molecular mechanism(s) of its protection. Continued studies will include *in vivo* modulation of this mitochondrial deacetylase to determine whether it can abrogate motor dysfunctions, as well as other components of SBMA pathology.

Disclosures: J. Mazuk: None. **E. Heine:** None. **K. Han:** None. **D. Simpson:** None. **D. Exler:** None. **D. Garcia-Castro:** None. **H.L. Montie:** None.

Poster

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Title: Efficacy of gene therapy using allele-specific RNAi in a precision humaninzed model of a dominantly-inherited peripheral neuropathy

Authors: K. H. MORELLI[1], L. B. GRIFFIN[2], N. K. PYNE[3], L. M. WALLACE[3], A. M. FOWLER[3], A. ANTONELLIS[2], S. Q. HARPER[3], *R. W. BURGESS[4]
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Abstract: Charcot-Marie-Tooth disease type 2D is a dominantly inherited axonal neuropathy that leads to loss of motor and sensory innervation in the periphery. The disease is caused by mutations in glycyl tRNA synthetase (*GARS*), the essential, non-redundant enzyme that charges glycine onto its cognate tRNAs for translation. Although the disease mechanism remains unclear,

previous results from mouse models of *Gars*/CMT2D suggest that effective treatments need to decrease the mutant forms of *GARS*, whereas bolstering wild type *GARS* does not mitigate the neuropathy. To accomplish this allele-specific-reduction, we have used RNAi, processed through a mir30 micro-RNA cassette, and delivered using adeno-associated virus 9 (AAV9). Testing an RNAi that targets an existing dominant allele of *Gars* in mice indicated that this strategy is successful. Delivery of the AAV9 into the central nervous system could almost completely prevent the disease if administered pre-onset, and still had significant benefit when delivered post-onset. These effects were dose-dependent, and persisted for at least a year. To test the translational potential of this approach, we introduced a human disease-associated allele of *GARS* into the mouse genome. This mutation is a *de novo* mutation and has not been previously described. The mice carrying this allele developed a neuropathy by a few weeks of age, validating its pathogenicity. Delivery of an RNAi specifically targeting this allele produced similar results, with an almost complete prevention when delivered pre-onset, and lesser, but still significant improvement when delivered at post-onset time points. This project demonstrates the utility of precision animal models for the validation of human genetic variants and for preclinical studies. It also demonstrates that viral delivery of RNAi for allele-specific knockdown is a viable therapeutic strategy for dominantly inherited neuromuscular diseases.

Disclosures: K.H. Morelli: None. **L.B. Griffin:** None. **N.K. Pyne:** None. **L.M. Wallace:** None. **A.M. Fowler:** None. **A. Antonellis:** None. **S.Q. Harper:** None. **R.W. Burgess:** None.

Poster

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Program #/Poster #: 380.06/L8

Topic: C.06. Neuromuscular Diseases

Support: Danish research council DFF-1333-00197

Title: Disturbances of the homeostasis of the neuro-muscular complex in contractures of individuals with Cerebral Palsy

Authors: *J. PINGEL[1], J. B. NIELSEN[2]
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Abstract: Abstract Muscle contractures are common in individuals with cerebral palsy (CP), but the mechanisms responsible for the development of contractures are still unclear. Here we propose that changes in tissue homeostasis within the neuro-muscular-tendon tissue-complex are at the heart of the development of contractures. In order to unravel the neural, mechanical and metabolic factors, as well as genetic and transcriptional factors in muscle contractures, several

different studies have been conducted. **Changes at tissue level:** Our recent results reveal that some individuals might be genetically predisposed to become contractures. Furthermore, a significant correlation was observed between the passive stiffness of skeletal muscle and the expression of HSPG2, PRELP, RYR3, COL5A3, ASPH and COL4A6. **Systemic differences:** When levels of CRP, TGF-β and IL-6 was measured in serum of children with CP, adults with CP and healthy adults, it was observed that Children with CP has significantly higher systemic levels of CRP and TGF-β. Whether inflammation affects the growth of the muscles or might have other negative adverse effects in children with CP needs further investigation **Effect of treatments:** While micro-architectural analyses still are under investigation in humans, our animal studies have shown, that BoNT/A injections damages the microstructure of both the non-fibrillar and the fibrillar tissue and impairs the motor control of the gait in rats, and causes an increased collagen turnover in the muscle tissue. In summary, the present results indicate that muscle contractures might be caused by multiple factors, and we therefore suggest that it is necessary to reconsider of how and why muscle contractures develop.

Disclosures: J. Pingel: None. **J.B. Nielsen:** None.

Poster

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Topic: C.06. Neuromuscular Diseases

Support: Danish Medical Research Council
Lundbeck Foundation

Title: Abnormal motor axon potassium currents in a mouse model of hereditary neuropathy

Authors: *C. KRARUP[1], S. ALVAREZ[1], D. KLEIN[2], R. MARTINI[2], M. MOLDOVAN[1]
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Abstract: Charcot-Marie-Tooth neuropathy type 1A (CMT1A) resulting from peripheral myelin protein 22 KDa (PMP22) overexpression is the most common hereditary motor and sensory neuropathy in humans. The transgenic PMP22 (PMP22tg) mouse line C61 carrying 4 copies of the human PMP22 gene, has a slowly progressing neuropathy with thin myelin, immature Schmidt-Lanterman incisures and supernumerary Schwann cells, phenotypically similar to CMT1A. PMP22tg nerves showed activated macrophages leading to axon-myelin compartment disruption and maldistribution of K+ channels (Kohl B et al, Am J Pathol. 2010; 176: 1390), although the pathophysiological significance of these changes remains unclear. The aim of this study was to investigate the motor axon excitability in PMP22tg heterozygotes as compared to wild-type (WT) littermates. Multiple measures of motor axon excitability under anesthesia were

carried out by stimulation of the tibial nerve at ankle and “threshold-tracking” of the plantar CMAP responses. At age 3 months, when the post-developmental maturation was nearly complete in the WT, the PMP22tg CMAP showed an increase in latency by 29%. The CMAP amplitude was decreased by 36% although the mean motor unit size (MScan motor unit number estimation method) appeared unchanged. These conduction abnormalities were paralleled by marked abnormalities in excitability. PMP22tg showed a larger rheobase, an increased strength-duration time constant, a reduced minimum I/V slope (input conductance) and larger than normal deviations during threshold electrotonus. At age 6 months, the CMAP latency of PMP22tg was increased by 70% as compared to WT. In contrast to this marked conduction slowing along the tibial nerve from 3 to 6 months of age, further progression of excitability changes at ankle appeared modest. Nevertheless, when pooling data from 3 to 6 months, the increase in PMP22tg latency was correlated (Spearman $P<0.05$) with an increase in accommodation half-time during depolarizing electrotonus (+40% of threshold) from 29 to 35 ms and a reduction of the late subexcitable period of the recovery cycle from 16 to 9% of threshold, both changes consistent with a redistribution of K+ currents. Our data suggest that in the PMP22tg CMT1A model, a functional, thus potentially reversible, nodo-paranodopathy, accumulates along the nerve and aggravates the conduction impairment due to demyelination.

Disclosures: C. Krarup: None. **S. Alvarez:** None. **D. Klein:** None. **R. Martini:** None. **M. Moldovan:** None.

Poster

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Topic: C.06. Neuromuscular Diseases

Support: Civitan International Research Center Emerging Scholar Award

Title: Mir-486 is an epigenetic regulator of duchenne muscular dystrophy disease pathology

Authors: *R. HIGHTOWER[1], G. V. HALADE[2], M. S. ALEXANDER[3]
[1]Pediatric Neurol., The Univ. of Alabama at Birmingham, Birmingham, AL; [2]Cardiovasc. Dis., Univ. of Alabama at Birmingham, Birmingham, AL; [3]Pediatric Neurol., Children's of Alabama, Birmingham, AL

Abstract: Duchenne muscular dystrophy (DMD) affects 1 in 5000 live male births making it the most common form of muscular dystrophy worldwide. Patients with this X-linked neuromuscular disorder develop muscle loss, ambulation loss, cardiac arrhythmias, and respiratory complications which irreversibly progress throughout their lifespan. DMD is caused by mutations in the *DYSTROPHIN* (*DMD*) gene that result in non-functional dystrophin protein.

Dystrophin is critical for maintaining muscle structure and integrity; therefore, loss of dystrophin ultimately results in neuromuscular junction disintegration, myofiber membrane breakdown, myofiber death, and whole muscle atrophy. Although all patients experience many similar clinical disease manifestations, there remains a wide spectrum of phenotypic variability between them regarding onset and severity of symptoms. This clinical variability suggests dysregulation of secondary signaling pathways that may be influencing progression of disease. Dysregulated secondary signaling pathways in DMD have yet to be elucidated. MicroRNAs have shown to play a key role in muscle development and maintenance and have been implicated as biomarkers in various neuromuscular diseases. Our laboratory has previously shown that expression of a muscle-enriched microRNA, miR-486, is significantly decreased in both DMD patients and mouse models. In addition, our lab has demonstrated that overexpression of miR-486 in dystrophin-deficient mice can ameliorate neuromuscular disease pathology. From this, we hypothesized that miR-486 is a significant epigenetic regulator of DMD disease progression. Locomotive, histological, and metabolic analyses were used to assess muscle-specific transgenic overexpression of miR-486 in dystrophin-deficient mice. Global knockout of miR-486 in both WT and dystrophin-deficient mice was also investigated. We observed that miR-486 overexpression improved serum creatine kinase levels, improved muscle architecture, reduced centralized myonuclei, and increased physical activity in dystrophin-deficient adult male mice. MiR-486 global knockout resulted in significant disruption of skeletal muscle architecture, decreased physical activity, and defects in brain, cardiac, and metabolic function compared to WT controls. Future experiments focused on the detailed mechanism of miR-486 and its mRNA targets that will help to define the role of miR-486 in dysregulated secondary signaling pathways in DMD pathology. Overall, our findings suggest that miR-486 is a valuable biomarker for DMD and may serve as a novel therapeutic target for the treatment of dystrophin deficiency.

Disclosures: R. Hightower: None. **G.V. Halade:** None. **M.S. Alexander:** None.

Poster

380. Neuromuscular Diseases: Other Neuromuscular Diseases

Location: SDCC Halls B-H

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Program #/Poster #: 380.09/L11

Topic: C.06. Neuromuscular Diseases

Title: Neuro-Cardio-Autonomic modulations in children with Duchenne muscular dystrophy

Authors: *T. N. SATHYAPRABHA[1], M. ADOOR[1], P.-K. VEERAMANI[2], A. JOHN[3], K. POLAVARAPU[2], S. NASHI[4], P. PRATHUYSHA[5], A. NALINI[4], T. RAJU[1]
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Abstract: Background: Duchenne muscular dystrophy (DMD) is an X-linked devastating progressive muscle disease. Death is usually secondary to cardiorespiratory complications. Preclinical /early detection of cardiac autonomic disturbances prior to identification by standard techniques of cardiac assessment may help in timely initiation of cardio-protective therapy. **Methods:** A prospective study in **36** DMD boys and 23 age-matched healthy controls was conducted. ECG analysis, Heart rate variability - HRV, blood pressure variability(BPV) and baroreceptor sensitivity -BRS, were analysed and correlated with disease severity and genotype according to Multiplex Ligation-dependent Probe Amplification (MLPA). **Results:** In the DMD group**,** the mean age at first presentation to neuromuscular clinic was 8.22 ± 2.3 years, mean age at onset of illness was 3.8 ± 2.1 years and mean duration was 4.6 ± 2.7 years. The mean age at onset was 3.94 ±2.2 years. MLPA showed deletions in 32 (89.1%) and duplications in 4 (11.1%) patients. The mutation was distal in 27 and proximal in 9 cases. The mean heart rate was significantly higher in DMD children compared to controls (100 ± 10.22 /min vs 86.23 ± 12min). All the BPV parameters assessed were significantly reduced except diastolic, mean BP and coefficient of variation with increase in aortic impedance in the DMD group. The BRS parameters were also significantly impaired. A positive correlation was found between duration of illness and abnormalities in BPV parameters. There were no significant changes between proximal and distal mutation pattern with ECG/ HRV/BPV/BRS changes. **Conclusion:** This study shows a definite early impairment of neuro-autonomic control in DMD before the onset of clinical symptoms. These simple non-invasive tests like HRV, BPV and BRS can be used to identify pre-clinical cardiac dysfunction.

Disclosures: T.N. Sathyaprabha: None. **M. Adoor:** None. **P. Veeramani:** None. **A. John:** None. **K. Polavarapu:** None. **S. Nashi:** None. **P. Prathuysha:** None. **A. Nalini:** None. **T. Raju:** None.

Poster

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Program #/Poster #: 380.10/L12

Topic: C.06. Neuromuscular Diseases

Title: Characterizing chemotherapy-related somatosensory impairment

Authors: *J. HOLST-WOLF, L. TURCOTTE, J. KONCZAK
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Abstract: Chemotherapy-induced peripheral neuropathy is an unwanted side effect of treatment affecting patients and survivors of pediatric cancers. Peripheral neuropathy is known to cause impairments in haptic perception and proprioception. Currently, there is no gold-standard means of measuring these somatosensory impairments. Consequently, the extent of sensory impairment

during chemotherapy and recovery after treatment is unknown. Given that these senses are crucial for motor development and for performing activities of daily life, it seems imperative to obtain accurate measures of how chemotherapy affects these senses in patients with pediatric cancers. We here present two novel, simple assessments that yield objective measures to characterize sensory impairment associated with chemotherapy. The first assessment measures haptic discrimination, specifically the ability of an individual to discriminate between curvatures explored by active touch with the index finger. The second assessment measures proprioceptive acuity of the elbow by measuring an individual's ability to match a passively presented forearm position with the contralateral forearm. Eleven individuals (ages 6 to 25 years old) diagnosed with extracranial cancers such as acute lymphoblastic leukemia, Hodgkin and non-Hodgkin lymphoma, and Ewing sarcoma who are undergoing or have recently completed chemotherapy have completed at least one of these assessments. Preliminary data demonstrate the efficacy of the haptic assessment for measuring somatosensory dysfunction in pediatric patients treated with chemotherapy as 9 of 11 patients have haptic acuity thresholds in the 4th quartile of an the age-matched normative cohort. Regression analysis has identified a relationship between haptic discrimination threshold and the cumulative dosage of chemotherapeutic agent types. These results indicate the haptic discrimination assessment has sufficient resolution to characterize the effects of chemotherapeutic agents on somatosensation.

Disclosures: J. Holst-Wolf: None. **L. Turcotte:** None. **J. Konczak:** None.

Poster

380. Neuromuscular Diseases: Other Neuromuscular Diseases

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Topic: C.06. Neuromuscular Diseases

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NIH-NIGMS TL4GM118971
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Title: MitCHAP-60 and SPG13 arise from an inactive hsp60 chaperonin that fails to fold the ATP synthase b-subunit

Authors: *J. M. BHATT, J. WANG, A. ENRIQUEZ, J. LI, R. BERNAL
Chem., Univ. of Texas At El Paso, El Paso, TX

Abstract: The human mitochondrial heat shock protein (hsp60) functions as a chaperonin, folding proteins that are destined towards the mitochondrial matrix. Mutations in Hsp60 have been shown to lead to neurodegenerative diseases, namely, MitCHAP60 and SPG13. A D3G mutation in hsp60 leads to MitCHAP60, an early onset neurodegenerative disease characterized by muscle weakness, limb spasticity and involuntary eye movement. Another hsp60 mutation, V98I, has been linked with SPG13, a form of hereditary spastic paraplegia, characterized by progressive weakness, spasticity in the lower limbs, impaired vision, deafness, and cognitive impairment. The β-subunit of the mitochondrial ATP synthase has been shown to interact with Hsp60, however, the biological effects of the interaction have not been studied. We hypothesized that hsp60 is important in folding the β-subunit and that the mutant hsp60 complexes would be deficient in folding the β-subunit; leading to the neurodegenerative diseases. We expressed and purified Wt. and mutant hsp60 complexes from bacteria and analyzed them via electron microscopy and biochemical assays. Our data suggests that hsp60 plays a crucial role in folding the ATP synthase β-subunit and that the mutant complexes are unable to fold it in *in-vitro* protein-folding assays.

Disclosures: J.M. Bhatt: None. **J. Wang:** None. **A. Enriquez:** None. **J. Li:** None. **R. Bernal:** None.

Poster

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Program #/Poster #: 380.12/L14

Topic: C.06. Neuromuscular Diseases

Title: *In vitro* recapitulation of the dysfunctional neuromuscular junction in Charcot-Marie-Tooth disease

Authors: *R. R. BESSER, R. MACIEL, I. CLAURE, A. ALASSAF, D. CARBONERO, M. SAPORTA, A. AGARWAL
Univ. of Miami, Miami, FL

Abstract: Neuromuscular junction (NMJ) dysfunction has been identified in several forms of Charcot-Marie-Tooth disease (CMT). We seek to design a multi compartment cell culture system comprised of CMT patient iPSC derived neurospheres, aligned axons, and engineered anisotropic skeletal muscle tissue to model NMJ dysfunction in CMT using a human *in vitro* platform. Upon validation, this platform will enable mechanistic studies of CMT, as well as discovery of novel therapeutics. Mouse skeletal muscle cells and human neurospheres were co-cultured on a 2-phased hydrogel in maturation media. The hydrogel was fabricated by crosslinking gelatin (10% w/v) with microbial transglutaminase (mTg) (4%) and micromolded. A solution of laminin (10 μg/mL) and mTg (4%) was then incubated on top of the hydrogels.

During hydrogel fabrication one of two patterns were stamped onto the gel: 20 µm x 10 µm grooves for standard co-culture or a compartmentalized design. Our 2-phase biomaterial allowed enhanced myotube growth and alignment. The muscle cells were immunostained for α-sarcomeric actinin, which revealed mature myotube formation with organized sarcomeres. Additionally, neurospheres adhered and extended axons after seeding on the gelatin-laminin hydrogel. The addition of neurosphere media to skeletal muscle cultures enhanced the percentage of myoblasts that differentiate into myotubes from 30% to 85%. Both co-cultures and compartmentalized systems were imaged using immunofluorescence. Co-localization of the terminal end of an axon and acetylcholine clusters indicates the presence of a neuromuscular junction. To confirm the existence of neuromuscular junctions, further functional studies are underway. Gelatin-laminin micromolded hydrogels, which utilize biomimetic extracellular matrix components, are an optimal substrate to co-culture iPSC-derived neurospheres and muscle cells. These engineered hydrogels promote myotube formation and axonal growth. Additionally, micromolded gelatin-laminin hydrogels can be utilized to engineer a multi compartment culture system.

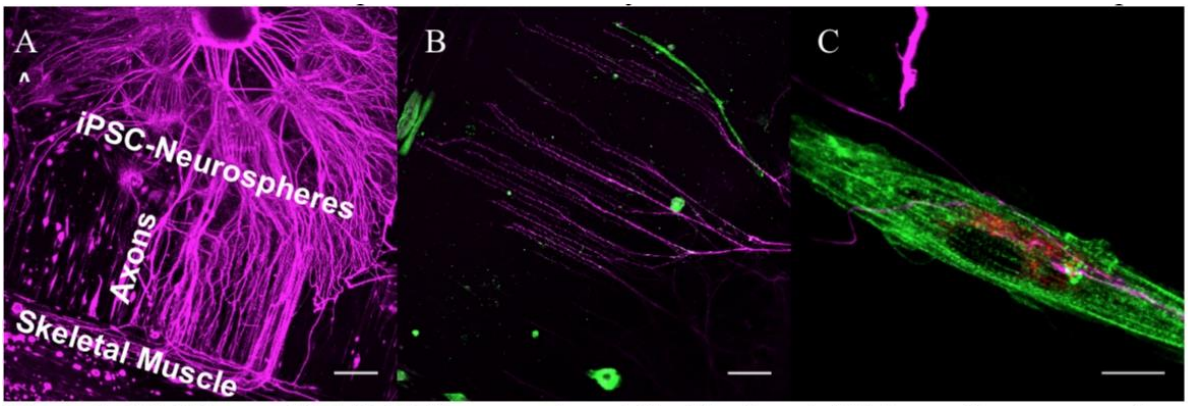

Figure 1. (A) Micromolded Gel-LN compartmentalized hydrogel. (B) Axons from neurosphere following channels and connecting with muscle cell. (C) Co-localization of acetylecholine receptor clusters (red) and terminal axon (magenta) indicates presence of a neuromuscular junction. Scale bar (A) and (B) is 500 µm, scale bar (C) is 50 µm.

Disclosures: R.R. Besser: None. **R. Maciel:** None. **I. Claure:** None. **A. Alassaf:** None. **D. Carbonero:** None. **M. Saporta:** None. **A. Agarwal:** None.

Poster

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Topic: C.06. Neuromuscular Diseases

Support: NSF - IIS1608147
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Title: The homeostatic adjustment of intrinsic excitability plasticity & spontaneous presynaptic neurotransmitter release in motor neurons and interneurons of severe spinal muscular atrophy mice

Authors: *J. SUN, M. A. HARRINGTON
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Abstract: Spinal muscular atrophy (SMA) is the leading genetic cause of death in infants and toddlers. Studies with animal models have demonstrated increased excitability and loss of afferent proprioceptive synapses on motor neurons in severe type SMA mice. To further understand the functional changes of the motor neural network in SMA, we studied the intrinsic excitability and synaptic transmission of both motor neurons and interneurons in the ventral horn of the lumbar spinal cord in the SMNΔ7 mouse model. We found that the resting membrane properties of motor neurons were significantly changed in SMA mice, including a hyperpolarized resting membrane potential, increased input resistance and decreased membrane capacitance, while more interneurons in SMA mice fired spontaneously and at higher rates than in littermate controls. The properties of the action potential (AP) in motor neurons were also changed, including a decreased rheobase current, increased amplitude and increased after depolarization potential. The relationship between AP firing frequency and current did not change in either motor neurons or interneurons, but the threshold current was reduced in motor neurons. These changes in the passive membrane and AP properties suggest an overall increase in the excitability of both motor neurons and interneurons. The plasticity of intrinsic excitability changed differently in motor neurons and interneurons. The percentage of neurons showing long-lasting potentiation was increased in motor neurons but decreased in interneurons, while long-lasting depression showed the opposite changes in SMA mice. For spontaneous inhibitory synaptic currents (sIPSC), the amplitude was significantly reduced in motor neurons, while the frequency was increased in interneurons of SMA mice. Interestingly, for miniature synaptic currents mEPSC frequency increased in motor neurons, and both mEPSC and mIPSC frequency increased in interneurons. These data suggest that in SMA-affected mice, defects in the network of interneurons induce homeostatic adjustments of intrinsic and synaptic properties in motor neurons. More research is needed to determine if this modulation contributes to the pathophysiology or is a compensatory response that helps preserve the function of the motor network.

Disclosures: J. Sun: None. **M.A. Harrington:** None.

Poster

380. Neuromuscular Diseases: Other Neuromuscular Diseases

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Program #/Poster #: 380.14/L16

Topic: C.06. Neuromuscular Diseases

Title: Cytoskeletal alterations in motor neurons derived from inducedcytoskeletal alterations in motoneurons derived from induced pluripotent stem cells (iPSCs) of patients with Brown-Vialetto-Van Laere (BVVL) syndrome can be rescued by riboflavin and N-acetyl-cysteine treatments

Authors: *A. NICEFORO[1], S. PETRINI[2], F. COLASUONNO[1], E. BERTINI[3], S. MORENO[1], C. COMPAGNUCCI[3]
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Abstract: Brown-Vialetto-Van Laere syndrome (BVVL) is a rare childhood disorder characterized by progressive sensorineural deafness and selective degeneration of ponto-bulbar motor neurons (MNs)[1]. The disease is caused by mutations in *SLC52A3* or *SLC52A2* genes, encoding for the human Riboflavin Transporters RFT2 and RFT3. Riboflavin (vitamin B2), as a precursor of FMN and FAD, is a crucial micronutrient for growth and normal cellular functions, particularly involving energy metabolism pathways driven by flavoproteins[2]. Induced pluripotent stem cells (iPSCs), derived from reprogramming of somatic cells are increasingly used to reproduce rare diseases. We therefore took advantage of this approach, using iPSCs obtained from fibroblasts of BVVL patients, to characterize molecular and cellular aspects of the disease. To study the cell type mostly affected in patients, we differentiated iPSCs into motor neurons, focusing on the cytoskeleton, whose dynamics is fundamental to drive the morphological and functional changes occurring in these cells, extending their neurites for long distances[3]. While patients' iPSCs showed no abnormalities in microtubule organization, when differentiated into MNs, these cells displayed deranged cytoskeletal components. Indeed, both immunofluorescence and RT-qPCR analyses demonstrated altered expression and distribution of α-/β-tubulin and NFH intermediate filaments in BVVL motoneurons. Considering the role of riboflavin in redox homeostasis[2], we explored possible beneficial effects of excess vitamin and/or the antioxidant molecule N-acetyl-cysteine (NAC). Treatment with riboflavin resulted in improved cytoskeletal features in patients' derived motor neurons, suggesting that redundancy of transporters may rescue neuronal functionality, when adequate concentrations of the vitamin are present in the microenvironment. This finding supports empirical data of patients' symptoms amelioration following riboflavin-based therapies[4]. Even more importantly, supplementation of culture medium with NAC, particularly in combination with riboflavin, restored normal cytoskeletal arrangement in patients' iPSCs-derived motoneurons. Our data, while supporting the use of

iPSCs for *in vitro* modeling of BVVL syndrome, highlights the pathogenic role of cytoskeletal abnormalities, which can be rescued by riboflavin and NAC treatments, thus suggesting possible therapies based on these molecules. 1. Van Laere J et al. Rev Neurol 1966, 115:289 2. Barile M et al. J Inherit Metab Dis 2016, 39:545 3. Compagnucci C et al. Cell Mol Life Sci 2014, 71:1623 4. Bosch AM et al. Orphanet J Rare Dis 2012, 7:83

Disclosures: S. Petrini: None. **F. Colasuonno:** None. **E. Bertini:** None. **S. Moreno:** None. **C. Compagnucci:** None.

Poster

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Title: Modeling hereditary spastic paraplegias with patient-specific stem cell-derived cortical and spinal neuron co-cultures

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Abstract: Hereditary spastic paraplegias (HSPs) are a heterogeneous group of neurogenetic disorders characterized by axonal degeneration of cortical motor neurons (MNs), a group of large projection neurons (PNs). How the connections between cortical PNs and their targets, spinal MNs, are affected in HSPs remain largely unknown. We have previously generated iPSCs from fibroblasts of an SPG3A patient with mutation of Atlastin-1 (*ATL1*, p.S342PP342S). Here, we first generated SPG3A isogenic human pluripotent stem (hPSC) cell (hPSC) lines by knocking in a mutation (*ATL*161 line, p.A161P) and correcting the mutation in the SPG3A hPSC line (p.S342P). The mutation of *ATL-1* (p.A161P) induced the axonal defects, including decreased axonal outgrowth, increased axonal swellings and impaired axonal synaptophysin transport in cortical PNs. Moreover, the correction of SPG3A mutation rescued the axonal defects, confirming the cause-effect relationship between the *ATL-1* mutations and disease phenotypes of cortical PNs. Next, we seek to determine the synaptic defects in HSP by establishing a co-culture model for SPG3A. We generated the channel rhodopsin 2 (ChR2)-EYFP expressing hPSC lines using CRISPR/Cas9-mdeiated homologous recombination. These normal, SPG3A iPSCs or isogenic cell lines were differentiated into cortical PNs (ChR2+), which were then co-cultured with spinal MNs derived from their corresponding regular pluripotent stem cells (without ChR2).

After the immunostaining, we observed a dramatic decrease in the numbers of the Synapsin+/EYFP+/PSD95+ synaptic clusters in the SPG3A (p.PS342PS) and ATL161 (p.A161P) co-cultures comparing to control coculture of cortical PNs and spinal MNs. Furthermore, the electrophysiological analysis revealed that the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) recorded in SPG3A spinal MNs decreased significantly comparing to control coculture group after the activation of ChR2-expressing cortical PNs using blue light stimulation, indicating the impaired functional synaptic connections between co-cultured cortical PNs and spinal MNs in SPG3A cell model. Taken together, our data reveal that the mutation of Atlastin-1 induced the axonal defects of cortical PNs and the impaired synaptic connections between cortical PNs and spinal MNs in a SPG3A co-culture model, which can serve as a unique system to study the pathogenic mechanism and explore the treatment for HSPs.

Disclosures: Y. Mou: None. **Y. Dong:** None. **S. Zhang:** None. **X. Li:** None.

Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 381.01/L18

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Practical Research Project for Rare/intractable Diseases from Japan Agency for Medical Research and Development, AMED to T.Y.

Title: RGMa inhibition ameliorates the severity of localized model of neuromyelitis optica

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Abstract: Background: Neuromyelitis optica (NMO) is an autoimmune disease associated with NMO-IgG, an antibody that selectively binds to aquaporin-4. Because of its association with this disease, passive transfer of NMO-IgG has been used to develop rodent models of NMO. But, previous models causes damage to multiple neuronal circuits, making it difficult to determine if the clinical deficits can be directly caused by a defined neuronal tract system. NMO and multiple sclerosis (MS) are immune-mediated neurodegenerative diseases with broadly comparable symptoms; thus, they may share some underlying molecular mechanisms. Systemically treating models of MS with RGMa-antibodies significantly improved function, reduced microglial lesion

size, enhanced axon regeneration into the lesion, and produced signs of remyelination.
Objectives: To study whether neutralizing the effect of RGMa could prevent the disease progression of a localized NMO model, and to obtain a deeper insight into the mechanism.
Rat model of NMO: Wistar rats (8 weeks) were used. Laminectomy was performed at thoracic 9/10 vertebral level and a micro-syringe attached to a pulled-glass micro-capillary needle was used to infuse a single injection of NMO-IgG or Control-IgG (20 μg) at the 10^{th} thoracic vertebrae. Animals were randomly and evenly divided (n = 10 per group): non-NMO rats, Control mAb-treated NMO rats, and anti-RGMa mAb-treated NMO rats. For the NMO groups, anti-RGMa mAb or Control-IgG was injected intravenously (10 mg/kg) after the NMO-IgG injection (day 0) and then every 7 days.
Immunohistochemistry: Primary antibodies includes: anti-AQP4, anti-GFAP, anti-Iba-1, anti-CD45, anti-MBP, and anti-SMI-312.
Results: The rats received a single 20-μg injection of NMO-IgG in the dorsal column of the thoracic spinal cord, which induced a well-demarcated single inflammatory lesion in the spinal cord. The weekly injections of anti-RGMa mAb significantly attenuated the severity of the clinical signs after day 12. We then examined the involvement of RGMa in T-cells in order to understand the mechanism of alleviated symptoms observed in our NMO rat. Interestingly, anti-RGMa mAb-treated NMO rats displayed a sharp reduction in the $IL17A^+$ T-cell population. The treatment might have brought about this effect by suppressing the enhanced production of $IL17A^+$ T-cells, which subsequently reduced axonal and astrocyte loss. Furthermore, the inhibition of RGMa promoted restoration of injured neural networks, presumably leading to a delay in the progression of the secondary phase of NMO.
Conclusions: We propose that humanized anti-RGMa mAb may help in preventing and attenuating the neurological symptoms of NMO.

Disclosures: K. Harada: None. **Y. Fujita:** None. **T. Okuno:** None. **S. Tanabe:** None. **Y. Koyama:** None. **H. Mochizuki:** None. **T. Yamashita:** None.

Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

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Program #/Poster #: 381.02/M1

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: MSU-Mankato Foundation
MSU-Mankato Biology

Title: The polyglutamine protein FAM171B localizes to neuronal cytoplasm

Authors: D. RAJAGURU, M. BAUER, D. S. SHARLIN, *G. M. GOELLNER
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Abstract: Expansion mutation within polyglutamine (polyQ) tract proteins is known to underlie a number of severe neurodegenerative disorders such as Huntington's Disease and Spinocerebellar Ataxia. Using a bioinformatics approach, we have identified a novel protein, FAM171B, that contains a stretch of 14 consecutive glutamines. Using *in situ* hybridization and immunohistochemistry experiments, our data strongly suggests that FAM171B is widely expressed in the brain with abundant expression in the hippocampus, cortex, and cerebellum. To begin elucidating FAM171B sub-cellular location we are using confocal fluorescence imaging of GFP-fusion tagged FAM171B and anti-FAM171B antibodies *in vitro*. Our findings indicate that FAM171B displays a punctate/vesicular staining pattern throughout the cytoplasm of human glioblastoma tissue culture cells and primary mouse cortical neurons. FAM171B localization is particularly enriched in the peri-nuclear region and adjacent to the plasma membrane. Current studies are utilizing organelle specific markers to verify sub-cellular locale and live-cell imaging to assay whether FAM171B may traffic between intracellular compartments.

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Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

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Program #/Poster #: 381.03/M2

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH/NIDA R01DA031429
Karolinska Institutet KID support
Swedish Research Council
Linnaeus environment grant
Swedish National Board of Forensic Medicine

Title: Hippocampal granule cell loss in human chronic alcohol abusers

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Abstract: Chronic alcohol abuse often causes cognitive impairment, which both experimentally and in human studies has been associated with neurodegeneration and volume loss in the hippocampus. Here, we hypothesize that alcohol reduces the number of granule cells in the human dentate gyrus and consequently contribute to the observed volume loss. Hippocampal samples were isolated from deceased donors with a history of chronic alcohol abuse and from controls with no alcohol overconsumption. From each case, a sample from the mid-portion of hippocampus was sectioned, immunostained for the neuronal nuclear marker NeuN, and counter stained with hematoxylin. Granule cell number and volume of granular cell layer in the dentate gyrus were estimated using the optical fractionator method of stereology. We found a considerable reduction in granule cell number and also a significantly reduced volume of the granular cell layer of chronic alcohol abusers as compared to controls. In controls there was a slight age-related decline in the number of granule cells and volume of granular cell layer in line with previous studies. This was not observed among the alcoholics, possibly due to a larger impact of alcohol abuse than age on the degenerative changes in the dentate gyrus. Loss of neurons in the alcoholic group could either be explained by an increase of cell death or a reduced number of new cells added to the granular cell layer. However, there is no convincing support for an increased neuronal death by chronic alcohol exposure, whereas a growing body of experimental data indicates that neurogenesis is impaired by alcohol. In a recent study, we reported that alcoholics show a reduced number of stem/progenitor cells and immature neurons in the dentate gyrus, hence that alcohol negatively affects hippocampal neurogenesis. The present results further suggest that such impairment of neurogenesis by chronic alcohol abuse also results in a net loss of granule cells in the dentate gyrus of hippocampus.

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Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

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Program #/Poster #: 381.04/M3

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Electrophysiological impairment and K^+ dyshomeostasis in retinal ganglion cell axonopathy

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Abstract: Axonopathy is a common feature among many neurodegenerative disorders. In axonopathy, functional deficits, such as axon transport dysfunction, occur prior to structural degeneration of the axon. Axon degeneration is then followed by degeneration of the soma. This temporal sequence of events is often accompanied by spatial progression, where clusters of neighboring neurons exhibit a similar rate of degeneration. Spatial progression of axonopathy suggests that external cues in the immediate milieu of degenerating neurons may play an important role in disease progression. To determine how neurons in the early stages of axonopathy differ from those in surrounding areas with no overt signs of axonopathy, we compared electrophysiological function with axon transport deficits in retinal ganglion cells (RGCs) in a mouse model of optic neuropathy. In this model, pressure-related stressors induce axonopathy in clusters that ultimately spread to encompass larger areas of the retina and optic nerve. We assessed electrophysiological function of RGCs with and without axon transport deficits, as measured by uptake and anterograde transport of the neural tracer cholera toxin beta (CTB). We found that RGCs with deficits in CTB uptake possess a more depolarized resting membrane potential (Vm), compared to RGCs with intact uptake ($p<0.05$). Despite similar K^+-induced depolarization of Vm ($p>0.05$), the K^+-induced spike rate decreases as the $[K^+]_e$ increases in RGCs with deficient CTB uptake. This is opposed to RGCs with intact CTB transport, where increased $[K^+]_e$ induces a reciprocal increase in spike rate. At the same $[K^+]_e$, the spike rate of RGCs with deficient CTB uptake is significantly lower than that for RGCs with intact CTB uptake ($p<0.05$). *In vitro* studies confirm a disrupted K^+ homeostasis surrounding RGCs exposed to pressure-related stressors. In primary, purified RGCs, elevated pressure leads to increased $[K^+]_e$ in the culture media ($p<0.005$) and a significant decrease in cation influx ($p<0.05$), as compared to control condition. Gene expression and protein localization studies in the optic neuropathy model indicate that this K^+ dyshomeostasis may arise from decreased expression of Na/K-ATPase, particularly in RGCs ($p<0.05$). Our data suggest that axon transport deficits in RGCs are accompanied by electrophysiological impairment that involves failure to re-establish the electrochemical gradients of ions required for maintenance of spiking frequency. This impairment may be linked to K^+ dyshomeostasis secondary to altered expression of the Na/K-ATPase and could contribute to spatiotemporal progression of pathology between neighboring RGCs.

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Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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JSPS KAKENHI Grants-in-Aid for Scientific Research JP15H04999

Title: Loss of neuritin accelerates RGC loss and retinal degeneration in adult mice following optic nerve injury

Authors: *Y. AZUCHI[1,3], K. NAMEKATA[1], T. SHIMADA[2], X. GUO[1], A. KIMURA[1], C. HARADA[1], A. NISHIGAKI[3], K. YAMAGATA[2], T. HARADA[1]
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Abstract: Glaucoma is one of the major causes of blindness and is characterized by progressive degeneration of retinal ganglion cells (RGCs) and their axons. The optic nerve injury (ONI) model mimics some aspects of glaucoma, including RGC death induced by excitotoxicity and oxidative stress, and therefore, it is a useful animal model for glaucoma. Previous studies have shown that neurotrophins, such as brain-derived neurotrophic factor, protect RGCs in an ONI model. Neuritin, also known as candidate plasticity gene 15 (CPG15), was first identified as one of the activity-dependent gene products in the brain. Neuritin is an extracellular, glycosylphosphoinositide-linked protein, which can be secreted as a soluble form by various cells including neuronal and glial cells. Neuritin induces neuritogenesis, neurite arborization, neurite outgrowth and synapse formation, which are involved in the development and functions of the central nervous system. In addition, it is recently thought to be a kind of neurotrophin that regulates neural cell survival. Here, we investigated the function of endogenous neuritin following ONI using *neuritin* knockout (KO) mice. We first examined *neuritin* mRNA expression levels in the mouse retina before and after ONI. ONI induced slight upregulation of *neuritin* mRNA in the retina of WT mice. Next, we investigated developmental abnormalities and severity of ONI-induced degeneration in the *neuritin* KO mouse retina. *In vivo* retinal imaging and histopathological analyses demonstrated that there is no difference in the retinal

structure and number of RGCs between *neuritin* KO and WT mice under normal condition. However, ONI caused more severe RGC death and inner retinal degeneration in *neuritin* KO mice compared with those in WT mice, over the course of 2 weeks following ONI. We also investigated if neuritin has any stimulatory effects on cell survival signaling pathways in the retina following ONI. Previous studies have reported that exposure of cerebellar granule neurons to neuritin markedly induced phosphorylation of Akt and ERK in part by activating the insulin receptor signaling. Akt and ERK signaling pathways are known to promote RGC survival after ONI. In present study, immunoblot analyses revealed that ONI-induced phosphorylation of Akt and ERK were suppressed in *neuritin* KO mice. Our findings suggest that neuritin has neuroprotective effects via Akt and ERK signaling pathways after ONI and may be useful for treatment of glaucoma.

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Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 381.06/M5

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: The Intramural Research Program of the National Eye Institute, NIH

Title: Possible involvement of vwc2l, a constituent of the AMPA receptor complex, in the functioning of neuronal and non-neuronal tissues

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Abstract: Vwc2l (also known as brorin-like) is a secreted protein considered to be a chordin-like BMP antagonist. Vwc2l is a component of the ionotropic AMPA-type glutamate receptor (AMPAR) complex but its function in the complex unknown. Previous proteomics data indicated that vwc2l, together with olfactomedin 1 (Olfm1), Olfm2, Olfm3, vwc2, and neuritin may interact with extracellular parts of the AMPAR core subunits. The objective of this study was to investigate possible functions of vwc2l using mouse and zebrafish models. Quantitative proteomics demonstrated that the amount of vwc2l assembled with AMPARs was strongly reduced in immunoprecipitates obtained with GluA1-4 antibodies from *Olfm1* null brain or retina

membrane fractions compared with wild-type samples, indicating that the integration of vwc2l in native AMPAR complexes is largely mediated by Olfm1. Indeed, mouse and zebrafish vwc2l were co-precipitated with Olfm1 from lysates of COS7 cells transiently transfected with corresponding expression constructs indicating direct interaction of Olfm1 and vwc2l. Overexpression of vwc2l during early zebrafish development induced by an injection of *vwc2l* mRNA into zebrafish eggs resulted in severe developmental defects, mainly in the posterior part of larvae. These defects were remarkably reduced when the *vwc2l* mRNA was injected into *olfm1* knockout eggs, indicating that the effects of vwc2l over- or mis-expression may be modulated by Olfm1. Overexpression of vwc2l may affect TNFα signaling as suggested by the results of RNAseq analysis of zebrafish larva heads 24 hours post-fertilization. TNFα signaling is known to be involved in neuronal degeneration in brain and eye diseases and glutamate toxicity. Moreover, glia-derived soluble TNFα was shown to be a major inducer of retinal ganglion cell death through the activation of Ca^{2+}-permeable AMPA receptor. Another potential target of vwc2l overexpression is SREBP, a transcription factor responsible for synthesis of cholesterol and other lipids. Cholesterol synthesis is known to be important for maintaining healthy neuronal membrane structure in axons and dendrites as well as lipid rafts in synapses. Our data sugest that vwc2l has multiple functions in both neural and non-neural tissues.

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Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

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Program #/Poster #: 381.07/M6

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01NS079166
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Title: Temporal tracking of microglia subtypes in HIV associated neurocognitive disorders by single-cell RNA sequencing

Authors: *J. ZHENG[1], W. RU[1], J. R. ADOLACION[3], R. X. LIANG[2], J. DONG[2], A. POTTER[4], S. S. POTTER[4], N. VARADARAJAN[3], S.-J. TANG[1]
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Abstract: HIV infection induces synaptodendritic injury in the brain. As HIV does not efficiently infect neurons, its influence on neurons is largely due to the effect of infected/activated glial cells. Previous studies implicate the contribution of activated microglia to neuronal damages. However, how microglia are activated in response to HIV remain elusive. We used the transgenic mice expressing gp120, a HIV glycoprotein contributing to neurocognitive disorders, to study microglia turnover. Single cell RNA-Seq was performed on 10000 cells dissociated from the cortex of 2-, 4- and 8- month wide type and gp120-transgenic mice. Our results indicate the chronologic differentiation of microglial subtypes with potential activity in phagocytosis, demyelination, gliosis, or proliferation in the transgenic mice. The findings reveal previously unknown microglial plasticity induced by HIV and the trajectory of potential microglial differentiation that may contribute to synaptodendritic injury.

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Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 381.08/M7

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIAAA

Title: Increased chemokines and chemokine receptors contribute to neurodegeneration in postmortem human alcoholic brain

Authors: *L. QIN, R. P. VETRENO, F. T. CREWS
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Abstract: Chemokines and chemokine receptors are associated with neuroinflammation and their expression is induced by inflammatory mediators. In the current study, qPCR and immunohistochemistry were used to study expression of chemokines and chemokine receptors in post-mortem human orbital frontal cortex (OFC) tissue samples from moderate drinking controls and alcoholics. In the alcoholic OFC, we found significant increases in mRNA levels of C-C motif chemokines (CCL2 [MCP1; 163%], CCL8 [MCP2; 346%], CCL7 [MCP3; 152%], CCL13 [MCP4; 289%], and CCL5 [RANTES; 237%]) and C-X-C motif chemokines (CXCL8 [IL-8; 306%] and CXCL12 [SDF-1; 291%]), compared to moderate drinking control subjects. We also observed an approximate two-fold increase in protein levels of IL-8 in the alcoholic OFC. We also observed significant increases in mRNA levels of C-C motif chemokine receptors (CCR1 [146%], CCR2 [245%], and CCR8 [221%]), C-X-C motif chemokine receptors (CXCR1

[252%], CXCR2 [264%], CXCR3 [210%], and CXCR4 [235%]), and the C-X3-C chemokine receptor for fractalkine CX3CR1 (337%) in the alcoholic OFC, relative to moderate drinking control subjects. Interestingly, expression of these chemokines and chemokine receptors in the human OFC were correlated with each other, and negatively correlated with age of drinking onset such that an earlier age of drinking initiation (~18 years of age) was associated with greater expression levels of these chemokine signaling molecules. Gene expression of the chemokines CCL3 (MIP-1α), CCL4 (MIP-1β), CCL19 (MIP-3β), CXCL10 (IP-10), and CX3CL1 (fractalkine) were unchanged in the alcoholic OFC. To evaluate the association of increased chemokines and chemokine receptors with neurodegeneration in the post-mortem alcoholic OFC, we investigated caspase-3, -7, -8, -9 mRNA, and activated caspase-3 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. Expression of caspase signaling molecules were negatively correlated with age of drinking onset, and positively correlated with expression of chemokines and chemokine receptors. Together, these data suggest that the enhanced expression of chemokines and chemokine receptors in human alcoholic brain could contribute to chronic ethanol-induced neuroinflammation and neurodegeneration.

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Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

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Support: NIH Grant R01NS086981
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ARCS foundation

Title: Axonal damage in spinal cord is associated with gray matter atrophy in sensorimotor cortex in mice with experimental autoimmune encephalomyelitis

Authors: *C. E. MEYER[1], J. L. GAO[2], J. CHENG[2], M. OBEROI[2], H. JOHNSONBAUGH[2], S. LEPORE[2], F. KURTH[2], M. J. THURSTON[2], N. ITOH[2], R. VOSKUHL[3], A. MACKENZIE-GRAHAM[4]
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Abstract: Gray Matter (GM) atrophy is one of the best predictors of long term disability in multiple sclerosis (MS) and recent evidence has further revealed that localized GM atrophy in MS is associated with clinically relevant disabilities. However, the mechanisms underlying GM atrophy remain largely unknown. In this report, we used voxel-based morphometry (VBM) to characterize the pattern of GM atrophy in the early stages of the most widely used animal model of MS, experimental autoimmune encephalomyelitis (EAE). We identified GM atrophy throughout the cerebral cortex, cerebellum, caudoputamen, and thalamus of mice with EAE compared to healthy controls, consistent with previous literature. Our investigation revealed that axonal damage and loss in the spinal cord was strongly correlated with voxelwise GM atrophy within motor and sensory regions of the cortex. We believe this is the first report to use VBM in EAE in mouse and to associate specific pathology with localized GM atrophy in EAE. This finding suggests that axonal damage and loss plays a key role in permanent GM atrophy. Understanding the relationship between cellular pathology and GM atrophy may lead to the development of better neuroprotective therapies for patients with MS.

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Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Sphingosine kinase 1-associated autophagy differs between neurons and astrocytes

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Abstract: Autophagy is a degradative pathway for removing aggregated proteins, damaged organelles, and parasites. Evidence indicates that autophagic pathways differ between cell types. In neurons, autophagy plays a homeostatic role, compared to a survival mechanism employed by starving non-neuronal cells. We investigated if sphingosine kinase 1 (SK1)-associated autophagy differs between two symbiotic brain cell types—neurons and astrocytes. SK1 synthesizes sphingosine-1-phosphate, which regulates autophagy in non-neuronal cells and in neurons. We found that benzoxazine autophagy inducers upregulate SK1 and neuroprotective autophagy in neurons, but not in astrocytes. Starvation enhances SK1-associated autophagy in astrocytes, but not in neurons. In astrocytes, SK1 is cytoprotective and promotes the degradation of an autophagy substrate, mutant huntingtin, the protein that causes Huntington disease. Overexpressed SK1 is unexpectedly toxic to neurons, and its toxicity localizes to the neuronal soma, demonstrating an intricate relationship between the localization of SK1's activity and neurotoxicity. Our results underscore the importance of cell type-specific autophagic differences in any efforts to target autophagy therapeutically.

Disclosures: J.F. Moruno Manchon: None. **N. Uzor:** None. **C.R. Ambati:** None. **V. Shetty:** None. **N. Putluri:** None. **C. Jagannath:** None. **L.D. McCullough:** None. **A.S. Tsvetkov:** None.

Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 381.11/M10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: PNSD 2015/005
PR26/16-11B PR26/16-11B-2

Title: Characterization of the brain innate immune system in an animal model of Wernicke-Korsakoff syndrome

Authors: *B. GARCIA-BUENO[1], M. MOYA[2], A. BALLESTA[2], A. RODRIGUEZ GONZALEZ[2], M. SANCIO[2], D. SAN FELIPE[2], M. LOPEZ-GALLARDO[2], R. GOMEZ DE HERAS[2], F. RODRIGUEZ DE FONSECA[2], E. M. MARCO[2], L. ORIO[3]
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Abstract: Wernicke-Korsakoff syndrome (WKS) is the consequence of a severe deficiency of thiamin (B1 vitamin) that is typically related to alcohol abuse. The clinical course of WKS begins with Wernicke's Encephalopathy (W), characterized by ataxia and cognitive dysfunction,

and may continue with the Korsakoff syndrome (WK), characterized by irreversible brain damage. The molecular mechanism responsible of the transition between both stages is unknown, although some evidences suggest a role for neuroinflammation and oxidative/nitrosative stress.
We characterized an animal model of both pathological states, investigating also alterations on innate immune Toll-like receptor 4 and tested a potential approach to manage such alterations based on the systemic administration of the anti-inflammatory oleylethanolamide (OEA).
W was induced by feeding the animals with a thiamine-deficient diet and administration of a thiamine antagonist (pyrithiamine, 0.5mg/Kg, ip,). WK was then triggered by the intravenous administration of a glucose overdose (5g/Kg). For the characterization of the model, general activity was analyzed in the open field and motor coordination in the rotarod test. OEA (10 mg/kg, i.p.) was daily administered during the last 6 experimental days.
W animals showed a loss of body weight starting from day 12, reaching around the 10% of the body weight loss at the end of the experiment. In the rotarod test, an impaired performance was exhibited by W animals. Some W and WK animals, by the end of the study displayed rigid movements, splayed legs, hunched posture and sometimes tremors and jerking seizures. We also shown a concomitant increase of the TLR4 ligand lipopolysaccharide plasma levels and an up-regulation of TLR4 signaling pathway in the WK animals. OEA recovered WK animals' performance in the rotarod test. Similarly, OEA seems also to revert the increased anxiety levels observed in W and WK animals in the elevated plus-maze (EPM).
The pharmacological modulation of innate immunity could be an attractive therapeutical strategy to manage the transition between reversible to irreversible structural and functional damage in the Wernicke-Korsakoff syndrome.

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Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 381.12/M11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Kuwait University grant RW01/14

Title: Lead exposure during lactation increases the levels of quinolinic acid in blood and quinolinic acid immunoreactive cells in the brain of young rats

Authors: *K. M. KHAN[1,2], M. S. RAO[3], A. RAHMAN[4]
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Abstract: Lead (Pb) is a neurotoxicant, which is particularly toxic to the developing brain. Quinolinic acid (QA), a metabolite of the kynurenine pathway of tryptophan metabolism, is also a known neurotoxicant, affecting hippocampal, striatal and cortical neurons. Pb and QA share certain features of neurotoxicity; both produce oxidative stress and induce tau hyperphosphorylation. Production of QA in the brain is increased by oxidative stress. We have previously shown that exposure to Pb or intraventricular infusion of QA lead to learning and memory deficits in young rats. The purpose of this study was to investigate whether exposure to low levels of Pb during lactation has an effect on the levels of QA in blood and QA immunoreactive neurons in different brain regions of young rats. Wistar rat pups were exposed to 0.2% Pb-acetate via their dams' drinking water from postnatal day (PND) 1 to 21(Pb-exposed group, n=10). The control group (C, n=10) was given regular tap water. QA level in serum was measured by ELISA on PND45 and PND60. QA-immunoreactive neurons in cerebral cortex, thalamus and hippocampus (dentate gyrus, CA3 and CA1 areas) of Pb-exposed and control rats were quantified in the brain sections immunostained for QA on PND45 and PND60. Pb exposure did not affect the level of QA in serum in the PND45 rats (5.74±0.77ng/ml in control vs 6.43±0.35 ng/ml in Pb-exposed, $p>0.05$), whereas, in PND60 rats, Pb exposure significantly increased QA levels as compared to control group (5.72±0.80 vs 9.01±1.0; $p<0.001$). Pb-exposed rats had significantly higher number of QA-immunoreactive cells in all the areas examined at both PND45 ($p<0.05$) and PND60 ($p<0.01$), with the exception of the thalamus in the PND45 rats, where the difference between Pb-exposed and control rats was not significant. These results show that Pb exposure increases the levels of QA in the blood and QA immunoreactive cells in brain regions. This increase in QA levels may be the cause for Pb-induced learning and memory deficits, which we reported earlier

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Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 381.13/M12

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: C9ORF72 poly-GA inclusions promote phosphorylated TDP-43 aggregation *in vitro* and *in vivo*

Authors: *T. NONAKA, M. HASEGAWA
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Abstract: Amyotrophic lateral sclerosis and frontotemporal lobar degeneration are neurodegenerative diseases characterized by accumulation of insoluble aggregates of phosphorylated 43-kDa TAR DNA-binding protein (TDP-43), and linked with abnormal expansion of a hexanucleotide repeat in an intron of chromosome 9 open reading frame 72 (*C9ORF72*). However, the relationship between *C9ORF72* mutations and TDP-43 aggregation remains unknown. Non-ATG-dependent translation of *C9ORF72* repeats produces dipeptide repeat (DPR) proteins, which form p62-positive aggregates in cerebral cortex and cerebellum of patients. Here, we show that the formation of poly-GA protein inclusions induced intracellular aggregation of endogenous and exogenous TDP-43 in cultured cells. Poly-GA aggregation preceded accumulation of phosphorylated TDP-43. These inclusions induced intracellular aggregation of phosphorylated TDP-43, but not tau or alpha-synuclein. Formation of phosphorylated TDP-43 aggregates depends on the number of poly-GA repeats. Detergent-insoluble fraction from cells co-expressing poly-GA and TDP-43 could function as seeds for further TDP-43 aggregation. We also present *in vivo* evidence that poly-GA inclusions elicit intracellular aggregation of endogenous TDP-43 and neurodegeneration in mice expressing poly-GA, without formation of nuclear RNA foci containing GGGGCC repeat expansion or loss-of-function of the C9ORF72 protein. These findings suggest a novel pathogenic mechanism that poly-GA protein aggregation directly promotes pathogenic changes of TDP-43, leading to disease-associated neurodegeneration in patients with *C9ORF72* mutations.

Disclosures: T. Nonaka: None. **M. Hasegawa:** None.

Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

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Program #/Poster #: 381.14/M13

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Neuroprotective role of sigma-1 receptors in amyotrophic lateral sclerosis relevant to nucleocytoplasmic transport

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Abstract: Expanded hexanucleotide G_4C_2 repeats lead to the nucleocytoplasmic transport defect in the nuclear pore complex (NPC). G_4C_2 RNA repeats were shown to interact with Ran GTPase-

activating protein 1 (RanGAP1) at the NPC and impaired the conversion of RanGTP to RanGDP, causing the former to accumulate in the cytosol of neurons from *C9orf72*-related amyotrophic lateral sclerosis (ALS) patients. Missense mutation at amino acid E102Q of sigma-1 receptor (Sig-1R) chaperons was reported in familial ALS. The molecular action of Sig-1Rs in ALS is unknown even though Sig-1Rs exist at the nuclear envelope. Here, we examined the relationship between G_4C_2 RNA repeats, Sig-1Rs, and RanGAP1. Immunoprecipitation assay and fluorescence confocal microscopy reveal that Sig-1Rs associate with RanGAP1 in the NPC. Sig-1Rs partly colocalize with Cy3-labeled C_4G_2 RNA probe in the perinuclear region by using RNA fluorescence *in situ* hybridization (i.e., RNA-FISH). Interestingly, the recombinant glutathione S-transferase (GST)-tagged full-length Sig-1R proteins (amino acids 1-223) from both human and mouse physically interact with biotin labeled G_4C_2 RNA repeats in the biotinylated RNA pull-down assay. To identify the G_4C_2 RNA-protein interacting domains on the Sig-1R, we purified three truncated fragments of GST-tagged Sig-1R including amino acids 1-70, 71-150 and 151-223. The results demonstrated that protein fragments including amino acids 71-150 and 151-223 of Sig-1R are important for the Sig-1R-G_4C_2 RNA interaction. Furthermore, the Sig-1R E102Q mutation, reported to cause ALS, causes a decrease in its affinity with G_4C_2 repeats. The overexpresson of Sig-1Rs reduces the cytoplasmic accumulation of Ran GTPase while knockdown or knockout of Sig-1Rs exacerbates the cytosolic accumulation. Further, we found that Sig-1Rs bind to FG-repeats-containing nucleoporins of NPC and stabilize those NPC proteins. Thus, our results suggest dual neuroprotective roles of Sig-1Rs in the G_4C_2 subtype of ALS: one by enhancing the nucleocytoplasmic transport by stabilizing the NPC and the other by sponging away the toxic G_4C_2 repeats. Sig-1R drugs may thus benefit the G_4C_2 subtype of ALS patients. (Supported by IRP, NIDA, NIH/DHHS)

Disclosures: T. Su: None.

Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 381.15/M14

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Carboxypeptidase E/neurotrophic factor-α1 is a novel neuroprotective protein functioning independently of its prohormone processing enzymatic activity

Authors: *L. XIAO, X. YANG, V. K. SHARMA, D. ABEBE, A. PELTEKIAN, Y. P. LOH
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Abstract: Carboxypeptidase E (CPE) also known as Neurotrophic factor-α1 (NF-α1), was first identified as an exopeptidase and is highly expressed in the nervous and endocrine systems. It

functions intracellularly as a prohormone/proneuropeptide processing enzyme. Recent studies show that CPE/ NF-α1 plays a critical role extracellularly, as a trophic factor to mediate neuroprotection, stem cell differentiation and neurite outgrowth *in vitro*. To show that these neurological functions are independent of its enzymatic activity, we generated a CPE/NF-α1 knockout (CPE-KO) and a mouse model with a CPE E342Q point mutation which obliterates the enzymatic activity. We found that both CPE-KO and E342Q mice exhibited increased body weight and glucose levels due to lack of processing of prohormones and neuropeptides to yield insulin and peptides that affect feeding. Brain morphology studies by Nissl staining revealed that the CPE-KO mice had significant degeneration of CA3 regions of hippocampus, while E342Q mice had an intact hippocampus, similar to wild-type (WT) animals. In addition, doublecortin positive cells (neuroblast marker) in CPE-KO mice were significantly decreased in dentate gyrus, but there was no difference between E342Q and WT mice. Microtubule-associated protein 2 staining showed degeneration of dendrites in the hippocampal CA3 region in CPE-KO mice, but there was no difference between E342Q and WT mice. To investigate the effect of E342Q mutation on various behaviors, open field, forced-swim test, and Morris water maze tests were performed on the mice. The results showed that WT and E342Q mice exhibited a gradual decrease in escape latency during the 5-day training in the Morris water maze test, while CPE-KO mice demonstrated an abnormal acquisition curve. In probe test, both WT and E342Q mice spent twice as much time in the target quadrant than other quadrants, unlike the CPE-KO mice. These results suggest E342Q mutant mice maintain normal learning and cognitive ability despite the loss of CPE enzymatic function; in contrast learning and memory function were compromised in CPE KO mice. This study provides *in vivo* evidence that CPE/NF-α1 is a novel trophic factor that functions in neuroprotection, independent of its enzymatic role in prohormone processing.

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Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

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Program #/Poster #: 381.16/M15

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: JSPS KAKENHI Grant Number 17J09810 to MN.
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Title: Layer-dependent time course of neuronal death following ultraviolet light irradiation of the cerebral cortex

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Abstract: Ultraviolet (UV) light irradiation applied to living cells induces a variety of reactions including inflammation, DNA damage, and cell death. We previously reported that the destructive features of UV light can be used to create focal brain lesions on the cerebral cortex. In that study, almost no neurons were observed within an inverted bell-shaped UV lesion at 5 days after irradiation. The present study investigated the processes underlying neuronal degeneration after UV exposure. Adult Wistar rats were anesthetized and irradiated with UV light (UV-A; wavelength 365 nm, 2.0 mWh) through an optic cannula (flat circle, 400 µm in diameter) that was in contact with the dura mater at a targeted brain site (anterior parietal cortex; bregma -3.7 mm, 2.0 mm from the midline). The rats were perfused for histological analyses at six different timepoints after the irradiation: 2 h, 6 h, 12 h, 24 h, 3 days, and 5 days. Immunohistochemistry for NeuN, which is a neuronal marker, revealed that neuronal degeneration began in the bottom half of the lesion within 6 h after irradiation. At 24 h after irradiation, almost all neurons in the bottom half of the lesion had disappeared and there was an evident decrease in the number of neurons in the top half. At 3 days after irradiation, only a cluster of neurons in layer II/III remained, in the central part of the lesion. At 5 days after irradiation, almost no surviving neurons were observed. Terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) staining to detect apoptotic cell death revealed that apoptosis began at 2 h and that the neurons surviving at 3 days had already initiated this process. Taken together, the present results indicate that UV irradiation induced apoptotic cell death in the cerebral cortex, and that not all of the neuronal degeneration within the lesion occurred simultaneously. These findings suggest that neuronal tolerance to UV irradiation varies among the cortical layers.

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Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

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Program #/Poster #: 381.17/M16

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India
Kerala State Council for Science, Technology and Environment, India
Department of Biotechnology, India
Council for Scientific and Industrial Research, India

Title: Attenuation of excitotoxicty induced molecular changes by NMDAR inhibitors

Authors: *M. KUMAR[1], S. PAUL[1], M. JOHN[1], M. MAYADEVI[1], J. JAMES[2], R. V. OMKUMAR[1]
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Abstract: Over activation of Ca^{2+} channels cause excitotoxicity induced neuronal death in various neurodegenerative diseases. Molecular mechanisms leading to cell death under excitotoxic conditions are still not completely understood. In this study we are observing the changes at the level of protein expression as well as their post translational modifications upon exictotoxic insults through acute NMDA treatment via intracerebroventricular (ICV) injection and chronic monosodium glutamate (MSG) treatment via intraperitoneal (i.p) injection in adult male rats. Activation of calcium signaling by NMDA receptor is expected upon the above mentioned treatments. Methodology involves bilateral ICV injections of NMDA to rats for the acute treatment that was accompanied with prior ICV administration of NMDAR inhibitor, MK801 or CaM kinase inhibitor, KN93 or the vehicle. Chronic excitotoxic insult was caused by 15 days of MSG injection by i.p route to adult male rats, which were also orally administered with either vehicle or an NMDAR inhibitory plant extract. A group of animals were also fed with a known NMDAR inhibitory drug, dextromethorphan. Analysis by Morris water maze (MWM) test showed that the behavioral impairment caused by MSG administration could be ameliorated by simultaneous treatment with one of the NMDAR inhibitors, either the plant extract or dextromethorphan. We have shown increased p-GluN2B by immunoblotting in hippocampal and cortical tissues in both NMDA and MSG treated animals which were found to be attenuated by MK801 and the NMDAR inhibitory plant extract respectively. We have seen reduction of p-AKT and p-TrkB upon excitotoxic insults. Changes in the levels of other proteins related to calcium signalling and cell survival such as p-CREB and caspases are also being analyzed. Data from this study might provide new insights on signaling pathways during excitotoxic insults.

Disclosures: M. Kumar: None. **S. Paul:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Soumya Paul is designated as an inventor in Indian Patent Application No. 3616/CHE/2013. **M. John:** None. **M. Mayadevi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); M Mayadevi is designated as an inventor in Indian Patent Application No. 3616/CHE/2013. **J. James:** None. **R.V. Omkumar:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr RV Omkumar is designated as an inventor in Indian Patent Application No. 3616/CHE/2013.

Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 381.18/M17

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: City University of Hong Kong grant 7200484
Research Grants Council grant 21106716

Title: Molecular mechanisms of neurodegeneration through the altered circadian clock

Authors: *Y. CHANG, M. CHOI, J. KIM
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Abstract: Circadian clock, a cell-autonomous oscillator, presents in most of the cells to drive a 24-h cycle in biological and behavioral processes. The core clock mechanism is composed of a transcriptional negative feedback loop, where the transcription factor BMAL1-CLOCK drives the expression of their target genes including their own inhibitors, Period (PER) and Cryptochrome (Cry), as well as other circadian output genes. As several lines of epidemiological evidence have indicated a bidirectional linkage between altered circadian rhythms and neurodegeneration, we take effort in exploring the underlying mechanisms in this association. In this study, we found that circadian clocks were altered in the presence of neurotoxic stimuli via a decline activity of transcription factors BMAL1-CLOCK in primary neurons. In addition, focal adhesion pathway and intracellular tension signaling relating to cytoskeleton were identified as the affected processes by neurodegeneration through RNA sequencing analysis. Taken together, our works aims to provide a comprehensive insight into a relationship between circadian clock and neurodegeneration.

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Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

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Program #/Poster #: 381.19/M18

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: IWT R&D grant 150003

Title: Image-based profiling of synaptic connectivity in primary neuronal culture

Authors: *P. VERSTRAELEN[1], M. VERSCHUUREN[1], R. NUYDENS[2], P. LARSEN[2], J.-P. TIMMERMANS[1], W. H. DE VOS[1]
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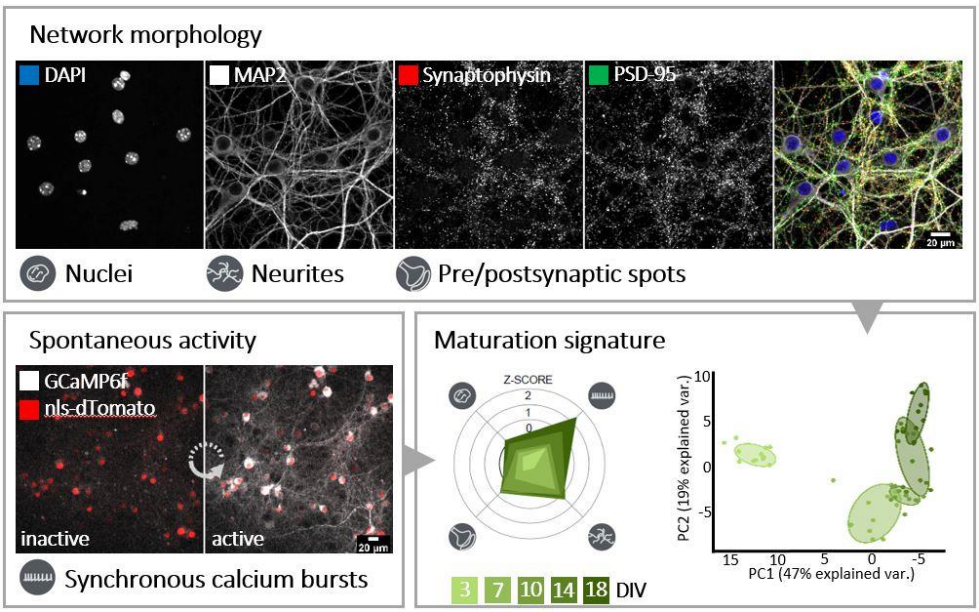
Abstract: Development of treatments for neurodegenerative disorders demands understanding the mechanisms that contribute to synaptic plasticity and the loss thereof. This in turn, requires methods to quantify the connectivity of a neuronal network with high accuracy and throughput. To meet this demand, we have established a microscopy-based assay that integrates morphological (nuclei, neurites, pre- and postsynaptic markers) and functional (spontaneous synchronous calcium activity) correlates of neuronal connectivity in mouse primary and human iPSC-derived neurons [1, 2, fig 1]. Using supervised and unsupervised classification approaches, we were able to establish a signature of neuronal maturation in primary cultures, that integrated a diverse set of connectivity features. We found that dilution of B27 medium supplement induced a gradual connectivity impairment as evidenced by a shift in the maturation signature. Dysregulation of microtubule stability, induced by pharmacological means or by MAPT-(P301L) overexpression, resulted in much more pronounced impairment [3]. Using a focused compound screen, we discovered that inhibition of dual leucine zipper kinase activity rescued the connectivity defects witnessed in both adverse conditions. This underscores the neuroprotective effect that has been described for this compound [4]. Our results illustrate that image-based profiling enables sensitive interrogation of neuronal connectivity. Therefore, the current approach holds promise for identifying pathways and treatments that preserve or rescue neuronal connectivity in neurodegenerative disorders.



References [1] Verstraelen et al., 2018, Front Neurosci Accepted as invited review. [2] Kuijlaars et al., 2016, Sci Rep, doi:10.1038/srep36529 [3] Verstraelen et al., 2017, Front Cell Neurosci, doi: 10.3389/fncel.2017.00173 [4] Le Pichon et al., 2017, Sci Transl Med, doi: 10.1126/scitranslmed.aag0394

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Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

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Program #/Poster #: 381.20/N1

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH AG023084 to BVZ
NIH NS034467 to BVZ
Cure Alzheimer's Fund to BVZ

Title: PICALM has crucial role in embryonal development

Authors: *D. LAZIC[1,2], Z. ZHAO[1], A. MONTAGNE[1], A. P. SAGARE[1], T. MAEDA[3], B. V. ZLOKOVIC[1]
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Abstract: Phosphatidylinositol-binding clathrin assembly protein, *PICALM*, has been validated to be associated with Alzheimer's disease (AD). PICALM has an important biological role in facilitating the clearance of amyloid-β through the blood-brain barrier, as well as maintaining brain homeostasis. In developing neurons, PICALM provides proper function of synaptic transmission by regulating the size and density of synaptic vesicles. Studies have shown that protective *PICALM*-associated single-nucleotide polymorphism (SNP) $rs3851179^{A}$ is correlated with increased enthorhinal cortical thickness when compared to $rs3851179^{G}$ SNP. Here, we crossed $Picalm^{+/-}$ male with $Picalm^{+/-}$ female mouse and collected brains from $Picalm^{-/-}$, $Picalm^{+/-}$ and $Picalm^{+/+}$ embryos at E12.5, E15.5 and E18.5 developmental stage. At E15.5 and E18.5, we observed microcephaly in $Picalm^{-/-}$ embryos when compared to their $Picalm^{+/+}$ littermates, leading to their premature death *in utero*. However, $Picalm^{+/-}$ embryos do not exhibit any signs of degeneration and have similar phenotype as $Picalm^{+/+}$ embryos. Furthermore, we

found drastic differences in distribution of special AT-rich sequence binding protein 2 (SATB2), COUP-TF-interacting protein 2 (CTIP2) and T-box brain 1 (TBR1), transcription factors involved in post-mitotic differentiation of developing neurons and the thickness of neocortex was decreased by 50% in *Picalm*$^{-/-}$ when compared with *Picalm*$^{+/+}$ embryos. *In vitro* experiments with primary neuronal cultures further showed different patterns in neuronal maturation in *Picalm*$^{-/-}$ and *Picalm*$^{+/+}$ cells. Because PICALM plays a crucial role in endocytosis, PICALM deficiency has strong negative effect on the developing brain that is highly sensitive to protein turnover. Overall, our data demonstrate that PICALM has crucial role in the embryonic brain development.

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Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

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Program #/Poster #: 381.21/N2

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01NS096176

Title: Smcr8 depletion disrupts lysosome function and induces inflammation in the immune system and nervous system

Authors: *C. LIANG[1], M. YANG[2], Q. SHAO[2], L. MA[2], J.-K. LEE[1], J. CHEN[2]
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Abstract: Hexanucleotide repeat expansion in the first intron of *C9ORF72* gene causes the most common forms of familial FTD and ALS. We and other groups have previously identified that SMCR8 forms a protein complex with C9ORF72 and regulates autophagy initiation and flux. However, the *in vivo* function of SMCR8 is unknown. Here, we report that *Smcr8* knockout mice developed splenomegaly and lymphadenopathy. Mutant spleens exhibited increased numbers of CD68-positive macrophages and increased expression of inflammatory cytokines, including IL-1β, IL-6, TNF-α and IFN-1β. These phenotypes resemble the phenotypes observed in *C9orf72* null mice, therefore suggesting that SMCR8 and C9ORF72 not only physically interact but may also have similar functions. Indeed, both *C9orf72*-/- and *Smcr8*-/- mice displayed increased numbers of astrocytes in the hypothalamus. SMCR8 was previously identified as a component of purified lysosomes and *Smcr8* null MEFs displayed defects in lysosome-dependent autophagy. Using *Smcr8* depleted peritoneal macrophages, we found accumulation of enlarged autolysosomes without tubulation. Furthermore, mutant macrophages failed to disassemble

autophagosomses during long-term starvation and displayed a significant increase in cells with Lamp1 and LC3 double-positive autolysosomes. This suggests that depletion of SMCR8 disrupts autophagy lysosome reformation and impairs lysosomal degradation. Similar results were found within the hypothalamus of *Smcr8* knockout mice, which displayed accumulation of Lamp1-positive organelles. SMCR8 was previously identified to form a stable cognate protein complex with C9ORF72 that protects each other from degradation. Therefore, it is tempting to speculate that haploinsufficiency of C9ORF72/SMCR8 in FTD/ALS diseases leads to impaired lysosomal degradation, dysfunction of macrophages and astrocytes, and contributes to neurodegeneration.

Disclosures: C. Liang: None. **M. Yang:** None. **Q. Shao:** None. **L. Ma:** None. **J. Lee:** None. **J. Chen:** None.

Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 381.22/N3

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant DE022757
VA Grant 5I01BX000638

Title: Transcriptomics analysis highlights the sexually dimorphic mechanical pain hypersensitivity mechanisms

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Abstract: Females more frequently than males suffer from chronic pain. The sexual dimorphism mechanisms underlying the experience of pain from non-painful tactile stimuli (mechanical allodynia) have started to emerge. Here, we report a novel murine model of sexually dimorphic mechanical allodynia induced in female, but not male mice, by sciatic nerve injection of myelin basic protein 84-104 fragment (IS-MBP). Our RNA-seq analysis of the sciatic nerve, dorsal root ganglia (DRG) and the dorsal spinal cord transcriptomes in female and male mice exposed to IS-MBP revealed the sexually dimorphic regulation patterns in: (1) lipid metabolism signaling; (2) cell-mediated immunity signaling; (3) nociceptive, including glutamate and Ca2d1/calcium, (4) anti-nociceptive, including opioid and cannabinoid, receptor, signaling; (5) X-chromosome-linked TIMP-1 gene; (6) maternally-imprinted IL6, H19 and IGF2 genes and (7) non-coding

RNAs, including, the X-linked XIST, microRNA. The overall high variation in the expression of gene isoforms suggests a possible regulatory role of splice variants and UTRs. The greatest number of the sexually dimorphic gene regulation was dominant in DRG and spinal cord. Mitochondrial dysfunction and unfolded protein response were differentially induced by IS MBP relative to the controls. The present study offers a novel model and, to our knowledge, the first global molecular database of sexual dimorphism of neuropathic pain.

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Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 381.23/N4

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01NS091519

Title: Regulation of microglial function in aging brains by CSF-1 and GM-CSF

Authors: *V. CHITU, P. WANG, D. ZHENG, H. KETCHUM, E. R. STANLEY
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Abstract: Adult onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is an adult onset dementia caused by dominantly-inherited inactivating mutations in the CSF-1 receptor kinase (CSF-1R). Our studies in the *Csf1r*$^{+/-}$ mouse model of ALSP suggest that aberrant activation of microglia by GM-CSF (also known as CSF-2) plays an important role in the pathology of ALSP. Indeed, removal of a *Csf2* allele rescued the cognitive and olfactory phenotypes of the *Csf1r*$^{+/-}$ mice, but not the loss of motor coordination. Unexpectedly, these studies showed that *Csf2*$^{+/-}$ mice exhibit olfactory, cognitive and motor phenotypes. However, these were milder than those of *Csf1r*$^{+/-}$ mice. To determine how reduction in *Csf1r* or *Csf2* expression alone, or in combination, affect microglial function in aged mice, we analyzed changes in the transcriptome of cerebral Tmem119^{+} microglia compared to wild type controls (n=3, females, 21 months of age). *Csf1r* heterozygosity led to the differential expression of 496 genes. Bioinformatic analysis indicated that *Csf1r* heterozygosity did not produce a neurotrophic defect or overt inflammatory activation of microglia. Rather, the transcriptional profile predicted a maladaptive phenotype characterized by changes in membrane proteins (40% of differentially expressed genes (DEGs)), unresponsiveness to deactivating stimuli and mitochondrial dysfunction that could result from changes in lipid metabolism. *Csf2* heterozygosity affected the

expression of 1168 genes in microglia. Similar to *Csf1r* heterozygosity, the neuropathology in *Csf2*$^{+/-}$ mice was not associated with a neurotrophic deficit or with inflammatory activation of microglia. Instead, GM-CSF insufficiency caused the activation of antioxidant and lipid metabolic pathways that are expected to be neuroprotective. However, it also promoted the expression of components of the complement system that has a well-established role in synapse loss in neurodegenerative disease. Examination of microglia isolated from *Csf1r*$^{+/-}$;*Csf2*$^{+/-}$ mice revealed a substantial reduction in DEGs (254) and restoration of most signaling pathways and biological processes affected by either single heterozygosity. Together, these data suggest that balanced signaling by CSF-1R and GM-CSF in microglia is critical for brain homeostasis during aging and that regulation of the redox status and lipid metabolism are converging targets.

Disclosures: V. Chitu: None. **P. Wang:** None. **D. Zheng:** None. **H. Ketchum:** None. **E.R. Stanley:** None.

Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 381.24/N5

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R15 NS101594

Title: Functional characterization of TMEM163 single nucleotide polymorphisms

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Abstract: Mucolipidosis type IV (MLIV) is a genetic lysosomal storage disorder caused by the dysfunction of Mucolipin-1 (TRPML1) ion channel. We previously reported an elevation of zinc within lysosomes of MLIV cells and identified Transmembrane (TMEM)-163 protein, a putative zinc transporter, as an interaction partner for TRPML1. Both proteins co-localize on the plasma membrane and lysosomal compartments. The function of TMEM163 is yet to be fully elucidated; however, it is predicted to have six transmembrane domains (TMD) and our data show that TMEM163 is an efflux transporter. To further study its function and how it might be involved in certain human diseases like MLIV, we used the single nucleotide polymorphism database (dbSNP) from the National Center for Biotechnology Information website to study specific sequence variations within the *TMEM163* gene. We identified SNPs that produce amino acid substitution (non-synonymous) when compared with the wild-type TMEM163 protein sequence. We then used bioinformatics to predict the topology of TMEM163 protein, and found that some non-synonymous SNPs are located in areas of the protein that could affect post-translational

modification and change its structure and function. Thus, we hypothesize that SNPs that affect post-translational modifications will disrupt the zinc efflux transport function of TMEM163 in cultured cells over-expressing the protein. To accomplish this study, we performed site-directed mutagenesis using In-Fusion homologous recombination cloning technique to systematically replace specific nucleotides corresponding to the SNPs located within or in proximity of N- and C-termini, as well as between TMD-1 and TMD-6. Upon sequence verification, we tested the effect of each SNP-associated mutant by transfecting them into cultured HEK-293 cells. Twenty-four hours post transfection, the cells were exposed to zinc chloride (10 μM) and zinc pyrithione (1 μM) and assayed for zinc flux using the zinc-specific, membrane impermeable FluoZin-3 AM dye. Our results confirmed that TMEM163 is a zinc efflux transporter and we found that the non-synonymous SNP mutants showed markedly lower zinc efflux relative to the wild-type control. Additional experiments using zinc specific dyes with different affinity are being conducted. Understanding the function of TMEM163 could explain the nature of its interaction with TRPML1 and its potential role on the observed zinc dyshomeostasis in MLIV cells.

Disclosures: V.B. Sanchez: None. **S. Ali:** None. **M.P. Cuajungco:** None.

Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 381.25/N6

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant AA020103
NIH Grant AA025328

Title: LncRNA NEAT1 in ethanol induced neuronal cell death

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Abstract: Background: Our previous work has demonstrated that ethanol increase the expression of Kruppel-like factor 11 (KLF11), a neuronal cell death mediator in brain of rodent treated with alcohol by binge or chronic feeding. Our preliminary data suggest that KLF11 functionally interacts with the Nuclear Enriched Abundant Transcript 1 (NEAT1), a long non-coding RNA playing roles in cell proliferation, growth. **Objectives**: The current work is to generate the stable expressing CRISPR/Cpf1 and NEAT1 dual gRNAs to decipher the role of the two isoforms of NEAT1 in this ethanol induced and KLF11 mediated neuronal cell death **Methods**: 1) Construct vectors that contain the nuclease AsCpf1 and gRNAs specifically

targeting to the interesting genes; 2) Establish stable NEAT1-1 or NEAT1-2 knockout cells by our recently established CRISPR/Cas mediated dual gRNAs technique; 3) Determine the role of NEAT1-1 and NEAT1-2 in ethanol treated cells; 4) Identify the interaction of NEAT1-1 and NEAT1-2 with KLF11 in ethanol induced cells death. **Results**: Our genotyping analysis indicated that we have successfully established a stable transfected CRISPR-Cpf1 SH-SY5Y cells which completely KO the NEAT1-1 from the genome with abolishing both transcripts of NEAT1-1 and NEAT1-2, suggesting that both isoforms share the same promoter; Treatment with 100mM or 150mM EtOH significantly increased the expression of NEAT1-2, but not NEAT1-1 in SH-SY5Y cells; There exists the interaction between NEAT1-2, but not NEAT1-1 with KLF11; The treatment of 100mM or 150mM EtOH disrupted the combination of KLF11 with NEAT1-2, without changing the co-localization between KLF11 and NEAT1-1. **Conclusion:** Our study demonstrates that this dual gRNA guided CRISP-Cpf1 system is a reliable approach to specifically knock out the lncRNA NEAT1. Establishment of NEAT1 isoform specific cell models provides a critical research tool for better understanding of how NEAT1 and its interaction with KLF11 are involved in ethanol induced neural cell death. As a result, NEAT1 may serve a therapeutic target for intervention.

Disclosures: L. Shi: None. **B. Zheng:** None. **Y. Mo:** None. **J. Wang:** None.

Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 381.26/N7

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01NS091519

Title: Contribution of the neuronal and microglial lineages to the behavioral phenotypes of $Csf1r^{+/-}$ ALSP mice

Authors: *F. BIUNDO[1], V. CHITU[2], G. S. SHLAGER[2], E. S. PARK[2], M. E. GULINELLO[2], K. SAHA[2], H. KETCHUM[2], E. R. STANLEY[2]
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Abstract: Adult onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is a dementia caused by dominantly-inherited mutations in *CSF1R* gene that result in CSF-1R kinase inactivation or disrupt expression. A *Csf1r* heterozygous mouse model of ALSP mimics the clinical symptoms, radiological and histological changes observed in human patients. In addition, we found an early olfactory impairment, as reported in other neurodegenerative disorders. Histological examination of brains from $Csfr^{+/-}$ mice revealed microgliosis and

elevation of several pro-inflammatory cytokines and chemokines, suggesting that microglial expansion and activation may have an important role in disease. Apart from microglia, the CSF-1R is also expressed by neural progenitors and subpopulations of cortical and cerebellar neurons. It is upregulated in hippocampal neurons in response to excitotoxic insult and cell-autonomously confers survival. Furthermore, the transient increase in cortical layer V neurons observed in young *Csf1r*$^{+/-}$ mice, followed by their normalization with age, suggest that CSF-1R supports the survival of aging neurons. Together, these data imply that *Csf1r* heterozygosity affects both microglial activation and neuronal survival. To explore the contribution of reduced neuronal and microglial CSF-1R signaling to neurodegeneration in ALSP, we employed two lineage-specific Cre deletion systems, *Nestin*^{Cre}, for the neural lineage and *Cx3Cr1*^{Cre}, for the microglial and monocytic lineages. *Csf1r*$^{fl/+}$*;Nes*^{Cre}/+ and *Csf1r*$^{fl/+}$*;Cx3Cr1*^{Cre}/+ mice were tested in a longitudinal battery of behavioral tasks to assess olfaction, memory, motor coordination and depression-like behavior (blinded studies of males and females, ≥14/genotype, followed from 7 to 18 months of age). Reduced *Csf1r* expression in microglia was sufficient to reproduce the olfactory and short-term memory deficits observed in *Csf1r*$^{+/-}$ mice. In contrast, neither lineage-specific deletion alone was sufficient to phenocopy the depressive behavior, long-term memory, or locomotor coordination deficits observed in *Csf1r*$^{+/-}$ mice. These data suggest that while dysregulation of microglia alone is sufficient to some behavioral phenotypes, alterations in both neuronal cells and microglia are necessary for others. Current studies are focused on addressing the cellular and molecular mechanisms involved.

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Poster

381. Neurotoxicity, Inflammation, and Neuroprotection: Mechanisms of Neurodegeneration II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 381.27/N8

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Microglial TREM2 mediatesTDP-43 protein aggregate clearance & neuroprotection in a mouse model of ALS

Authors: *M. XIE[1], Y. LIU[1], M. P. MATTSON[2], L. WU[1]
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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). In WT mice, overexpression of hTDP-43 resulted in progressive motor neuron loss, motor dysfunction and microglia activation. Reactive microglia cleared TDP-43 aggregates, preventing the spread of hTDP-43 proteinopathy to nearby neurons. On the other hand, the absence of TREM2 significantly increased neuronal loss and worsened motor dysfunction. Using two-photon *in vivo* imaging, we observed the direct response of microglia to hTDP-43 protein following a stereotaxic injection into the motor cortex, dramatically increasing their process motility after hTDP-43 injection. 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

382. Neurotoxicity, Neuroinflammation, HIV, and Infections I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 382.01/N9

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH
PCOM DO/PhD program

Title: Extracellular vesicular transport in HIV-1 Tat protein and morphine directed microRNA changes in astrocytes on HIV-associated neurocognitive disorders (HAND)

Authors: *K. CHEN[1,2], L. SARDO[1], S. MITA[1], Z. A. KLASE[1]
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Abstract: HIV-associated neurocognitive disorder (HAND) impacts nearly one-half of HIV-infected patients. Astrocytes harbor latent HIV-1 that can produce appreciable amounts of

neurotoxic viral proteins, such as HIV-1 Tat. Evidence for Tat protein and morphine have both been implicated in exacerbation of HAND. We predict aberrant microRNA intracellular changes in astrocytes impair their ability to support neurons and moreover act as a driver for ongoing neuroinflammation through extracellular vesicles (EVs) to bystander cells. Tat protein can inhibit Dicer endoribonuclease cleavage necessary for miRNA maturation. Our objective is to determine the role of miRNAs altered by Tat and morphine in HAND through EVs. We cultured astrocytes and 293Ts, transfected with Tat plasmid, and treated with morphine. Luciferase reporter assay was used to quantify β-catenin activity, a neuroprotective pathway in astrocytes and biological activity of Tat EVs in TZMbl cells. We then profiled changes of 380 miRNAs by RT-qPCR on Tat and morphine treated cells. Cell supernatant was collected and processed for EVs. In astrocytes, morphine increased the suppressive ability of Tat on β-catenin activity compared to Tat alone in a dose-specific manner. We found that 90 miRNAs were uniquely dysregulated by a 3-fold change or more in combined Tat and morphine treatment. We found significant activation of the HIV-1 LTR in our TZMbl assay after treatment with our extracellular Tat-EV isolates and found Tat to be stable in EVs for over 20 days stored in 4 degrees Celsius. We observed morphine's ability to increase the suppressive ability of Tat on β-catenin activity in a dose-dependent manner. Our finding suggests worsening neuropathology in joined Tat and morphine interactions. Continuing experimentation and data will include miRNA profile in EVs, the relationship between intracellular and extracelluar miRNA changes, and biological impact of these EVs on neuroinflammatory related pathways in uninfected cells.

Disclosures: K. Chen: None. **L. Sardo:** None. **S. Mita:** None. **Z.A. Klase:** None.

Poster

382. Neurotoxicity, Neuroinflammation, HIV, and Infections I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 382.02/N10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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Institute of Organic Chemistry and Biochemistry of the Academy of Sciences of the Czech Republic (RVO 61388963)
PhRMA Foundation Postdoctoral Fellowship in Pharmacology/Toxicology

Title: Targeting glutamate metabolism for the treatment of hiv-associated neurocognitive disorders

Authors: *L. E. LOVELL[1,2], M. NEDELCOVYCH[2], L. TENORA[4], B.-H. KIM[5], J. KELSCHENBACH[5], W. CHAO[6], A. JANCARIK[4], E. PRCHALOVA[3], R. DASH[3], A.

GADIANO[7], J. ALT[3], P. MAJER[4], D. VOLSKY[8], R. RAIS[2], B. S. SLUSHER[3]
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Abstract: Despite combined antiretroviral therapy (cART) effectively inhibiting HIV viral replication and extending life, about 50% of all HIV+ individuals develop HIV-Associated Neurocognitive Disorders (HAND). HIV infection of perivascular macrophages, microglia, and astrocytes in the brain occurs early in disease, and has been linked to persistent neuroinflammation and cognitive impairment even when peripheral viral loads are suppressed. HAND symptoms have also been linked to upregulated expression of the primary glutamate-synthesizing enzyme, glutaminase, which may contribute to observed excess glutamate production in the brain leading to aberrant excitatory neurotransmission, excitotoxicity, and impaired cognitive function. Glutaminase inhibition may thus represent a novel drug target to confirm treatment of HAND. We recently developed JHU083, an orally available, brain penetrant prodrug of the glutaminase inhibitor 6-diazo-5-oxo-l-norleucine (DON). Here, we show that JHU083 reduces excess glutamate levels in the central nervous system, normalizes microglia glutaminase activity and restores cognitive function in HIV-infected mouse models. Therefore, glutaminase inhibition can reduce toxic glutamate levels and may serve as a novel target for HAND treatment.

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Poster

382. Neurotoxicity, Neuroinflammation, HIV, and Infections I

Location: SDCC Halls B-H

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Program #/Poster #: 382.03/N11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant RO1 DA02446

Title: HIV-1 and HIV proteins interact with morphine to induce loss of KCC2 and GABAergic dysfunction in primary human neurons in a NMDAR and CCR5 dependent manner

Authors: *A. J. BARBOUR[1], J. M. BALINANG[1], A. R. MCQUISTON[1], K. F. HAUSER[2], P. E. KNAPP[3]
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Abstract: Despite the introduction of combined antiretroviral therapy, the CNS remains highly susceptible to insult from HIV-1 and inflammatory factors which cause sublethal damage to bystander neurons, providing the neural basis of HIV-associated neurocognitive disorders (HAND). Opiate use is often comorbid with HIV infection and these patients show exacerbated HAND symptomology. Little is known about electrophysiological changes associated with HIV ± morphine co-exposure. We addressed this question by developing a dissociated primary human model derived from differentiating human neural progenitor cells (hNPC) into a mixed neuron-astrocyte culture containing glutamatergic and gamma-aminobutyric acid-(GABA)ergic neurons. Optical techniques were used for electrophysiological experiments, thus circumventing the biohazard of sharp electrodes in the presence of HIV. With genetically encoded voltage indicators (GEVI), Flicr1/Archon1, and genetically encoded calcium indicator (GECI), GCaMP6f, we measured primary human neuron electrophysiological and calcium activity to elucidate changes in excitatory-inhibitory balance due to HIV ± morphine exposure. We determined that HIV and morphine both dysregulate neuronal $[Cl^-]_i$ resulting in hyperexcitability. K-Cl cotransporter 2 (KCC2) maintains low $[Cl^-]_i$ necessary for $GABA_AR$-induced hyperpolarization. Thus, we hypothesized that both HIV and morphine decrease expression/activity of KCC2 leading to dysregulated $[Cl^-]_i$ and loss of subsequent $GABA_AR$ hyperpolarization. This was confirmed by immunostaining experiments that showed significant loss of KCC2 in neurons exposed to supernatant from HIV-infected monocytes (250-500 pg/mL p24) and 500nM morphine in the absence of neuron death. We have further determined that the viral proteins transactivator of transcription (Tat) and glycoprotein 120 (gp120; R5-tropic) contribute to KCC2 loss. These results correlate with significant defects of GABA-ergic signaling in primary human neurons exposed to HIV, or HIV proteins ± morphine. KCC2 expression and response to GABA were rescued by co-exposure with KCC2 activity enhancer, CLP257. Our data identify KCC2 and upstream activity as a promising, novel target for therapeutic intervention to alleviate functional changes underlying HAND ± opiate use.

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Poster

382. Neurotoxicity, Neuroinflammation, HIV, and Infections I

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Program #/Poster #: 382.04/N12

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01 DA018633

Title: $GABA_A$ signaling reduces morphine and tat-induced intracellular calcium spikes in hippocampal neurons through AMPAR-dependent mechanism

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Abstract: Opiates exacerbate HIV-induced neuropathogenesis within vulnerable regions of the central nervous system. Within the hippocampus of mice expressing doxycycline-inducible HIV-1 protein *transactivator of transcription* (Tat), 2 weeks of Tat exposure reduced apical dendritic spine density on dentate gyrus (DG) granule cells ($p = 0.008$), CA1 pyramidal neurons ($p < 0.001$), and CA3 pyramidal neurons ($p < 0.001$) *in vivo*, while simultaneous morphine exposure (escalating s.c. injection b.i.d., 10-40 mg/kg) did not have a significant effect. *In vitro*, prolonged (72 h) exposure to Tat (100 nM) reduced primary murine hippocampal neuron survival, particularly GAD67+, GABAergic cells ($p < 0.001$). Acute Tat exposure increased neuronal intracellular calcium concentration ($[Ca^{2+}]_i$) by 193.9 ± 53.9% compared to controls, which was exacerbated by morphine (312.0 ± 31.5%) and reversed by $GABA_A$ agonist muscimol (100 nM, three-way interaction, $p < 0.001$). $[Ca^{2+}]_i$ returned to baseline within 20 min in Tat-exposed cells (114.4 ± 30.3%); however, $GABA_A$ antagonist bicuculline (20 µM) prolonged Tat-induced elevation of $[Ca^{2+}]_i$ in the presence of morphine (208.0 ± 22.8%, $p < 0.001$). To identify key sources of Tat-induced calcium influx, potential extracellular [NMDAR (MK-801), AMPAR (CNQX), L-type Ca^{2+} channels (nimodipine, isradipine)] and intracellular [$RyR_{1,3}$ (dantrolene), σ_1R (BD-1063)] calcium sources were blocked pharmacologically. Tat-induced $[Ca^{2+}]_i$ was significantly reduced by nimodipine (10 µM, $p < 0.001$) and MK-801 (20 µM, $p < 0.001$), but not BD-1063 (1 µM, $p = 0.381$). Morphine-induced calcium influx was reduced by isradipine (5 µM, $p = 0.009$) and BD-1063 ($p = 0.038$). Bicuculline and morphine interactive effects within Tat-exposed cells were reduced by CNQX (500 nM, $p < 0.001$). Overall, these data indicate morphine, $GABA_AR$, and HIV-1 Tat elevate $[Ca^{2+}]_i$ within hippocampal neurons through both independent (i.e., AMPAR, NMDAR, RyR, σ_1) and shared (i.e., $Ca_v1.2$) calcium sources. While 2 wks morphine exposure showed limited additive impact on dendritic spine density in vivo, the morphine-dependent alterations in calcium homeostasis may ultimately contribute to structural deficits within hippocampal neurons. GABAA signaling ameliorates Tat-induced [Ca2+]i as shown by muscimol, which could ultimately contribute to structural and behavioral outcomes in vivo. We hypothesize that the potential loss of the vulnerable GABAergic interneuron subpopulation within the hippocampus may exacerbate opiate- and HIV-induced neuropathology.

Disclosures: V.D. McLane: None. **M.D. Denton:** None. **P. Vo:** None. **P.E. Knapp:** None. **K.F. Hauser:** None.

Poster

382. Neurotoxicity, Neuroinflammation, HIV, and Infections I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 382.05/O1

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01DA039005
T32 MH079795

Title: Role of dopamine in the modulation of macrophage-mediated inflammation: Implications for NeuroHIV and neurodegenerative disorders

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Abstract: Despite the success of combination anti-retroviral therapy (cART), around 50% of HIV-infected individuals still display a variety of neuropathological and neurocognitive sequelae known as NeuroHIV. Current research suggests these effects are mediated by long-term changes in CNS function in response to chronic infection and inflammation, and not solely due to active viral replication. Among these changes are alterations in dopaminergic signaling and increased inflammation in dopamine-rich brain regions. In the post-cART era, drug abuse is one of the most prominent risk-factors for the development of NeuroHIV. All drugs of abuse increase extracellular dopamine in the CNS, and our lab has shown that dopamine can increase HIV infection of primary human macrophages and increase the production of inflammatory cytokines. These and other studies showing correlations between dopaminergic dysfunction and severity of NeuroHIV suggests that elevated dopamine could enhance the development of HIV-associated neuropathology. However, the precise mechanism(s) by which elevated dopamine could exacerbate the progression of NeuroHIV, particularly in chronically-infected, virally suppressed individuals, remain unclear. Within the CNS, the primary targets for HIV are myeloid lineage cells, such as microglia and perivascular macrophages. Thus, to determine the connection between dopaminergic alterations and HIV-associated neuroinflammation, we have examined the impact of dopamine exposure on inflammatory functions of macrophages and microglia. Our data show that dopamine treatment of human macrophages isolated from healthy and cART-treated donors promotes a inflammatory phenotype in these cells by inducing production of inflammatory mediators including IL-1β, IL-6, CCL2, CXCL8, CXCL9, and CXCL10. Further, dopamine-mediated modulation of specific cytokines is correlated with macrophage expression of dopamine-receptor transcripts, particularly DRD5, which we show to be expressed at significantly higher levels than other dopamine-receptor subtypes. Dopamine exposure in human macrophages also activates inflammatory pathways such as caspase1, leading to downstream

activation of the inflammasome, potentially via epigenetic modulation. Moving forward we are focused on elucidating the effect of dopamine on production of cytokines and neurotrophic factors, as well as inflammasome activation in iPSC-derived microglia. Overall, these data will provide more understanding of the role of dopamine in the development of NeuroHIV, and may suggest new molecules or pathways that can be useful as therapeutic targets during HIV infection.

Disclosures: R. Nolan: None. **R. Muir:** None. **S. Matt:** None. **E. Nickoloff:** None. **K. Runner:** None. **E.K. Haddad:** None. **P.J. Gaskill:** None.

Poster

382. Neurotoxicity, Neuroinflammation, HIV, and Infections I

Location: SDCC Halls B-H

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Program #/Poster #: 382.06/O2

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 5R01DA039005

Title: Dopamine increases macrophage susceptibility to HIV infection through activation of non-canonical signaling pathway

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Abstract: Despite the success of antiretroviral therapy (ART), the neurological effects of HIV infection remain a major health issue. The constellation of neuropathologies, behavioral, cognitive and motor impairments now collectively known as NeuroHIV, affects around 50% of infected individuals. These effects are driven by infection and subsequent dysfunction in myeloid cells, such as macrophages and microglia, which are the primary targets for HIV in the CNS. Our studies show myeloid cells exposed to the neurotransmitter dopamine have an increased susceptibility to HIV infection, potentially enhancing the spread of infection within the brain. The number of myeloid cells exposed to dopamine is greatly increased in the CNS of drug abusers, a common comorbidity with HIV infection. Although drugs have different mechanisms of action, all drugs of abuse exert their addictive and reinforcing effects through elevation of CNS dopamine. In addition, dopaminergic therapeutics, such as those for depression, Parkinsons, Alzheimers, diabetes and some cancers could also elevate CNS dopamine. As myeloid cells are exposed to dopamine due to spillover during neurotransmission, the effects of exogenously induced changes in dopaminergic tone could exacerbate the development of NeuroHIV. Our research demonstrates that dopamine concentrations of 10^{-8}M or higher significantly increase the entry of HIV into primary human macrophages. This increase is due to the activation of either D1-like or D2-like dopamine receptors, suggesting that in MDM, both types of receptors may act

through a common pathway. Canonically, D1-like receptors couple to $G_{\alpha s}$ to activate cyclic AMP (cAMP), resulting in phosphorylation of PKA, while D2-like receptors couple to $G_{\alpha i}$ to inhibit cAMP. Our data show that in MDM, this pathway is not active, and that dopamine receptors act through an alternative pathway mediated by $G_{\alpha q}$ or $G_{\beta\gamma}$ activation of PLC and PKC, increasing IP_3 and Ca^{2+} release. This pathway seems to be mediated, at least partially, by DR5, which is the most prominently expressed DRD transcript in these cells. Further, inhibition of this pathway by blocking Ca^{2+}release with Dantrolene completely abrogates the impact of dopamine on HIV infection in these cells. These data indicate dopamine mediates its effects on HIV infection through elevations in intracellular Ca^{2+}, and indicate that HIV infection involves previously unknown cellular pathways. Further, these data indicate that dopamine signaling in macrophages is biased toward a non-canonical signaling pathway and suggest that this pathway could be a novel therapeutic target to ameliorate the impact of drug abuse on NeuroHIV.

Disclosures: P.J. Gaskil: None. **E.A. Nickoloff:** None. **R. Nolan:** None. **K. Runner:** None.

Poster

382. Neurotoxicity, Neuroinflammation, HIV, and Infections I

Location: SDCC Halls B-H

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Program #/Poster #: 382.07/O3

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant DA013137
NIH Grant HD043680
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NIH Grant NS100624

Title: Effects of S-Equol on norepinephrine and dopamine receptor mRNA expression in the HIV-1 transgenic rat prefrontal cortex

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Abstract: HIV-1 infection affects over 36 million people worldwide, and despite combination antiretroviral therapy, neurocognitive disorders such as mild cognitive impairment persist along the trajectory of the disease. HIV-1 is known to alter neurochemistry, however, there is no known treatment for this neurochemical disruption. Forming the gut-brain axis, gut microbiota acts through neurotransmitter systems and immune interactions to affect emotional and social behavior as well as cognitive processing. S-Equol is produced naturally by gut microbiota in most individuals, acting as a beta estrogen receptor agonist. It is thought to alter neurochemistry

through the mesolimbic noradrenergic system and dopamine system, producing a neuroprotective effect. Previous studies have shown that S-Equol treatment is effective in promoting neurorecovery against Tat, an HIV-1 viral protein, as well as preventing HIV-1 Tat induced apoptosis. Testing the effectiveness of S-Equol on HIV-1 transgenic rats, treatment was given during a formative period of PD 28 through PD 90 at a dose of 0.2 mg/day. After a history of operant training, an innovative RNA *in situ* hybridization assay (RNAScope) was used to label mRNA expression of the norepinephrine receptor, alpha-2, and the dopamine receptor, DrD1, in the prefrontal cortex. Preliminary results suggest a sex dependent shift towards higher cell scores in alpha-2 mRNA transcript levels in females, independent of genotype; an effect not observed in males. A sex and genotype dependent shift in DrD1 mRNA levels was also found, shifting the cell scores of the HIV-1 transgenic rats treated with S-Equol towards their placebo treated control counterparts. By examining the neural mechanism of S-Equol, potential therapeutic uses of this treatment may be identified. Funded by NIH grants DA013137, HD043680, MH106392, NS100624.

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Poster

382. Neurotoxicity, Neuroinflammation, HIV, and Infections I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 382.08/O4

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DA044939 (PEK and KFH)

Title: HIV-associated cognitive deficits; sex and regional specific outcomes correlate with changes in activity-regulated cytoskeleton (Arc) expression

Authors: *Y. K. HAHN[1], W. D. MARKS[2], S. R. NASS[2], S. KIM[1], J. J. PARIS[3], K. F. HAUSER[2], P. E. KNAPP[1]
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Abstract: The introduction of combined and highly active anti-retroviral therapies (cART) has transitioned HIV from a disease with short-term survival into a chronic disease and has changed the profile of HIV-associated neurocognitive disorders (HAND). While severe neurocognitive deficits leading to dementia are now rarely found, the prevalence of mild and moderate cognitive and motor deficits has remained constant or increased, even among patients with systemic viral suppression. This phenomenon likely reflects inefficient penetration of current antiretroviral drugs through the blood brain barrier, which allows the central nervous system (CNS) to exhibit

low levels of persistent infection. HIV-infected patients commonly show neurocognitive deficits that affect memory, attention/concentration, mood, and fine motor skills. Furthermore, although the percentage of women in the HIV-infected population has increased, sex-related effects on memory/cognition deficits in HIV patients remain unclear. We utilized a transgenic mouse model of HIV (conditionally expressing HIV-1 Tat_{1-86} protein in CNS) and examined both males and females for changes in cognitive behavior and for expression of biochemical markers related to memory and learning, especially the Arc protein. Arc is an immediate early protein, and its expression can be induced by any environmental experience leading to learning and memory. Altered Arc expression is involved in disruption of memory after radiation therapy. The induction of Arc occurring after contemporaneous acoustic/odor stimuli was reduced by HIV-1 Tat exposure in both sexes, although Arc expression remained significantly higher in Tat^+ females compared to males. Multiple cognitive behavioral tests showed that only Tat^+ males exhibited significant deficits in spatial memory, increased anxiety and altered extinction of fear. Sex-specific differences were also found in both Arc cell signaling pathway proteins and other memory-associated proteins such as Homer1 and Zif268 in hippocampal lysates. We also examined spine density in different brain regions, male Tat^+ mice showed significant reductions of spine density in hippocampus and striatum, but not in amygdala. Parallel results were seen in levels of Arc protein expression *in vivo*. Interestingly, treatment with BDNF for 4 h significantly increased Arc levels in hippocampal organotypic slice cultures +/- Tat exposure. Overall, our results indicate that sex may influence HIV-1 Tat protein-related effects on cognition and anxiety, and that some sex-related outcomes correlate with regionally specific changes in Arc-associated cell signaling.

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Poster

382. Neurotoxicity, Neuroinflammation, HIV, and Infections I

Location: SDCC Halls B-H

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Program #/Poster #: 382.09/O5

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: MH087332
MH104131
MH105330

Title: Sexual dimorphism in the gene expression of cerebrocortical neurotransmitter systems in hiv gp120 transgenic mice

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Abstract: Human immunodeficiency virus (HIV)-associated neurocognitive disorder (HAND) is estimated to affect around half of the HIV-positive population. The neurocognitive symptoms of HAND affect not only survival and quality of life but can also impact everyday functioning. Despite advancing strategies to suppress the virus through combination antiretroviral therapy (CART), HAND has persisted in these individuals. Studies in our laboratory utilize transgenic mice expressing the viral envelope protein gp120 under the control of a modified GFAP promotor in astrocytes (gp120tg mice). These mice (C57BL/6 x SJL background) exhibit neuronal damage and differential gene expression resembling that observed in brains of patients with HAND.

In humans, males and females exhibit neurochemical sex differences which have been implicated in diseases that differentially affect men and women. The global proportion of men vs women has been approximately 50% (UNAIDS) since the late 1990s. However, the predominance of HIV among men in the United States (CDC: 76% vs 24%) has led to an underrepresentation of women in clinical and preclinical studies. Evidence from previous small-scale studies indicates that HIV-positive women may have an increased risk for neuropsychological impairment. Thus, increasing the understanding of how biological sex affects HAND can provide essential information required for future development of improved personalized treatment strategies.

In this study, we describe the sex differences observed at RNA level in both the dopamine/serotonin and GABA/glutamate neurotransmitter systems in the cerebral cortex of 6 month old mice with and without transgenic expression of viral gp120 protein. Among wild type (WT) males without gp120, there is a lower expression of many genes in the dopamine/serotonin pathway (Drd5, Slc18a1, Arrb1, etc; p&lt0.05) compared to WT females. In gp120+ males, there is a similar downregulation of the dopamine/serotonin pathway but with discrete changes in the genes (Drd4, GFAP, Htr6, etc; p&lt0.05). In the GABA/glutamate system, there were significant differences in WT males; both upregulation (Gria2, Gabra1, Gabra5, etc; p&lt0.05) and downregulation (Gabre, Grin2c, Grik5, etc; p&lt0.05) when compared to WT females. In gp120+ males, most alterations were upregulated compared to gp120+ females (Gria2, Gabrg1, Gabra1; p&lt0.05). These findings indicate that there are substantial sex differences in the cerebrocortical RNA expression profiles of major neurotransmitter systems in both WT and gp120 transgenic animals.

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Poster

382. Neurotoxicity, Neuroinflammation, HIV, and Infections I

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 382.10/O6

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DA043268
DA041750

Title: Homeostatic changes in CA1 pyramidal neurons of the hippocampus from HIV transgenic rats

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Abstract: We used HIV transgenic (Tg) rats and the patch-clamp technique to study the effect of chronic low-level expression of HIV viral proteins on CA1 pyramidal neurons of the hippocampus. Electrophysiological properties of CA1 pyramidal neurons in acute slices from both dorsal and ventral areas of the hippocampus from HIV rats were compared to those in their counterparts from wild-type control rats. We found increased excitatory inputs onto pyramidal neurons in the two hippocampal regions. However, these increases were mediated through distinct mechanisms in the dorsal and ventral hippocampi. CA1 pyramidal neurons in the dorsal hippocampus of HIV Tg rats displayed elevated rate of excitatory postsynaptic currents (EPSCs), while CA1 pyramidal neurons in the ventral hippocampus of HIV Tg rats showed increased EPSC amplitudes. The enhanced excitatory input onto CA1 pyramidal neurons in HIV rats was associated with overall decrease of neuronal intrinsic membrane excitability. Neurons of HIV Tg rats were significantly more hyperpolarized than the control neurons. In experiments using conventional protocol of neuronal stimulation by current injection, the minimal stimulation current in neurons of HIV Tg rats was significantly higher than that in control neurons. The action potential (AP) rise time and the AP ½ width in HIV neurons were increased. The finding of reduced excitability was confirmed in experiments in which the dynamic clamp protocol was used to stimulate neuronal spiking activity. In both areas of the hippocampus, the minimal synaptic conductance required to trigger neuronal firing was higher in HIV neurons. We used neuronal modeling to examine whether diminished I_h in HIV neurons could account for their reduced excitability. Enhanced glutamatergic inputs and reduced excitability in CA1 pyramidal neurons of HIV rats may represent coordinated homeostatic changes aimed at stabilizing firing rates in the hippocampus of HIV Tg rats.

Disclosures: I.V. Sokolova: None. **A. Szücs:** None. **P.P. Sanna:** None.

Poster

382. Neurotoxicity, Neuroinflammation, HIV, and Infections I

Location: SDCC Halls B-H

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Program #/Poster #: 382.11/O7

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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NIH Grant 5T32GM081740

Title: Voltammetric analysis of escitalopram treatment in the HIV-1 transgenic rat: Implications for comorbid HIV and depression

Authors: *A. DENTON[1], S. BERGER[2], C. F. MACTUTUS[3], S. B. HARROD[4], P. HASHEMI[1], R. M. BOOZE[5]
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Abstract: HIV-1 infection is a serious condition affecting approximately 37 million people as of 2015. Despite the advent of combination antiretroviral therapy (cART), approximately 50% of seropositive individuals report some degree of clinical depression. The HIV-1 transgenic (Tg) rat contains seven of the nine genes that comprise the HIV viral genome and represents a non-infectious rodent model of HIV-1. The present study examined the effects of chronic escitalopram treatment on dopamine (DA) and serotonin (5-HT) in the HIV-1 Tg rat. Escitalopram is a selective serotonin reuptake inhibitor (SSRI) that is commonly prescribed for the treatment of clinical depression. In order to evaluate the release and reuptake kinetics of DA and 5-HT, fast-scan cyclic voltammetry (FSCV) was employed. Adult male and female HIV-1 Tg and F344/N control animals were subcutaneously implanted with an escitalopram pellet (4 mg) or placebo pellet. At least 2 weeks following pellet implantation, animals were anesthetized and a stimulating pin was placed in the medial forebrain bundle and a recording carbon-fiber microelectrode was placed in the nucleus accumbens for DA. For 5-HT, the recording carbon-fiber was placed in the hippocampus. Consistent with our previous findings using ex vivo brain slice preparations, impaired DA release and reuptake was found in HIV-1 Tg rats relative to controls; however, chronic treatment with escitalopram did not attenuate the dopaminergic deficits. Impaired 5-HT release was found in the HIV-1 Tg rat hippocampus. Although escitalopram treatment did not increase 5-HT function in HIV-1 Tg rats, a temporal shift in peak 5-HT was observed. Collectively, these findings suggest that escitalopram treatment in HIV-1 Tg rats did not alter DA kinetics, but alterations in 5-HT suggested a mechanism for antidepressant actions in HIV-1 infected patients.

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Poster

382. Neurotoxicity, Neuroinflammation, HIV, and Infections I

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant DA013137
NIH Grant HD043680
NIH Grant MH106392
NIH Grant NS100624

Title: Disruption of timing: NeuroHIV progression in the post-cART era

Authors: *K. A. MCLAURIN, H. LI, R. M. BOOZE, C. F. MACTUTUS
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Abstract: By 2030, approximately 73% of HIV-1 seropositive individuals will be 50 years or older, heralding an examination of the progression of HIV-1 associated neurocognitive disorders (HAND). A longitudinal experimental design was used to assess progression of the core components of executive function across the functional lifespan in the HIV-1 transgenic (Tg) rat, resembling HIV-1 seropositive individuals on lifelong combination antiretroviral therapy. At the earliest assessment (i.e., approximately 2 months of age), the factor of biological sex was the driving factor for observed differences in task acquisition, tapping learning, and signal detection, tapping sustained attention [HIV-1 Tg (*N*=20 litters; male: *n*=37, female: *n*=33); F344/N Control (*N*=17 litters; male: *n*=34, female: *n*=33)]. Despite the "savings" afforded by repeated testing, HIV-1 Tg animals exhibited progressive, relative impairments in the completion of signal detection and the detection of shorter signal durations; deficits which became most prominent in male HIV-1 Tg animals at 18 months of age. A reversal task, tapping flexibility and inhibition, revealed marked impairment in HIV-1 Tg rats, with sex-dependent deficits in task acquisition and the detection of shorter signal durations. Examination of dendritic spine branch order in layers II-III pyramidal neurons of the medial prefrontal cortex revealed a profound distributional shift, with a greater relative frequency of spines on lower order branches, in HIV-1 Tg animals relative to controls, supporting an alteration in synaptic connectivity. Both neurocognitive alterations and synaptic dysfunction were independent of neuroinflammation. A differential relationship between dendritic spine branch order and signal detection during reversal in HIV-1 Tg and control animals supports a primary mechanism for HAND. Thus, even in the absence of comorbidities, HAND is a neurodegenerative disease characterized by sex dependent, progressive neurocognitive impairments across the functional lifespan; impairments which may be due, at least in part, to alterations in synaptic connectivity. Funded by NIH grants DA013137, HD043680, MH106392, NS100624.

Disclosures: K.A. McLaurin: None. **H. Li:** None. **R.M. Booze:** None. **C.F. Mactutus:** None.

Poster

382. Neurotoxicity, Neuroinflammation, HIV, and Infections I

Location: SDCC Halls B-H

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Impaired neurogenesis in the HIV-1 transgenic rat and recovery with physical activity

Authors: J. LAPOINTE, M. CRANSTON, V. MADORMO, C. MACTUTUS, S. HARROD, *R. M. BOOZE
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Abstract: Prior evidence suggests that the HIV-1 associated protein Tat affects neuronal proliferation and substantially inhibits neurogenesis. Similarly, the HIV-1 envelope protein gp120 can affect progenitor proliferation, potentially affecting neurocognitive processes involved in HIV-1 Associated Neurocognitive Disorders (HAND). Physical activity is known to increase dentate gyrus hippocampal adult neurogenesis. In the current study, we examined if voluntary nocturnal wheel running would promote neurogenesis and neuronal maturation of the dentate gyrus in HIV-1 Tg rats compared to F344/N controls. Adult HIV-1 Tg female animals (n=20) and F344/N female control animals (n=20) were compared to adult HIV-1 Tg male animals (n=20) and F344/N male controls (n=20). All animals were provided nocturnal access to a running wheel for 67 minutes/day for a minimum of 85 consecutive days, with half of the running wheels immobilized (sedentary condition). Following wheel running, animals were immediately sacrificed and brain slices were evenly divided, with alternating hippocampal slices processed for either doublecortin (DCX) immunostaining or with ballistically-delivered DiI, followed by 3-dimensional dendritic spine analysis. In female HIV-1 Tg animals, wheel running significantly increased the number of DCX+ granule cells in the dentate gyrus and decreased DCX+ numbers in HIV-1 Tg males ($p \leq .05$). The number of less mature DCX+ cells (without dendrites) was significantly decreased in HIV-1 Tg animals ($p \leq .05$). DCX+ granule cells with dendrites (more mature than DCX+ soma-only granule cells) showed significant differences between the sexes ($p \leq .05$), and a significant interaction between running condition and animal sex ($p \leq .05$). Dentate granule cell spine morphology differed between HIV-1 Tg and control animals, with increased spine length, head diameter, and volume in the HIV-1 Tg wheel running

animals compared to sedentary HIV-1 Tg animals. Thus, HIV-1 impairs adult hippocampal dentate granule cell neurogenesis and interferes with the integration of newborn cells into the dentate gyrus and this integration may, in part, be restored by physical activity.

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Poster

382. Neurotoxicity, Neuroinflammation, HIV, and Infections I

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Title: Behavioral analysis of escitalopram treatment in the HIV-1 transgenic rat

Authors: A. U. LATEEF, A. K. COOK, N. G. QUAN, A. R. DENTON, *C. F. MACTUTUS, S. B. HARROD, R. M. BOOZE
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Abstract: As of 2015, 37 million people worldwide are living with HIV-1, with nearly forty-thousand new cases of HIV-1 infection occurring in the United States alone. Despite the relative success of combination antiretroviral therapy (cART) in treating the illness, roughly half of all individuals suffering from HIV-1 infection will experience comorbid depression. The present study seeks to examine the effects of chronic exposure to escitalopram (14.76 mg pellet) upon behavioral markers of depression and apathy in the HIV-1 transgenic (Tg) rat. Escitalopram is a selective serotonin reuptake inhibitor (SSRI) that is commonly prescribed for depression and is recognized as a safe addition to cART treatment. In order to evaluate depressive symptoms in HIV-1, a battery of behavioral tests were administered to adult male and female HIV-1 Tg and F344/N control rats. To evaluate startle response, prepulse inhibition of the acoustic and visual startle was employed. Apathy, a core component of depression was evaluated using a five-bottle sucrose preference test. Both a modified hole board test and an elevated plus maze test was used to evaluate exploratory behaviors. Consistent with our previous reports, significant prepulse inhibition deficits were found in HIV-1 Tg, relative to F344/N control rats. Escitalopram had limited effectiveness in attenuating this deficit. Sex differences were observed in exploratory behaviors, but escitalopram did not appear to modify exploratory behaviors in either HIV-1 Tg

or F344/N control rats. Finally, although escitalopram treatment did not appear to shift sucrose preference in HIV-1 Tg or F344/N control rats, a curvilinear dose-response shift across sucrose concentration was observed in the HIV-1 Tg rats, suggesting motivational alterations. Collectively, these findings show that escitalopram may have limited effectiveness in mitigating behavioral markers of depression in the HIV-1 Tg rat.

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Poster

382. Neurotoxicity, Neuroinflammation, HIV, and Infections I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 382.15/O11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01 DA018633
NIH R01 DA045588
NIH K02 DA027347

Title: HIV-1 Tat alters anterior cingulate cortex morphology and executive functioning in a transgenic mouse model of neuro-HIV

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Abstract: Although combined antiretroviral therapies (cART) have improved the mortality and quality of life of HIV patients, approximately 30-50% develop neurocognitive impairments, including deficits in executive functioning, such as attention problems and impulsivity. In the central nervous system HIV-1 proteins, such as HIV-1 trans-activator of transcription (HIV-1 Tat), can be expressed leading to deleterious effects on neuronal morphology and function, and may underlie the development of neurocognitive deficits. The present study tested the hypothesis that HIV-1 Tat disrupts behavior and neuronal dendrite morphology associated with executive functioning. CNS expression of HIV-1 Tat under the control of a glial fibrillary acidic protein-driven, *tet*-on promoter was induced by administering doxycycline for 2-8 weeks in adult male, transgenic mice. A relatively short duration (2 weeks) of HIV-1 Tat induction decreased the density of dendritic spines on apical, but not basal dendrites, on layer 5 pyramidal neurons in the anterior cingulate cortex (ACC). Apical dendrites in the ACC receive “top-down” afferents from higher cortical areas; whereas basal dendrites receive “bottom-up” afferents from lower cortical and sensory areas, suggesting HIV-1 Tat may interfering with cortical feed-back input.

Interestingly, the morphological changes at 2 weeks of HIV-1 Tat expression did not coincide with changes in compulsive or coping behaviors. However, longer-term HIV-1 Tat exposure (6-8 weeks) increased feeding in the novelty suppressed feeding test, while decreasing prepulse inhibition, indicating that Tat may cause impulsivity in situations of competing motivation (e.g. drive to feed vs. fear of novel brightly lit areas) and attention deficits. Further, long-term Tat exposure affects coping behavior in the forced swim test. Ongoing experiments examining the effects of HIV-1 Tat on dendritic morphology in interconnecting brain areas and neuronal function are being conducted to elucidate the structural and functional deficits in specific neurocircuits underlying HIV-1 Tat-induced alterations in executive functioning.

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Poster

383. Ischemia II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 383.01/O12

Topic: C.08. Ischemia

Support: Inova Health system #222865

Title: Association of SERPINE1 gene polymorphism, haplotype and aneurysmal subarachnoid hemorrhage

Authors: *M. LIN[1,2], C. GRIESSENAUER[3], R. STARKE[4], P. FOREMAN[5], P. HENDRIX[6], M. HARRIGAN[5], W. FISHER[5], N. VYAS[2], B. WALTERS[2], J.-F. PITTET[5], M. MATHRU[5], R. H. LIPSKY[7,8]
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Abstract: Cerebral aneurysm formation is a complex interplay among environmental exposures, biomechanical features, cellular and molecular characteristics, and genetic predisposition. Aneurysmal subarachnoid hemorrhage (aSAH) is usually the result of these mixed factors and the triggers for aneurysmal aSAH remain poorly understood while mounting evidence suggests that genetic factors contribute both to aneurysm formation and aneurysm rupture. In this study, we focused on the association of SERPINE1 gene with aSAH and its sequelae, clinical vasospasm, and delayed cerebral ischemia (DCI). Six single nucleotide polymorphisms (SNP) of

SERPINE1 gene (rs2227631, rs1799889, rs6092, rs6090, rs2227684, rs7242) were investigated. The SERPINEI gene encoded PAI-1 protein, which is antifibrinolytic and responsible for the controlled degradation of blood clots.
Blood samples from 195 subjects (145 aSAH patients and 50 controls) ages 21-88 years were collected for genetic analysis. Exclusion criteria were age under 19 years and abnormalities in cerebral vasculature. The control group was composed of trauma patients with unremarkable CT angiograms of cerebral aneurysm or other vascular malformation, and without known genetic risk factors for cerebral aneurysm formation. Both aSAH patients and controls were enrolled within 72 hours of admission. Patients were treated in accordance with guidelines for the management of aSAH and surveillance for clinical vasospasm and DCI. Weight of evidence (WOE) and information value (IV) are used for initial screening of variables for aSAH, clinical vasospasm and DCI. Follow up logistic regression showed that individuals with diabetes ($P < 0.0001$), hypertension ($P=0.015$), rs2227631 G allele ($P=0.017$), and higher age ($P=0.035$) are associated with aSAH.
Individuals with hypertension (>140/90) ($P=0.02$), hyponatremia ($P=0.03$), and ventriculoperitoneal shunt (VPS) treatment ($P=0.04$) were associated with increased risk of DCI. Haplotype analysis showed that G5AGGT carriers (4% haplotype frequency) were associated with aSAH ($P=0.05$), smaller aSAH size ($P=0.004$), lower Hunt and Hess scale ($P=0.05$) and lower Fisher CT scale ($P=0.01$). Recessive G5GGGT carriers (32% haplotype frequency) were associated with DCI ($P=0.01$) and clinical vasospasm ($P=0.02$).
Recessive A4GGAG carriers (32% haplotype frequency) were associated with larger aneurysm size ($P=0.016$) and has longer hospital stay ($P=0.05$). Haplotypes are in the order of rs2227631, rs1799889, rs6092, rs6090, rs2227684 and rs7242. These results demonstrate the importance of SERPINE1 genetic polymorphism in predicting aSAH.

Disclosures: M. Lin: None. **C. Griessenauer:** None. **R. Starke:** None. **P. Foreman:** None. **P. Hendrix:** None. **M. Harrigan:** None. **W. Fisher:** None. **N. Vyas:** None. **B. Walters:** None. **J. Pittet:** None. **M. Mathru:** None. **R.H. Lipsky:** None.

Poster

383. Ischemia II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 383.02/O13

Topic: C.08. Ischemia

Support: The Regents of the University of California (University of California Davis) Award ID: 550884

Title: Gene expression profiling in whole blood of patients with ischemic stroke and cigarette smoking

Authors: *X. CHENG, F. HAMADE, N. SHROFF, H. HULL, G. JICKLING, B. ANDER, B. STAMOVA, F. SHARP
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Abstract: Though previous studies suggested that cigarette smoking-related genes may be linked to ischemic stroke (IS), no specific biomarkers have been identified in whole blood for patients who suffer IS and also smoke.
In this study, we performed whole genome mRNA expression study on Affymertrix HTA 2.0 microarrays using whole blood from 219 subjects (including 42 IS current smoker patients, 68 IS never smoker patients, 23 control smokers, 86 control never smokers). The significantly regulated genes were identified using ANOVA with p-value of 0.005 and |fold change|>1.2. The related functional pathway of identified genes was analyzed using Exploratory Gene Association Networks (EGAN) software.
Our data showed 63 (51 up and 12 down) genes were significantly altered in IS smoker patients *vs* IS never smoker patients, and 58 (48 up and 10 down) genes significantly altered in control smokers *vs* control never smokers. We also found three genes (GPR15, LRRN2 and CLDND1) associated with IS smoker patients were overlapped with non-IS control smokers. Based on 60 genes specifically associated with smoking and IS, the significantly related functional pathways include chemokine signaling pathway, T-cell receptor signaling pathway, cytokine-cytokine receptor pathway, iCOS-iCOSL signaling in T helper cells, as well as Th2 pathway.
In summary, we found that the alteration of inflammatory genes may provide direct evidence for explanation of health hazard of smoking before and/or after ischemic stroke and significance of smoking cessation, especially for people at high risk of IS.

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Poster

383. Ischemia II

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Topic: C.08. Ischemia

Support: NINDS Grant R01NS076012
NIDA Grant R01DA8292121

Title: Nicotine & e-Cig exposure alters brain glucose utilization in ischemic stroke

Authors: *A. E. SIFAT, B. VAIDYA, M. A. KAISAR, L. CUCULLO, T. J. ABBRUSCATO
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Abstract: Use of electronic cigarettes (e-Cig) is a growing health concern in both smoking and nonsmoking populations and rigorous studies are needed to investigate the effects of the nicotine exposure via e-Cig on the neurovascular unit (NVU) and stroke outcome. Previous studies by our lab have shown that nicotine exposure significantly decreases glucose transport across the blood-brain barrier (BBB) in ischemia-reperfusion. In the present study, we investigated the effects of both short-term and long-term nicotine exposure on neuronal glucose utilization in ischemic conditions. To extend this study, we also looked at the effects of in vivo e-Cig vaping on ischemic brain glucose utilization. In vitro primary cortical neurons were exposed to nicotine (10 μM) & cotinine (5 μM) for 1 or 5 days and then subjected to 2 h oxygen-glucose deprivation (OGD) followed by 24 h reoxygenation to mimic ischemic conditions. Neuronal glucose utilization was measured by radiolabeled deoxy-D-glucose uptake. Immunocytochemistry (ICC) was done to investigate glucose transporter 1 & 3 (glut1, glut3) and nicotinic acetylcholine receptors (nAChRs) expression while MTT assay measured neuronal viability. Neuronal glycolytic flux and mitochondrial respiration were measured by seahorse flux analyzer. Nicotine was also administered to male 6 months old mice by e-Cig vapor (2.4% nicotine) delivered by an electronic nicotine delivery system for 7 days. Brain deoxy-D-glucose uptake was also determined in brain slices exposed to 30 min OGD followed by 2 h reoxygenation utilizing an acute brain slices (ABS) technique. Glucose transporters expression in brain slices were investigated by western blotting. Nicotine & cotinine did not show any neuronal toxicity except for 1000 μM nicotine. 1 & 5 days of nicotine & cotinine exposure significantly decreases neuronal glucose utilization in OGD-reoxygenation conditions which were reversed by a non-specific nicotinic acetylcholine receptor (nAChR) antagonist, mecamylamine (20 μM). Nicotine & cotinine also decreases neuronal glut1 expression which were correlated with α7 nAChR upregulation in OGD-reoxygenation. Further, this decreases neuronal glycolytic flux and mitochondrial respiration in ischemic condition. E-cig exposure for 7 days also decreases glucose uptake under normoxic and OGD-reoxygenation conditions with corresponding decrease of glut1 & glut3. These data support, from a cerebrovascular perspective, that nicotine & e-Cig vaping exposure creates an enhanced glucose deprived state and dysregulates glucose metabolism at the NVU which could lead to enhanced ischemic brain injury.

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Poster

383. Ischemia II

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Program #/Poster #: 383.04/O15

Topic: C.08. Ischemia

Support: Grants-in-Aid and by special coordination funds from Grants-in-Aid for Scientific Research (C) [grant number 16K10988] from the Ministry of Education, Culture, Sports, Science and Technology of Japan
Kobe Gakuin University joint research (C)

Title: Influence of the cerebral and spinal nicotine signaling in the development of central post-stroke pain

Authors: *S. TOKUYAMA[1], W. MATSUURA[2], S. HARADA[1]
[1]Kobe Gakuin Univ., Kobe, Japan; [2]Kobe Gakuin Univ., Kobe City/Hyogo, Japan

Abstract: 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. Nicotine and its receptor have recently been shown to be critical in the modulation of nociceptive transduction following peripheral neuropathy. Especially, it has been reported that a nicotinic acetylcholine receptor (nAchR) subtype as α4β2 and α7 have regulated the induction of several pain behaviors. We have been suggested that cerebral or spinal nicotine might be involved in the induction of CPSP. The aim of this study is to determine that the involvement between nicotine signaling and CPSP.
Male ddY mice were subjected to 30 min of bilateral carotid artery occlusion (BCAO). The development of hind paw mechanical allodynia was measured using the von Frey test. On day 3 after BCAO, mice were intracerebroventricular (i.c.v.) or intrathecal (i.t.) injected nicotin (10, 20 nmol/mouse), dihydro-β-erythroidine (a selective α4β2 nAchR antagonist; 20 nmol/mouse) and/or methyllycaconitine (a selective α7 nAchR antagonist; 20 nmol/mouse).
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. The BCAO-induced mechanical allodynia was significantly suppressed by i.c.v. and i.t. injection with nicotine. Suppressive effect of nicotine (i.c.v. or i.t.) was significantly channeled by i.c.v. or i.t. injection of dihydro-β-erythroidine or methyllycaconitine. On the other hand, expression levels of α4 nAchR did not changed by BCAO as compared with sham operation.
Taken together, we conclude that nicotine and its receptors such as α4β2 and α7 nAchR are directly involved in the pathogenesis of CPSP. Our findings are helpful for better understanding of the pathological mechanism in CPSP.

Disclosures: S. Tokuyama: None. **W. Matsuura:** None. **S. Harada:** None.

Poster

383. Ischemia II

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Program #/Poster #: 383.06/P1

Topic: C.08. Ischemia

Support: NIH Grant R01NS095359

Title: CD36 deficiency alters the composition of monocyte-derived macrophages in the brain following ischemic stroke

Authors: *K. PARK[1], M.-S. WOO[1], M. BALKAYA[1], J. YANG[1], M. FEBBRAIO[2], S. CHO[1]
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Abstract: Although the inflammatory nature of CD36 expressed in monocytes/macrophages (MM) in acute stroke has been documented, their role during recovery phase of stroke is less clear. The present study investigates a role of MM CD36 in trafficking into the injured brain and MM subset composition in chronic stroke. C57 male mice (12 weeks old) were subjected to transient focal ischemia and brain immune cells were collected at 7day (d) and 2 month (m). Using a flow cytometer, CD11b+ cells were further distinguished by $CD45^{Hi}$ and $CD45^{Low}$ subsets, which conventionally represent infiltrating MMs and resident microglia respectively. The extent of MM trafficking was determined by an intravenous infusion of GFP+ splenocytes in the stroked animals 1d prior to sacrifice. Stroke caused the presence of $CD45^{Hi}$ subset at 7d and 2m only in the ipsilateral hemisphere. $CD45^{Low}$ populations were presented in both hemispheres with significant increase in the stroked hemisphere. Adoptive transfer of GFP+ splenocytes into stroked mice resulted in GFP+ cells in both $CD45^{Hi}$ and $CD45^{Low}$ subsets at 7d and 2m. Protein expression of CD36 and lysosomal acid lipase (LAL), M2 and phagocytic markers, were increased at 7d and the increase was higher at 2m. Immunohistochemical analyses showed CD36 expression in infiltrated GFP+ MMs in the ipsilateral hemisphere. In mice with myeloid specific deletion of CD36 ($CD36KO^{MM}$), GFP+ cells were absent in $CD45^{HI}$ subset but largely presented in $CD45^{Low}$ subsets, although the total number of GFP+ cells into the stroked brain were similar between the genotypes. There was no difference in infarct size at 3d (Wt vs cKO, n= 11/group, ns). The present study showed the persistent infiltration of MMs during acute and recovery stages. The absence of infiltrated GFP+ cells in $CD45^{Hi}$ subset in $CD36KO^{MM}$ mice further suggest an involvement of MM CD36 in balancing pro-inflammatory ($CD45^{Hi}$) and microglia-like ($CD45^{Low}$) subsets in the post-stroke brain. <!--EndFragment-->

Disclosures: K. Park: None. **M. Woo:** None. **M. Balkaya:** None. **J. Yang:** None. **M. Febbraio:** None. **S. Cho:** None.

Poster

383. Ischemia II

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Topic: C.08. Ischemia

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Title: TRIM9-mediated resolution of neuroinflammation confers neuroprotection against ischemic stroke in mice

Authors: *J. ZENG[1], Y. WANG[2], Z. LUO[3], L.-C. CHANG[4], X. XIE[2], B. DEVERMAN[5], V. GRADINARU[5], S. GUPTON[6], B. ZLOKOVIC[2], Z. ZHAO[2], J. JUNG[4]
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Abstract: Excessive and unresolved neuroinflammation is a key component of the pathological cascade in brain injuries such as ischemic stroke and timely resolution of neuroinflammation is critical for the recovery and repair after brain injury. The nuclear factor-κB (NF-κB) signaling plays a central role in neuroinflammation through transcriptional induction of proinflammatory genes. In the aged brain, without any evident disease, there are chronically increased levels of NF-κB activity and pro-inflammatory cytokines. We report that TRIM9, a brain-specific TRIpartite motif (TRIM) protein, is highly expressed in the peri-infarct areas shortly after ischemic insults and governs the resolution of NF-κB-mediated neuroinflammation, which is considerably abrogated in aged brain. Mechanistically, TRIM9 sequestered β-TrCP from Skp-Cullin-F-box ubiquitin ligase complex, blocking IκBα degradation and thereby dampening NF-κB-dependent proinflammatory mediator production and immune cell infiltration. Consequently, *Trim9* deficient mice were highly vulnerable to ischemia, manifesting uncontrolled neuroinflammation and exacerbated neuropathological outcomes. Systemic administration of recombinant TRIM9 adeno-associated virus-PHP.B, allowing brain-wide enriched TRIM9 expression, effectively resolved neuroinflammation and alleviated neuronal death, especially in aged mice. This reveals that TRIM9 is essential for resolving NF-κB-dependent neuroinflammation for the recovery and repair after brain injury and TRIM9-potentiation based therapy may offer a new treatment of stroke and inflammation-related neurological disorders.

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Poster

383. Ischemia II

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Program #/Poster #: 383.08/P3

Topic: C.08. Ischemia

Support: Grants-in-Aid for Scientific Research (C) [grant number 16K10988]
Kobe Gakuin University joint research (C)

Title: Specific action mediated by spinal HMGB1 signaling in the ischemic stress-induced mechanical allodynia in mice

Authors: *W. MATSUURA[1], S. HARADA[1], K. LIU[2], M. NISHIBORI[2], S. TOKUYAMA[1]
[1]Kobe Gakuin Univ., Kobe, Japan; [2]Okayama Univ., Okayama, Japan

Abstract: [Aims]We have previously shown that spinal high-mobility group box-1 (HMGB1) plays a key role in the induction of central post-stroke pain (CPSP). It has been also reported that HMGB1 exacerbates inflammation and pain condition through its receptors such as toll-like receptor 4 (TLR4) or receptor for advanced glycation end products (RAGE). Furthermore, it has been reported that HMGB1 regulates activation of glial cells and nitric oxide synthetase (NOS) involved in pain through TLR4 and RAGE. In this study, we investigated whether the interaction between spinal glial cells and HMGB1 signaling, including its receptors, is directly involved in the induction of CPSP. **[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, anti-HMGB1 monoclonal antibody (mAb), lipopolysaccharides from *Rhodobacter sphaeroides* (LPS-RS, a TLR4 antagonist), low-molecular-weight heparin (LMWH, a RAGE antagonist) and N^G-nitro-L-arginine methyl ester (L-NAME, a nonselective NOS inhibitor) intrathecally (i.t.) injected. Glial cells expression levels and NOS activity on day 3 after BCAO were measured using immunostaining and colorimetric assay, respectively. **[Result]**On day 3 after BCAO, intrathecal injection of anti-HMGB1 mAb, LPS-RS and LMWH significantly blocked mechanical allodynia. BCAO-induced upregulation of spinal Iba1-positive cells (microglial marker) and GFAP-positive cells (astrocyte marker) were suppressed by i.t. injection of anti-HMGB1 mAb and LPS-RS, but not LMWH. In addition, i.t. injection of L-NAME significantly blocked mechanical allodynia on day 3 after BCAO. Furthermore, i.t. injection of anti-HMGB1 mAb, LPS-RS and LMWH significantly inhibited the increase of NOS activity in the spinal cord on day 3 after BCAO.

[Conclusion]These results showed that spinal HMGB1/TLR4/glial cells/NOS and HMGB1/RAGE/NOS signaling are directly involved in the induction of CPSP.

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Poster

383. Ischemia II

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Program #/Poster #: 383.09/P4

Topic: C.08. Ischemia

Support: ERA-Net Neuron (01EW1501A, A.E.)
Vascular Dementia Research Foundation (SyNergy)

Title: Study the meningeal vessels and neuroinflammation after stroke in the intact transparent organisms by using panoptic imaging

Authors: *C. PAN[1,2], R. CAI[1,2], A. GHASEMIGHARAGOZ[1], M. I. TODOROV[1,2], B. FÖRSTERA[1], S. ZHAO[1], A. XAVIER[3], B. KRESS[3,4], C. BENAKIS[1], A. LIESZ[1,5], M. NEDERGAARD[3,4], A. ERTÜRK[1,2,5]
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Abstract: Stroke is one of the leading cause of death and disability in today's world[1]. Apart from the acute neuron death and degeneration, neuroinflammation, which involves with infiltration of peripheral immune cells, has been recognized as a major secondary injury mechanism after stroke[2]. The lymphatic system is critical for immune responses although until recently, the brain was considered to be devoid of any lymphatic vessels. The discovery of brain lymphatic vessels[3] indicates a potential immune cell trafficking route between the CNS and the rest of the body. However, standard histology is not an ideal method to study meningeal vessels as their connections are largely destroyed when the brain is harvested.
To overcome this hurdle and to image details of intact meningeal vessels, we used DISCO clearing of whole mouse body, which allows us to image the subcellular details through bones and highly autofluorescent tissues. Using DISCO panoptic imaging, we readily visualized intact brain meningeal vessels with immune cells in their native environment. Applying middle cerebral artery occlusion (MCAO) model of stroke in LysM-EGFP line transgenic mice (labeling monocytes and macrophages), we observed the invasion of LysM GFP+ cells into the brain

parenchyma especially in the peri-infarct region. Furthermore, we detected significant increase of LysM GFP+ cells in the brain meningeal structures after stroke in MCAO mice. This finding suggests that the meninges might be an additional invasion route of immune cells differently from the disrupted blood brain-barrier and the choroid plexus[4]. In summary, DISCO panoptic imaging of intact mouse bodies provides a powerful and unique methodology for studying the meningeal structures both in health and disease and it is readily applicable in diverse fields of biomedical research.

References

1 Lee, Y. *et al.* Therapeutically targeting neuroinflammation and microglia after acute ischemic stroke. *BioMed research international* **2014**, 297241, doi:10.1155/2014/297241 (2014).
2 Kim, J. Y., Kawabori, M. & Yenari, M. A. Innate inflammatory responses in stroke: mechanisms and potential therapeutic targets. *Current medicinal chemistry* **21**, 2076-2097 (2014).
3 Louveau, A. *et al.* Structural and functional features of central nervous system lymphatic vessels. *Nature* **523**, 337-341, doi:10.1038/nature14432 (2015).
4 Llovera, G. *et al.* The choroid plexus is a key cerebral invasion route for T cells after stroke. *Acta neuropathologica* **134**, 851-868, doi:10.1007/s00401-017-1758-y (2017).

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Poster

383. Ischemia II

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Program #/Poster #: 383.10/P5

Topic: C.08. Ischemia

Support: AHA Predoctoral Fellowship
Aldelson Foundation
R01 Neurovascular

Title: Pericyte contribution to scar after stroke

Authors: *T. PHAM, S. CARMICHAEL
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Abstract: Stroke is the leading cause of adult disability. Pericyte, a less-well-understood cell type of the brain, is a heterogeneous population of perivascular cells that regulates development and maintenance of structural elements of the blood brain barrier, vascular stability, and angiogenesis. The role of pericytes after stroke has not been well characterized. Most work on

pericytes in the brain in general and in stroke have focused on their role in regulating blood flow. Our previous data indicates that pericytes after stroke experience bursts in proliferation and unique migration patterns away from blood vessels to form populations of fibroblast-like cells at the infarct margin. This suggests that pericytes take part in active tissue remodeling adjacent to the stroke. Pericyte contributions to fibrotic scar after injury has been a topic of debate in recent years. The controversy stems from the use of different transgenic mouse lines to fate-track and different injury models with different biology. Here, using novel dual-pericyte-specific viral systems that tightly label pericyte populations before and after stroke, we phenotyped fate-tracked post-stroke pericytes. We found that post-stroke pericytes migrate away from vasculature, heavily proliferate, start to express fibroblast markers, and take residence inside the infarct core. Surprisingly, meningeal fibroblasts sharing the same pericyte markers did not contribute to the formation of the fibroblast scar in the infarct core. Our result confirms the pericyte origin of fibrotic scar in the central nervous system after stroke, and suggest the multiple roles this heterogeneous cell type plays in tissue repair and regeneration.

Disclosures: T. Pham: None. **S. Carmichael:** None.

Poster

383. Ischemia II

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 383.11/P6

Topic: C.08. Ischemia

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NARSAD Young Investigator Award
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NIH Grant NS100047

Title: Inhibition of miR-34b/c rescues hippocampal CA1 neurons and memory deficits in global ischemia

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Abstract: Transient global ischemia arising as a consequence of cardiac arrest in humans causes selective, delayed death of hippocampal CA1 pyramidal neurons and cognitive impairment. Effective treatments to ameliorate the neurodegeneration and cognitive dysfunction associated with global ischemia are an unmet need. Emerging evidence points to a widespread role for microRNAs (miRNAs) as key modulators of target gene expression in neurons. Accordingly, dysregulation of miRNAs are implicated in the pathophysiology of neurodegenerative disease

and neurological disorders. Our findings, derived *via* miRNA-seq, indicate that expression of a subset of microRNAs are altered in postischemic CA1 including miR-34b/c, miR-21, miR-331, miR-181 and miR-29. Ingenuity pathway analysis reveals that miR-34b/c is the leading miR candidate implicated in cell death and survival. Dysregulation of miR-34 has been implicated in pathophysiology of neurological disorders such as Parkinson's disease and epilepsy. However, a role for miR-34 in the pathogenesis of global ischemia is, as yet, unclear. Here we show ischemia induces p53-dependent activation of miR-34b/c and downregulation of its target genes, which together promote neuronal death in selectively vulnerable hippocampal CA1 *in vivo*. Consistent with this, inhibition of miR-34b/c affords neuroprotection, rescues impaired synaptic plasticity and reduces memory deficits in global ischemia. These findings document a causal role for p53-dependent activation of miR-34b/c in neuronal death and identify a novel therapeutic target for amelioration of the neurodegeneration and cognitive deficits associated with ischemic stroke.

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Poster

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Title: miRNA miR-7a-5p ameliorates ischemic brain damage by targeting alpha-synuclein

Authors: *T. KIM[1], S. MEHTA[1], M. LOPEZ[1], R. SULLIVAN[2], K. MORRIS-BLANCO[1], H. KIM[1], C. KIM[1], R. VEMUGANTI[1]
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Abstract: Transient focal ischemia is known to induce extensive temporal changes in rodent cerebral miRNAome. We previously showed that of those altered, miR-7a-5p (miR-7) was found to be significantly decreased in a sustained manner during the acute phase after focal ischemia. Functionally, miR-7 was shown to inhibit the expression of several proteins including alpha-synuclein that play detrimental roles in neurodegenerative diseases. Thus, we presently evaluated the therapeutic efficacy of miR-7 after cerebral ischemia in rodents as per Stroke Treatment Academic Industry Roundtable criteria. Rodents were subjected to transient middle cerebral

artery occlusion. miR-7 was injected via either intracerebrally or systemically through the retro-orbital sinus. miR-7 levels and protein levels were measured by either qPCR or Western Blots. The post-ischemic motor deficit was evaluated with rotarod, beam walk, and adhesive removal test, and brain damage was measured on cresyl violet stained brain sections. Cellular changes after ischemia were examined using immunofluorescence staining. We found that miR-7 expression was decreased in both young/adult and aged rats of both sexes after ischemia. Pre- or post-ischemic treatment with miR-7 mimic decreased the infarct volume and promoted functional recovery in both sexes and ages studied. Furthermore, systemic injection of miR-7 mimic into post-ischemic mice resulted in significant decrease in the infarct volume with minimal peripheral toxicity. The miR-7 mimic treatment significantly reduced the post-ischemic induction of alpha-synuclein which is known to induce mitochondrial fragmentation, apoptosis, oxidative stress and autophagy that promote post-stroke neuronal death. Therefore, out studies indicate that miR-7 is a potential therapeutic agent to minimize post-stroke brain damage.

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Poster

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Topic: C.08. Ischemia

Support: NIH Grant NS103017

Title: The microRNA-210/TET2 axis mediates neuroinflammation in neonatal hypoxic-ischemic brain injury

Authors: *Q. MA, C. DASGUPTA, L. ZHANG
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Abstract: Neonatal hypoxia-ischemia (HI) is a leading cause of acute mortality and chronic brain injury in newborns with a high risk of hypoxic-ischemic encephalopathy. MicroRNA-210 (miR-210) is "*the Master Hypoxamirs"* and has been reported to be involved in physiological and pathological processes in response to hypoxia. Our previous study demonstrated that the inhibition of miR-210 provided neuroprotection in neonatal HI brain injury. However, the underlying mechanisms remain elusive. Our data shows that Tet methylcytosine dioxygenase 2 (TET2) is a putative downstream target of miR-210, which plays a critical role in regulating inflammatory gene expression after neonatal HI in mice. The miR-210 inhibitor complementary locked nucleic acid oligonucleotides (LNA-miR-210) or LNA scramble was stereotaxically injected into the ipsilateral hemisphere of postnatal day 7 (P7) male and female mouse pups

intracerebroventricularly. Twenty-four hours later, mouse pups were subjected to ligation of unilateral common carotid artery followed by hypoxic treatment (8% O_2). The immunoblot assay found that miR-210-LNA rescued brain TET2 protein levels after HI insult, as compared to LNA scramble. Moreover, the delivery of miR-210 mimic into the mouse brain resulted in downregulation of brain TET2 protein levels as compared to scramble control. To investigate the role of TET2 in regulating inflammatory response in neonatal HI brain injury, mouse pups (P7) were injected with TET2 silencing RNA intracerebroventricularly followed by HI insult 48 hours later. The results showed that TET2 knockdown significantly increased the vulnerability of the neonatal brain to HI insult and resulted in exacerbated neurological deficits. Moreover, TET2 knockdown time-dependently upregulated the transcription levels of pro-inflammatory cytokines, as compared to scramble control. Thus, our results demonstrate that the miR-210/TET2 axis mediates the regulation of neuroinflammatory response in neonatal HI brain injury. (Supported by NIH NS103017)

Disclosures: Q. Ma: None. **C. Dasgupta:** None. **L. Zhang:** None.

Poster

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Topic: C.08. Ischemia

Support: NIH Grant NINDS R01NS086945

Title: Do microRNAs change expression in oligodendrocytes after perinatal hypoxia ischemia in mice?

Authors: M. H. BASHIR[1], S. C. CHAPMAN[2], *R. W. DETTMAN[3], M. L. V. DIZON[4]
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Abstract: In the postnatal brain, neural progenitors within the cortex and subcortical white matter predominantly produce cells of oligodendroglial lineage. These cells are particularly vulnerable to hypoxic-ischemic injury (HI) resulting in the loss of mature oligodendrocytes (OL) or defects in myelination. MicroRNAs (miRs) are short, non-coding RNAs that modify gene expression. We previously observed increased expression of miR-138 and mir-338 after perinatal HI in P7 mouse pups. Conditional ablation of Dicer in NG2-expressing neural progenitors protected against myelin loss and loss of motor function in mice indicating that miRs play a role in the response of the brain to perinatal HI. Here, we further evaluated changes to expression of miR-9, mir-21, miR-138, miR-338 after perinatal HI using quantitative real-time polymerase chain reaction (qRT-PCR) 24h, 48h, 72h, 7d, 14d and 28d post-HI compared to age-matched

shams. We observed that in total hemispheric RNA, pri-miR-9 peaked at 24h (Fold Change (FC)=2.06) and mature miR-9 peaked at 7d (FC=2.91) relative to sham. For miR-21, pri-miR-21 peaked at 24h (FC=1.82) and mature miR-21 peaked at 72h (FC=4.78) relative to sham. For miR-138, pri-miR-138 peaked at 72h (FC=1.87) and mature miR-138 peaked at 7d (FC=3.76) relative to sham. For miR-338, pri-miR-338 peaked at 72h (FC=1.48) and mature miR-338 peaked at 7d (FC=2.3) relative to sham. Thus, these miR's all increased in response to perinatal HI. To test if these miRs increased in neural progenitors we employed MIRAP (**mi**cro**R**NA tagging and **a**ffinity **p**urification). We generated NG2Cre^{ERTM}; tAgo2 mice and subjected them to perinatal HI at P7. MIRAP was performed at 72h post-HI. Here, qRT-PCR was performed using the locked nucleic acid technology developed by Exiqon using miR-103 as the control transcript. We observed that mature miR-9 and miR-138 were decreased relative to sham (FC=0.53 and FC=0.57). miR-338 was not detectable by MIRAP at 72h. Mature miR-21 was increased in neural progenitors at 72h (FC=2.2). These findings indicated that 72h after HI, only mature miR-21 is increased in neural progenitors. To test this further we performed *in situ* hybridization for mature miR-21 and observed that miR-21 was increased in the corpus callosum of mice exposed to perinatal HI 48h after injury. Together, we found increases to miRs at different times after perinatal HI. The presence of mature miRs always followed accumulation of pri-miRs indicating that tissues undergo a period of time before RNAs are packaged into RISC. MIRAP provides a basis for distinguishing between miRs that accumulate in neural progenitors versus other cell types of the perinatal brain.

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Poster

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Topic: C.08. Ischemia

Support: NIA (AG033720)
NINDS (NS094881)

Title: Anti-cancer drugs (CX-4945 and MS-275) protective of WM against ischemic injury differentially regulate miRNA expression

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Abstract: We have shown that two cancer drugs (CX-4945 and MS-275) are protective of white matter (WM) against ischemic injury. These compounds have two different mode of action. CX-4945 is a selective Casein Kinase 2 (CK2) inhibitor and MS-275 is a selective Class I HDAC inhibitor. Could alteration in miRNAs, noncoding RNAs that regulate gene expression, mediate some of the protective actions of these cancer drugs on WM from ischemic injury? The aim of this study was **1)** to identify miRNA expressed in mouse optic nerve, **2)** to determine miRNA regulated by OGD and **3)** to determine if CX-4945 and MS-275 alter the miRNA expression in a mouse model of WM ischemic injury. RNA was isolated from the mouse optic nerve (MON) from control, following oxygen glucose deprivation (OGD) with and without CX-4945 or MS-275. A quantifiable miRNA profiling technique (NanoString) was used to determine the expression of 610 miRNAs. Mean expression levels and fold change were determined. In control condition, NanoString analysis revealed that 280 miRNAs were expressed above background levels and that 12 miRNAs were expressed at high levels (> 10 000 sequence counts) in MONs. OGD resulted in the up-regulation of four miRNAs (miR-501-3p, miR-201, miR-1959, miR-146b) and the down-regulation of two miRNAs (miR-1937a and miR-1937b) compared to control conditions. OGD with CX-4945 treatment resulted in the up-regulation of two miRNAs (miR-1937a, miR-1937b) and the downregulation of three miRNAs (miR-501-3p, miR-200b and miR-mo1-2). Note that miR-501-3p, miR-1937a and miR-1937b showed differential expression between OGD and OGD with CX-4945. Finally, OGD with MS-275 resulted in the alteration of more miRNAs. Specifically, the upregulation of five miRNAs (miR-376b, miR-2140, miR-2141, miR-2146 and miR-487b) and the downregulation of five miRNAs (miR-501-3p, miR-190, miR-1959, miR-27a and miR-M1-7-3p). Note that miR-501-3p and miR-1959 showed differential expression between OGD and OGD with MS-275 treatment. Interestingly miR-501-3p was a common target but differentially regulated between OGD and OGD with CX-4945 or OGD with MS-275 . Our findings provide the first evidence that WM ischemia modulates miRNA and that CX-4945 and MS-275 regulate WM miRNA, providing a common mechanism by which CX-4945 and MS-275 confer protection to axon function and WM integrity against an ischemic injury. In addition, these findings may pave the way for treating WM by targeting specific miRNAs, leading to improved axonal function, decreased disability and ultimately increased quality of life of stroke patients.

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Poster

383. Ischemia II

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Title: Adrenergic receptor antagonism induces neuroprotection and facilitates recovery from acute ischemic stroke

Authors: *H. MONAI[1,2,3], K. YAHAGI[1,3], X. WANG[1,3], N. LOU[4], H. MESTRE[4], Q. XU[4], Y. ABE[5], M. YASUI[5], Y. IWAI[1,3], M. NEDERGAARD[4,6], H. HIRASE[1,3,6]
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Abstract: Repetitive waves of cortical spreading depression/depolarization (CSD) are spontaneously evoked in acute ischemic injury. CSD is linked to a sharp increase of extracellular K^+ that induces a long-lasting suppression of neural activity. CSD induces secondary irreversible damage in the ischemic brain suggesting that K^+ homeostasis might constitute a novel therapeutic strategy. Here, we found that asynchronous astrocytic Ca^{2+} activity appears after CSD**.** Adrenergic receptor (AdR) antagonism suppressed this aberrant astrocytic Ca^{2+} activity and accelerated normalization of extracellular K^+, resulting in faster recovery of neural activity. Remarkably, systemic adrenergic blockade before or after stroke facilitated functional locomotor recovery and reduced infarct volume, paralleling to the preservation of the water channel aquaporin-4. Our results suggest that AdR blockers promote cerebrospinal fluid exchange and rapid extracellular K^+ clearance, representing a novel and potent intervention for acute stroke.

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Poster

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Support: NIH grant R01MH086638

Title: Multiscale simulation of spreading depolarization in ischemic stroke

Authors: *A. NEWTON[1,2], A. H. SEIDENSTEIN[4], M. L. HINES[1], R. A. MCDOUGAL[1,5], W. W. LYTTON[2,6,3]
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Abstract: Occlusion of a blood vessel in the brain triggers a cascade of changes, including: 1. synaptic glutamate release, related to excitotoxicity; 2. elevated extracellular potassium, leading to spreading depolarization (depression); 3. edema due to cell swelling, reducing the extracellular volume and increasing the tortuosity; 4. production of reactive oxygen species, which gives rise to inflammation and direct cellular damage. These cascades occur over multiple time-scales, with the initial rapid changes in cell metabolism and ionic concentrations triggering several damaging agents that may ultimately lead to cell death. At the tissue scale coincident diffusion may divide ischemic tissue into distinct patterns of pathology, with the ischemic core surrounded by several types of ischemic penumbrae, with cells that are damaged but salvageable.
We developed models of ischemic stroke at molecular, cellular and tissue scale, using multiscale coupling of electrophysiology, intracellular molecular alterations, neuronal network activity, and bulk tissue alterations mediated by extracellular diffusion. We used these models to evaluate the hypotheses of multiple penumbrae and patterning of cell damage.
We considered several toxic substances released from the ischemic core as contributors to penumbral damage, on top of a background ischemic field in the watershed areas. Differences in the rates of production, release, diffusion and clearance of the substances could give rise to multiple distinct penumbrae about the ischemic core. In addition to the tissue patterning, the individual cells may show differential susceptibility to damage due to differences in morphology and physiology, such as the surface area to volume ratio or the intracellular calcium dynamics. We investigated this by placing biophysically detailed models of cortical pyramidal neurons in the penumbrae, with intracellular Ca^{2+} dynamics and Na^{+}/K^{+}-ATPase pumps. Such differential cellular damage could also lead to alterations in cell morphology and function in contexts of low-level repeated ischemia, potentially leading to ischemic dementia.

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Poster

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Title: Zn^{2+} entry through the mitochondrial calcium uniporter (MCU): Critical role in mitochondrial dysfunction and neurodegeneration after neuronal Zn^{2+} loading and during ischemia

Authors: *S. G. JI, Y. V. MEDVEDEVA, J. H. WEISS
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Abstract: In addition to Ca^{2+}, Zn^{2+} contributes to ischemic neurodegeneration. During ischemia, Zn^{2+} is released at synapses, where it can enter postsynaptic neurons, and from cytosolic buffering proteins (where it is normally bound). Prior studies suggest that Zn^{2+} can enter mitochondria through the mitochondrial calcium uniporter (**MCU**) and disrupt their function. Yet key questions remain. **(1) Validation of MCU dependent mitochondrial Zn^{2+} entry and dysfunction.** Prior studies were limited by the incomplete specificity of MCU blockers. Using MCU knockouts (**KO**), we were able to confirm Zn^{2+} entry through this route. We further examined effects of MCU deletion on Zn^{2+}-induced mitochondrial dysfunction. In cultured neurons subjected to mild exogenous Zn^{2+} loads combined with partial disruption of endogenous buffering, we found Zn^{2+}-induced mitochondrial depolarization and swelling both to be attenuated in MCU KO. **(2) MCU dependence of Zn^{2+}-triggered reactive oxygen species (ROS) production.** Despite prior studies showing that acute Zn^{2+}-triggered ROS generation largely depends upon mitochondrial Zn^{2+} uptake, we found increased Zn^{2+}-induced ROS production in MCU KO, likely reflecting induction of the cytosolic ROS generating enzyme NADPH oxidase as a previously unknown compensatory effect. **(3) MCU targeted interventions.** To assess the potential therapeutic utility of targeting the MCU, we examined effects of adding MCU blockers immediately after Zn^{2+} exposure, and found this to significantly attenuate mitochondrial dysfunction. **(4) MCU in Zn^{2+}-induced and ischemic cell death.** Finally, we examined the neuroprotective utility of targeting the MCU. In cultured neurons, both MCU KO and delayed MCU blockade attenuated Zn^{2+}-triggered cell death. Additionally, in hippocampal slices we found evidence that Zn^{2+} entry through the MCU contributes to cell death in a model of ischemia, likely via disrupting mitochondrial function. The strong correlations between preservation of mitochondrial function and neuroprotection in both models support the idea that mitochondrial Zn^{2+} uptake via MCU contributes directly to injury. These findings show a new role of MCU in Zn^{2+}-triggered and ischemic neurodegeneration and support the utility of delayed MCU blockade as a novel neuroprotective strategy.

Disclosures: Y.V. Medvedeva: None. **J.H. Weiss:** None.

Poster

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AHA Grant 17GRNT33410181

Title: Transient global ischemia induces early progressive Zn^{2+} accumulation in CA1 mitochondria that may contribute to mitochondrial structural disruption and neurodegeneration

Authors: H.-L. WANG, H. Z. YIN, G. TIAN, S. G. JI, Y. V. MEDVEDEVA, A. BAZRAFKAN, N. MAKI, Y. AKBARI, *J. H. WEISS
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Abstract: Despite high morbidity of ischemic stroke, there are as of yet no neuroprotective interventions with efficacy in humans. Whereas most prior efforts targeted Ca^{2+}, recent findings have highlighted contributions of Zn^{2+}. *In vitro* studies by us and others support the hypothesis that mitochondria are important sites of injurious Zn^{2+} effects; after entering neurons, Zn^{2+} can enter mitochondria, triggering mitochondrial dysfunction (including reactive oxygen species production, depolarization, and swelling), contributing to cell death. We have also used hippocampal slices subjected to oxygen glucose deprivation (**OGD**) to model ischemia, and found that early neuronal Zn^{2+} accumulation and entry into mitochondria both appear to precede and contribute to sharp Ca^{2+} rises associated with acute neurodegeneration. In addition, after sublethal OGD, there is long lasting Zn^{2+} accumulation in CA1 mitochondria that appears to contribute to delayed mitochondrial dysfunction. To address the above hypothesis in an *in vivo* translational model of global ischemia, rats were subjected to 8-9 min asphyxial cardiac arrest, resuscitated, then perfused after either 1 or 4 h recovery. Examining the hippocampal slices, we observe the following: (1) Vanadium acid fuchsin staining revealed substantial neuronal injury to hippocampal CA1 and CA3 pyramidal neurons after global ischemia, with significantly greater impact on CA1. (2) Examination of immunolabeled mitochondria under confocal microscopy indicated mitochondrial swelling in both CA1 and CA3 neurons. (3) To assess redistribution of labile (or loosely bound) Zn^{2+} after ischemia, we labeled reactive Zn^{2+} using the Timm's sulfide silver technique (in which Na_2S precipitates reactive Zn^{2+}, and electron dense silver associates with the precipitates), and examined the slices using electron microscopy. We found that ischemia caused substantial Zn^{2+} accumulation in CA1 mitochondria, which correlated strongly with disruption of mitochondrial structure and was far worse with 4 h than with 1 h recovery. This study provides the first ultrastructural examination of Zn^{2+} redistribution after *in vivo* global

ischemia, and lends support to the hypothesis that early and progressive mitochondrial Zn^{2+} accumulation after ischemia contributes to mitochondrial dysfunction and neuronal death.

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Poster

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Topic: C.08. Ischemia

Support: RO1NS076715

Title: The role of mitochondrial fission in neurons post cardiac arrest

Authors: *K. MAHERAS[1], A. R. ANZELL[2], T. H. SANDERSON[3]
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Abstract: Cardiac arrest is the leading cause of natural death in the United States. Patients successfully resuscitated from cardiac arrest will often die from the neurologic damage induced by lack of blood flow to the brain. Reperfusion of the brain, set in motion by the requisite resuscitation of the patient, causes significant mitochondrial dysfunction, exacerbating neuronal death. Mitochondria quality control depends on the delicate balance of fission, fusion, and targeted autophagy of unthrifty mitochondria (mitophagy). Proper mitochondrial dynamics can be perturbed by a variety of factors and are an imperative determinant of cell viability, often faltering under the stress of reperfusion. To better understand the molecular mechanisms of mitochondrial fragmentation during reperfusion injury, we have put forth a focused strategy to manipulate the mitochondrial fission protein, Dynamin-related protein-1 (Drp1) in a mouse model of cardiac arrest and resuscitation.
Mitochondrial fission is regulated by Drp1, which is recruited to the outer mitochondrial membrane to constrict mitochondria. Under duress, such as ischemia-reperfusion, the mitochondrial dynamic state becomes unbalanced, tending towards fragmentation, resulting in cell death. Using our novel mouse model of cardiac arrest/resuscitation in combination with a neuron specific Cre recombinase (CamK2a-ERT2) and floxed Drp1, we are able to induce Drp1 knockout in vulnerable neurons to elucidate the role of Drp1 induced fission in neuronal injury following cardiac arrest.

Disclosures: K. Maheras: None. **A.R. Anzell:** None. **T.H. Sanderson:** None.

Poster

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Topic: C.08. Ischemia

Support: NIH Grant R01NS076715

Title: Disruption of mitochondrial quality control following ischemic brain injury

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Emergency Med., The Univ. of Michigan, Ann Arbor, MI

Abstract: The primary cause of morbidity and mortality in post-cardiac arrest patients is brain injury caused by global ischemia/reperfusion injury. While the return of spontaneous circulation is essential to preserve the ischemic tissue, it also significantly exacerbates brain injury. Mitochondria play a central role in the pathophysiology of post-cardiac arrest injury. Furthermore, maintenance of mitochondrial integrity through quality control is critical to maintaining mitochondrial function. This process is achieved through the specific fragmentation and removal of dysfunctional mitochondria or mitochondrial segments. Although this process is known to play a pivotal role in the maintenance of healthy neurons, much controversy exists with regards to the role of mitochondrial quality control in the progression of post-ischemic brain injury. Recent development of a novel binary-based fluorescence assay for mitophagic flux (mito-QC) has permitted a rigorous analysis of mitochondrial quality control in cells. Expression of tandem mCherry and GFP fused to the targeting sequence of the outer mitochondrial membrane protein, Fis1 allows temporal resolution of this process. During stable conditions, both red and green fluorophores are localized to mitochondria. When a mitochondrion undergoes mitophagy, mCherry fluorescence remains stable, while GFP is quenched. Thus, the red/green fluorescence ratio can be utilized to quantify mitochondrial quality control flux in physiologic and pathologic contexts. Utilizing this transgenic mouse model in conjunction with our novel mouse model of cardiac arrest/resuscitation, we investigated disruption of the mitochondrial network and mitochondrial quality control balance after ischemic brain injury. Global brain ischemia during cardiac arrest induces extensive mitochondrial network fragmentation during reperfusion, which is followed by induction of regulated cell death. This mitochondrial fragmentation is associated with disruption of normal mitochondrial quality control flux in the CA1 hippocampus of mito-QC mice. These novel models allow a refined analysis of mitophagy in the brain during ischemia/reperfusion injury and suggest the manipulating mitophagic flux after ischemia could provide a novel means of therapeutic intervention.

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Poster

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Title: Pharmacological calcium/calmodulin-dependent kinase (CAMKII) inhibition protects against Purkinje cell damage following cardiac arrest and cardiopulmonary resuscitation in mice

Authors: *N. QUILLINAN[1], N. CHALMERS[1], O. PATSOS[1], K. STEKLAC[2], P. S. HERSON[3]
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Abstract: Introduction: Ischemic brain damage is triggered by glutamate excitotoxicity resulting in neuronal cell death. While the specific cascade of events leading to injury is complex, previous research has demonstrated that NMDA receptor activation triggers downstream calcium-dependent signaling pathways, specifically Ca2+/calmodulin-dependent protein kinase II (CaMKII). Focal and global ischemia studies have shown that inhibiting CaMKII is protective against hippocampal damage, but there is little known about cerebellar cell death mechanisms. The aim of this study is to examine the neuroprotective potential of inhibition of CaMKII in Purkinje cells using a global ischemia model. **Methods:** C57BL/6 male adult mice were subjected to 8 minutes of cardiac arrest followed by cardiopulmonary resuscitation (CA/CPR). Mice were randomized to receive tat-CN19o or control peptide (tat-SCR), 30 minutes after CA/CPR. We performed a dose-response for tat-CN19o and cerebellar injury was analyzed at 7 days after CA/CPR. Acute signaling was assessed at 6 hours after CA/CPR using western blot analysis of cerebellar homogenates. Antibodies recognizing phosphorylated and total CAMKII and DAPKI were used, and integrated volume of bands was quantified. Statistical analyses were performed using ANOVA tests, with a $P<0.05$ considered significant. **Results:** We observed increased phosphorylation of the T286 residue of CAMKII at 6 hours after CA/CPR, suggesting increased autonomous activation. Analysis of Purkinje cell density revealed a decrease in cell density at 7 days after CA/CPR that was prevented with tat-CN19o at doses of 0.1 and 1 mg/kg. A dose of 0.1 mg/kg was able to prevent gait abnormalities 7 days after CA/CPR. Western blot analysis of cerebellum revealed that cardiac arrest reduced DAPK1 phosphorlyation, which was not observed in mice that received tat-CN19o (0.1 mg/kg). **Conclusions:** These data demonstrate that autonomous activity of CAMKII is increased by ischemia in the cerebellum that contributes to cell death. Neuroprotection in the cerebellum with tat-CN19o required doses that were 10-fold higher than what was needed for neuroprotection in

the hippocampus (Deng, Cell Reports, 2017). Importantly, we have identified DAPK1 pathway was activated following CA/CPR and CAMKII inhibition reduced this activation. Future studies will address whether cerebellar injury is DAPK1-dependent and whether these pathways are also activated in a CAMKII-dependent manner in the hippocampus. In summary, our findings indicate that inhibition of autonomous CaMKII activity is a promising therapeutic approach that is effective across multiple brain regions.

Disclosures: N. Quillinan: None. **N. Chalmers:** None. **O. Patsos:** None. **K. Steklac:** None. **P.S. Herson:** None.

Poster

383. Ischemia II

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Program #/Poster #: 383.23/Q3

Topic: C.08. Ischemia

Support: K111923
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Title: Contribution of large-conductance Ca^{2+}-activated potassium (BK) channels to the evolution of spreading and anoxic depolarization

Authors: *R. FRANK, Á. MENYHÁRT, F. BARI, E. FARKAS
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Abstract: Anoxic depolarization (AD) is a permanent event, and indicates primary brain damage in the ischemic core. In contrast, spreading depolarization (SD) is a transient wave of mass depolarization that causes secondary lesion growth in the penumbra. Although the profound extracellular K^+ elevation has served as the hallmark of these events for decades, further investigation is needed to identify the ion channels that mediate the cellular K^+ efflux during AD and SD. Our aim was to examine the contribution of large-conductance Ca^{2+}-activated potassium channels (BK channels) to AD and SD evolution.
Anesthetized C57Bl/6 mice (n=15) were used for the in vivo assessment of cerebrocortical local field potential (LFP), $[K^+]_e$, and local cerebral blood flow (CBF); or Sprague-Dawley rats (n=13) to prepare in vitro oxygen-glucose deprived (OGD) or control brain slice preparations for LFP recording. SDs were evoked by high concentration K^+ in vivo or electric stimulation in vitro, while AD occurred spontaneously in response to OGD in vitro. Half of the preparations were topically treated with paxilline (500 nM).
Paxilline decreased SD amplitudes both in vivo (11.00±5.3 vs. 19.3±4.5 mV, paxilline vs.

control) and in vitro (9.1±5.3 vs. 17.2±5.5 mV paxilline vs. control), and AD amplitude in vitro (2.4±1.4 vs. 5.8±2.6 mV, paxilline vs. control). The relative shift of $[K^+]_e$ with SD was also markedly reduced by paxilline in vivo (12.7±7.5 vs. 22.2±2.7 mM, paxilline vs. control). Finally, paxilline completely diminished SD-related hypoperfusion in 6 of 7 mice, in which $[K^+]_e$ prior to SD initiation was experimentally elevated over 10 mM.
These observations indicate the fundamental role of BK channels in the evolution of SD and AD. Moreover, we propose that K^+ efflux via BK channels effectively contributes to vasoconstriction in response to SD.

Disclosures: R. Frank: None. **Á. Menyhárt:** None. **F. Bari:** None. **E. Farkas:** None.

Poster

383. Ischemia II

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Program #/Poster #: 383.24/Q4

Topic: C.08. Ischemia

Support: NRF Grant NRF-2017R1A2B4002704

Title: Altered expression pattern of gene associated with retinoid-interferon induced mortality-19 (GRIM-19) following transient global cerebral ischemia in the mouse hippocampus

Authors: S.-N. HWANG[1,2], J.-C. KIM[1,2], *M.-Y. LEE[3,2], S. KIM[1,2]
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Abstract: Gene associated with retinoid-interferon-induced mortality-19 (GRIM-19) has been well known as a tumor suppressor gene in cancer research and a subunit of the mitochondrial respiratory chain complex I, required for mitochondrial ATP production. Brain ischemia, the leading cause of death and disability in developed countries, results in an abrupt increase of reactive oxygen species (ROS) that finally leads to cell death. GRIM-19 is a composition of the mitochondrial respiratory chain complex I where ROS is predominantly produced. In addition, ROS overproduction after ischemic insult has been considered as one of major causes of ischemia/reperfusion injury. Thus, the role of GRIM-19 in brain ischemia will help to clarify the mechanism of ischemia-induced brain damage. However, to date, there has been no report about the immunohistochemical expression pattern of GRIM-19 following brain ischemia, which is necessary to understand the pathophysiological role of GRIM-19 in cerebral ischemia. Therefore, in the present study, we examined the expression pattern of GRIM-19 in the adult mouse hippocampus after transient global cerebral ischemia. Male C57BL/6 mice (11 weeks old) were subjected to a 40-min of bilateral common carotid artery occlusion (BCCAO) followed by 3 days of reperfusion. Sham-operated animals received the same procedure without occlusion of the

common carotid arteries. To identify the cell types expressing GRIM-19, double immunofluorescence staining was performed using antibodies against GRIM-19 and either NeuN (a neuronal cell marker), GFAP (an astrocyte marker) or Iba-1 (a microglia marker). In sham-operated animals, GRIM-19 was expressed in most NeuN-positive cells but not in GFAP- and Iba-1-positive cells. In the BCCAO group, neuronal cell death and reactive astrocytes and microglial cells were observed in the hippocampal CA1 regions. It is noteworthy that the numbers of double-labeled cells for GRIM-19 and astrocytes or microglial cells were significantly increased in the non-pyramidal cell layer of the hippocampus, whereas there was a substantial loss of GRIM-19 immunoreactivity in the pyramidal cell layer. This is the first report showing immunohistochemical expression pattern of GRIM-19 in the hippocampus following transient global cerebral ischemia. These results raised the possibility that GRIM-19 plays a role in the reactive astrocytes and/or microglia following transient global cerebral ischemia. Further studies are needed to clarify the function of GRIM-19 in astrocytes and microglia under this pathophysiological condition.

Disclosures: S. Hwang: None. **J. Kim:** None. **M. Lee:** None. **S. Kim:** None.

Poster

383. Ischemia II

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Program #/Poster #: 383.25/Q5

Topic: C.08. Ischemia

Support: Grant MOST 104-2320-B-010-015, from Ministry of Science and Technology, Taiwan

Title: TrkB activation attenuates gephyrin misfolding induced by activity blockade and hypoxia ischemia in developing neurons

Authors: C.-C. HUNG, S.-P. HSU, W.-H. CHIEN, C.-Y. LEE, Y.-L. GAN, *Y.-H. LEE
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Abstract: Gephyrin is a scaffolding protein involved in clustering postsynaptic $GABA_A$ receptors for mediating inhibitory neurotransmission. Our previous study revealed that gephyrin misfolding occurs in protein kinase C (PKC) inhibition or neuronal activity blockade, as observed in neonatal hypoxia-ischemia (HI), due to the dephosphorylation of growth-associated protein 43 (GAP43) at serine 41 (S41). Activation of tyrosine receptor kinase B (TrkB) by brain-derived neurotrophic factor is known to mediate activity-dependent neuronal survival and plasticity involving PKC activation. Here, we investigated whether TrkB activation could rescue the activity blockade-induced gephyrin misfolding. We found that activity blockade-induced misfolded gephyrin was transported to aggresomes through association with histone deacetylase 6 and ubiquntin-K63. TrkB-activator, LM22A, attenuated S41 dephosphorylation of GAP43,

GAP43-gephyrin interaction, and gephyrin misfolding caused by blocking neuronal activity in developing neurons. Moreover, LM22A attenuated GAP43 dephosphorylation induced by oxygen-glucose deprivation in cultured cortical neurons and by hypoxia-ischemia in neonatal rat brains. Thus, these results suggest that TrkB activation is effective in ameliorating activity blockade-induced GAP43-gephyrin interaction and subsequent gephyrin misfolding in developing neurons, which may protect GABAergic synapse formation against neonatal HI injury.

Disclosures: C. Hung: None. **S. Hsu:** None. **W. Chien:** None. **C. Lee:** None. **Y. Gan:** None. **Y. Lee:** None.

Poster

383. Ischemia II

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Program #/Poster #: 383.26/Q6

Topic: C.08. Ischemia

Support: NIH K08NS101122
UVA Children's Hospital Start-Up

Title: Neuronal activity associated with hypoxic-ischemic injury in neonatal mice

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Abstract: Background: Hypoxic-ischemic encephalopathy (HIE) affects 1.5/1000 newborns annually and is associated with deficient cerebral blood flow (ischemia) and oxygen supply (hypoxia). HIE is a major cause of neonatal seizures, cerebral palsy, behavioral and cognitive defects. Neuronal circuits involved in HI-induced acute seizures are unknown. Further understanding of neuronal activation following HIE may lead to improved prognostic tools and new therapeutic strategies. **Objective:** Examine neuronal activation following HI-induced seizures in a neonatal "TRAP" mouse. **Methods:** HI was created using the Vannucci model (unilateral carotid ligation+45 min of 8% FiO2) in Cre-tamoxifen transgenic mice (TRAP) on postnatal day (p)10. 30 min later, mice were injected with 4-hydroxytamoxifen (4OHT) to allow expression of fluorescent protein in activated cells during the 1-2 hrs prior. Sham mice (neck incision+anesthesia, no ligation or hypoxia) and HI mice were perfused 7 days after injection. 200µm tissue sections were processed using the tissue clearing CLARITY method, then immunostained for NeuN. Imaging was performed on Zeiss 780 confocal microscope. Imaris 9.1 software was used to analyze images using the "ImarisColoc" function to determine the percentage of green (NeuN) and red (tdTomato) voxels that overlap. **Results:** CLARITY and

confocal microscopy allowed detection of activated neurons in the TRAP mice following HI-induced acute seizures. We observed selective neuronal activation in bilateral somatosensory cortex, ipsilateral (to carotid ligation) entorhinal cortex, dentate gyrus, CA3, lateral thalamic structures and striatum in HI mice compared to sham mice. Our data show that the ipsilateral hemisphere contains significantly more colocalized voxels compared to the contralateral hemisphere ($p<0.00001$) and sham mice ($p<0.00001$). **Conclusions:** CLARITY tissue clearing and confocal microscopy allowed visualization of neuronal activation following HI-induced seizures in neonatal "TRAP" mice. More activated neurons were present in the cortex ipsilateral to ligation as well as the hippocampal structures. The future goal is to examine if the neuronal activation in ipsilateral hippocampus is associated with deficits in memory function in neonatal HI-treated "TRAP" adult mice.

Disclosures: D. Skwarzynska: None. **P. Wagley:** None. **J. Kapur:** None. **J. Burnsed:** None.

Poster

383. Ischemia II

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Program #/Poster #: 383.27/Q7

Topic: C.08. Ischemia

Support: NIH Grant R01 NS081149

Title: Hyperglycemia drives increased superoxide production by peri-lesional microglia after permanent ischemia

Authors: *S. WON, S. GHOSH, A. HUYNH, R. A. SWANSON
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Abstract: Hyperglycemia commonly occurs after stroke as part of a systemic stress response, even in non-diabetic patients. Hyperglycemia after stroke worsens outcome both in patients and in animal models of stroke, but the mechanism(s) of this effect are not defined and it remains uncertain whether patients should have hyperglycemia aggressively normalized. In other settings, hyperglycemia can promote tissue injury by fueling production of superoxide by NADPH oxidase. Here we evaluated superoxide production in a mouse photothrombotic model of permanent ischemia. Forty-eight hours after ischemia onset, hyperglycemia (blood glucose of 300-400 mg/dL; 15-20 mM) was induced by i.p. injection of glucose. Dihydroethidium (DHE) was infused at the same time. Three hours later brains were harvested for assessment of superoxide formation in the peri-infarct region by evaluating oxidation of DHE to fluorescent species. Hyperglycemia was found to significantly increase superoxide formation. The oxidized DHE was localized primarily to Iba1/CD11b positive cells (microglia/macrophages). The experiment was then repeated in $p47^{phox-/-}$ mice, which cannot form an active NADPH oxidse-2

complex. In contrast to wild-type mice, hyperglycemia did not produce an increase in peri-lesional superoxide formation in the $p47^{phox-/-}$ mice. These results suggest that hyperglycemia occurring many hours after ischemia can increase oxidative stress in peri-infarct tissues by fueling NADPH oxidase activity in reactive microglia/macrophages, and by this mechanism may contribute to worsened outcome.

Disclosures: S. Won: None. **S. Ghosh:** None. **A. Huynh:** None. **R.A. Swanson:** None.

Poster

383. Ischemia II

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Program #/Poster #: 383.28/Q8

Topic: C.08. Ischemia

Support: NIH grant NIH/NINDS R01NS080844
Division of Newborn Medicine, University of Mississippi Medical Center

Title: Intranasal insulin attenuates hypoxic-ischemic brain damage and sensorimotor behavioral disturbances in neonatal rats

Authors: C. P. TALATI, J. W. LEE, S. LU, N. B. OJEDA, Y. PANG, *A. J. BHATT, L.-W. FAN
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Abstract: Hypoxic-ischemic (HI) encephalopathy (HIE) causes significant morbidity and mortality in affected newborns. Current therapies have limited use and efficacy. There is an urgent need for additional therapies to improve these outcomes. Recent animal and clinical studies suggest that insulin may function as a neuroprotective agent, but its effect against HI brain injury is unexplored. Intranasal insulin (InInsulin) has been shown to have neuroprotective effects in adult stroke and Alzheimer's disease studies. The intranasal route is beneficial as it has a more direct CNS distribution and minimal systemic side effects. We tested a novel hypothesis that InInsulin is neuroprotective against HI brain injury in newborn rats. The objective of the current study is to examine whether InInsulin attenuates HI-induced brain injury and neurobehavioral dysfunction in neonatal rats.
At postnatal day 10 (P10), Sprague-Dawley rat pups were randomly divided into four groups of 8 pups per group: HI+Insulin; HI+Vehicle (Veh); Sham+Insulin; Sham+Veh. The male to female ratio was kept equal. Pups either had HI exposure by permanent ligation of right carotid artery followed by 90 min of hypoxia (8% oxygen) or sham surgery followed by room air exposure. Immediately after HI or Sham, pups received either intranasal recombinant human insulin (25 μg) or an equivalent volume of Veh in each nare. The Sham+Veh served as control. A panel of sensorimotor neurobehavioral tests was performed by a blinded observer on P11. A blinded

examiner evaluated the microscopic brain injury by estimations of brain damage following Nissl staining and Fluoro-Jade C staining (a marker for degenerating neurons) at P11. Statistical analysis was performed via two-way ANOVA followed by Student-Newman-Keuls method. The sample size was determined to find a difference of 30% between means with the power of 85% and significance of $p < 0.05$.
Our results showed that InInsulin attenuated HI-induced sensorimotor behavioral disturbances as seen in negative geotaxis, wire hanging, hind limb suspension, and righting reflex tests at P11 ($p<0.002$). There were no differences between the sexes. Rat pups exposed to HI had ipsilateral brain damage and Fluoro-Jade C positive cells compared with Sham+Veh group ($p<0.001$). InInsulin treatment reduced the HI-induced ipsilateral brain damage volume ($p<0.001$) and the Fluoro-Jade C positive cells in the neonatal brain ($p<0.002$). These results suggest that InInsulin may provide protection against neonatal HI exposure-induced sensorimotor behavioral disturbances and that these protective effects are associated with reduced brain damage.

Disclosures: C.P. Talati: A. Employment/Salary (full or part-time):; University of Mississippi Medial Center. **J.W. Lee:** A. Employment/Salary (full or part-time):; University of Mississippi Medical Center. **S. Lu:** A. Employment/Salary (full or part-time):; University of Mississippi Medical Center. **N.B. Ojeda:** A. Employment/Salary (full or part-time):; University of Mississippi Medical Center. **Y. Pang:** A. Employment/Salary (full or part-time):; University of Mississippi Medical Center. **A.J. Bhatt:** A. Employment/Salary (full or part-time):; University of Mississippi Medical Center. 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.; Shire Human Genetic Therapies, Inc, Regeneron Pharmaceuticals, Inc., MedImmune, Inc., Mallinckrodt Pharmaceuticals, Inc. **L. Fan:** A. Employment/Salary (full or part-time):; University of Mississippi Medical Center.

Poster

383. Ischemia II

Location: SDCC Halls B-H

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Topic: C.08. Ischemia

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R03 NS099920
T35 AG044303
R01 GM09040
R01 CA163743
Opera Therapeutics

Title: Endothelial activation of caspase-9 during hypoxia-ischemia regulates vascular integrity and neuronal survival

Authors: *M. I. AVRUTSKY[1], Y. Y. JEAN[1], C. W. CHEN[1], A. J. WHITE[1], S. K. YUEN[1], J. M. LAWSON[1], F. N. MORALES[1], A. M. POTENSKI[1], E. CANEPA[1], S. SNIPAS[2], G. S. SALVESEN[2], C. M. TROY[3]
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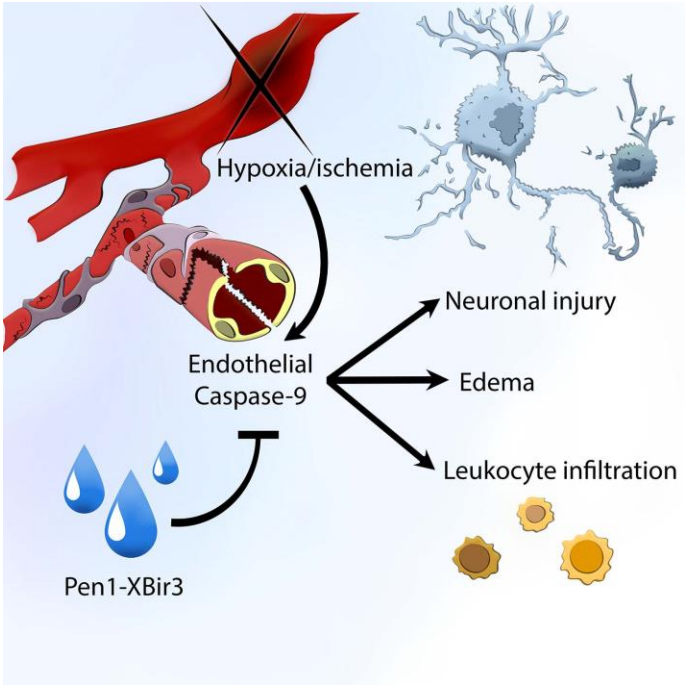
Abstract: Introduction: Ischemic injury in central nervous system (CNS) tissues features neuronal dysfunction, inflammation and breakdown of vascular integrity.
Methods: To determine the mechanistic relation of these phenomena, we employed clinically relevant *in vivo* imaging—fluorescein angiography, optical coherence tomography (OCT) and focal electroretinogram (ERG)— in a mouse model of retinal vein occlusion (RVO). RVO was achieved by tail-vein injection of rose bengal, followed by laser photocoagulation of retinal veins using the Micron Phoenix IV system.
Results: RVO induced neuronal cell death and nonapoptotic activation of caspase-9 and caspase-7 in endothelial cells. Human brain tissue from patients with cerebral ischemia showed caspase-9 activation in blood vessels. Inhibition or genetic deletion of endothelial caspase-9 in RVO provided morphologic and functional neuroprotection. Topical application (eyedrops) of a cell penetrating specific caspase-9 inhibitor attenuated retinal edema, reduced leukocyte infiltration and preserved neuroretinal function following RVO.
Conclusion:These results reveal a nonapoptotic function of caspase-9 in endothelial cells which regulates blood-retinal barrier and neuronal survival and identify endothelial caspase-9 as a tractable target for treatment of hypoxic and ischemic injury.



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Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

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Program #/Poster #: 384.01/Q10

Topic: C.09.Stroke

Support: NIH NINDS R03 NS101246
Veterans Affairs Merit Award I01 BX000589
SENSHIN Medical Research Foundation

Title: Temporal therapeutic window for calcium release-activated calcium (CRAC) channel inhibition in experimental stroke

Authors: *R. KACIMI[1], A. MIZUMA[2], K. KURISU[2], K. STAUDERMAN[3], M. J. DUNN[3], S. HEBBAR[3], M. A. YENARI[2]
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Abstract: Purpose: Inflammatory responses after ischemic stroke contribute to the worsening of brain injury. Calcium release-activated calcium (CRAC) channels contribute to inflammation in brain ischemia and injury. We previously reported that CM-EX-137, a novel small molecule CRAC channel inhibitor improved outcomes in experimental stroke. In that study, treatment was begun minutes after stroke onset. In order to understand the translational potential of this compound, we now explore whether this inhibitor can be delayed hours after stroke onset.
Subjects and Methods: C57/BL6 male mice, aged 2months were exposed to distal middle cerebral artery occlusion (dMCAO). Some were treated with CM-EX-137 (5mg/kg/d IP) or vehicle (maximum 7days). CM-EX-137 treatment was delayed 1, 4 and 6h after dMCAO. Mice treated with vehicle or inhibitor given immediately after dMCAO were included for comparison. Mice were survived 14d. Neurological functions were evaluated using a modified Bederson score, elevated body swing test, and corner test before surgery and 1, 3, 7, 14d post dMCAO. Ischemic lesion was evaluated from hematoxylin & eosin staining. Microglia/monocyte/macrophage activation was assessed via isolectin B4 and CD68 staining.
Results: All mice exposed to dMCAO survived out to 14 days (n=6-7/ group). Neurological functions were significantly improved in the 1h and 4h delayed treatment group ($p<0.001$), but not at 6h. CM-EX-137 treatment within 4h also significantly reduced infarct volume ($p<0.05$) and microglia/monocyte/macrophage activation in the 1h and 4h delay groups($p<0.01$), but not in

the 6h delay group. **Conclusion**: CRAC channel inhibition by CM-EX-137 appears to have a temporal therapeutic window between 4-6h. As acute interventions in humans appears to be effective for recombinant tissue plasminogen activator (rt-PA) within 4.5h, and mechanical thrombectomy within 24h, future studies should include whether combination treatment with CM-EX-137 can further improve outcomes or lengthen the temporal therapeutic window of revascularization treatments.

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Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

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Topic: C.09.Stroke

Support: NIH NINDS R03 NS101246
Veterans Affairs Merit Award I01 BX000589
SENSHIN Medical Research Foundation

Title: Suppression of inflammation by a novel calcium release-activated calcium (CRAC) channel inhibitor improves outcome in female mice exposed to experimental stroke

Authors: *A. MIZUMA[1], R. KACIMI[2], K. STAUDERMAN[3], M. J. DUNN[3], S. HEBBAR[3], M. A. YENARI[1]
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Abstract: Purpose: The calcium release-activated calcium (CRAC) channel is a novel channel identified on immune and other cells. They comprise two subunits, STIM1 and Orai1, which sense changes in intracellular calcium, and have been studied indirectly through non-specific calcineurin inhibitors such as cyclosporine A and FK506. In immune cells, these calcium changes lead to activation of immune responses. Acute immune responses following stroke are known to worsen outcome. We have studied a new small molecule class of CRAC channel inhibitor that in preclinical and pilot clinical studies appears to be safe, well-tolerated and lacks the toxicities seen with calcineurin inhibitors. We previously reported that such a novel CRAC channel inhibitor specific to this channel improved outcome in male animals subjected to experimental stroke. Here, we explore responses in female animals. **Methods**: C57/BL6 female mice (n=7/group), aged 2 months were subjected to distal middle cerebral artery occlusion (dMCAO) and some were given CRAC channel inhibitor CM-EX-137 (5mg/kg/d IP, CalciMedica; dMCAO-CM) or vehicle (dMCAO). Mice were studied during the same phase of

the estrus cycle (diestrus). CM-EX-137 was injected immediately after dMCAO and at 24 & 48h. Mice were survived 3d. Neurological functions were evaluated by modified Bederson score, elevated body swing test, adhesive removal test, and corner test before surgery, 24 and 72 hours post dMCAO. Lesion volumes were evaluated by cresyl violet staining. Adjacent sections were stained with isolectin B4 (IB4), macrophage marker CD68 and inducible nitric oxide synthase (iNOS) to assess inflammation as well as CRAC channel components STIM1 and Orai1. **Results**: Neurological assays at 1 and 3d post-dMCAO were significantly improved in dMCAO-CM group ($p<0.001$), compared with dMCAO-vehicle. CM-EX-137 also significantly reduced infarct volume ($p<0.001$). Stroke-induced microglia & monocyte activation was suppressed by CM-EX-137 ($p<0.001$; IB4, CD68). iNOS expression was induced after brain ischemia and was significantly reduced by CM-EX-137 ($p<0.01$). Double staining with CRAC channel components STIM1 and Orai1 plus the macrophage marker CD69 showed reduction of these components with CM-EX-137 treatment ($p<0.001$). **Conclusion**: Female mice respond to CRAC channel inhibition in a manner similar to males.

Disclosures: A. Mizuma: None. **R. Kacimi:** None. **K. Stauderman:** None. **M.J. Dunn:** None. **S. Hebbar:** None. **M.A. Yenari:** None.

Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

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Program #/Poster #: 384.03/Q12

Topic: C.09.Stroke

Support: Swedish Medical Center

Title: Regional responses of the brain water accumulation and blood-brain barrier disruption to conivaptan treatment after experimental stroke in mice

Authors: *E. ZEYNALOV[1], S. M. JONES[1], J. ELLIOTT[2]
[1]Swedish Med. Ctr., Englewood, CO; [2]Colorado Brain and Spine Inst., Englewood, CO

Abstract: Background: Stroke is associated with multiple devastating complications such as brain edema, disruption of blood-brain barrier (BBB), hyponatremia, and others. Conivaptan is a mixed V1a and V2 vasopressin receptor antagonist used to correct syndrome of inappropriate anti-diuretic hormone (SIADH)-induced hyponatremia. Our recent studies in mice showed that conivaptan can reduce stroke-evoked brain edema formation and BBB disruption in mice. Possible mechanisms of this beneficial effect are: 1) V1a receptor blocking effect may prevent vasoconstriction and improve cerebral blood flow; and 2) V2 receptor blocking can facilitate excretion of water by the kidneys, rise of plasma sodium creating osmolar gradient between the brain and the vascular compartment. Study rationale and objective: Stroke patients do not receive

treatment until the confirmation of diagnosis. Stroke-triggered severity of changes in the brain may be variable depending on the proximity to the occluded vessel. This study was designed to evaluate regional stroke-evoked brain edema formation and blood-brain barrier disruption in the context of the timing of conivaptan therapy initiation in mice. Methods: Mice underwent a 60-minute MCAO followed by reperfusion. Continuous infusion of conivaptan was initiated via an IV catheter at 3 and 5 hours after MCAO and administered for 48 hours. Animals were sacrificed, and the brains were harvested and divided into two hemispheres. Each hemisphere was further separated into three regions: anterior penumbra (AP), core (C) and posterior penumbra (PP). Brain edema was evaluated by measuring brain water content (BWC) using wet-to-dry ratio. Blood-brain barrier (BBB) disruption was assessed by the Evans Blue (EB) extravasation technique at the end point as well. Results: Continuous IV infusion with conivaptan initiated 3 hours of reperfusion reduced BWC in all regions of the brain: AP, C, and PP when compared to NS. However, 5-hour delay of conivaptan treatment was ineffective at reducing BWC in those regions. Conivaptan reduced BBB disruption after MCAO only in the AP at 3- and 5-hour delay. C and PP regions were unaffected by the treatment. Conclusions: Conivaptan treatment initiated at 3 hours after stroke reduces brain edema in all brain regions: AP, C, and PP. Further delay in treatment initiation up to 5 hours after stroke loses this beneficial effect of conivaptan. Conivaptan treatment initiated at 3 and 5 hours after MCAO attenuates BBB disruption only in AP, but not in C or PP regions. This diverse effect of conivaptan on BBB could be due to the vascular anatomy in mice. These findings may potentially be used in patients to improve recovery after stroke.

Disclosures: E. Zeynalov: None. **S.M. Jones:** None. **J. Elliott:** None.

Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 384.04/Q13

Topic: C.09.Stroke

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Title: Circular RNA TLK1 aggravates brain infarction and long-term neuronal dysfunction via miR-335-3p/TIPARP after ischemic stroke

Authors: *F. WU, B. HAN, S. WU, H. YAO
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Abstract: Circular RNAs (circRNAs) are highly expressed in the brain and involved in regulating physiological and pathophysiological processes. However, the potential role of circRNAs in neuronal damage and functional recovery in stroke remains largely unknown. Here, we demonstrated that circular RNA TLK1 (circTLK1) levels were significantly increased in ischemic brain tissues after transient middle cerebral artery occlusion (MCAO) in mice and up-regulated in plasma from acute ischemic stroke (AIS) patients. Knockdown of circTLK1 expression significantly decreased infarct areas, attenuated neuronal deficits, and promoted neuronal plasticity and long-term recovery in ischemic stroke mice. Mechanistically, circTLK1 functions as an endogenous microRNA-335-3p sponge to inhibit miR-335-3p activity, which subsequently caused the increased expression of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-inducible poly (ADP-ribose) polymerase (TIPARP), with subsequent inhibition of neuronal survival and plasticity. Taken together, our results indicate that circTLK1 and its coupling mechanisms are involved in cerebral ischemia, thus indicating that circTLK1 may serve as a novel biomarker or therapeutic target for stroke.

Disclosures: F. Wu: None. **B. Han:** None. **S. Wu:** None. **H. Yao:** None.

Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

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Program #/Poster #: 384.05/Q14

Topic: C.09.Stroke

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63402797 Pilot award

Title: A novel mouse model with motor and cognitive deficit

Authors: *M. MORENO GARCIA, O. PATSOS, C. SCHROEDER, N. QUILLINAN
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Abstract: Objective: Each year in the U.S. there are an estimated 20,000 strokes resulting in cerebellar infarction. The neurological impairments observed in these patients include motor coordination and motor learning impairments. Surprisingly, cerebellar stroke patients also exhibit cognitive impairments that affect memory and language. Establishing a model of cerebellar stroke that recapitulates aspects of human cerebellar infarction will allow for us to perform mechanistic studies of brain injury and identify therapeutic targets to improve recovery in stroke

patients. The goal of this study was to develop and characterize a photo-thrombotic mouse model of focal cerebellar stroke.
Methods: Adult male and female mice were head-fixed in a stereotaxic frame, were administered Rose Bengal (150 ug/g) and the superior cerebellar artery (SCA) was illuminated for 15 minutes with a cold white LED light source. Stereological analysis of lesion volume was performed at 1 and 7 days after surgery. Behavioral testing of motor and memory function was performed in mice subjected to sham procedure or photo-thrombosis at 7 days following surgery. Motor testing included gait, balance beam and paw preference analysis. Cognitive performance consisted on evaluation of spatial memory via a contextual fear conditioning assay.
Results: Cerebellar stroke volumes were larger at 1 day (2.4 mm^3) than 7 days (0.964 mm^3), likely do to edema at the earlier time point. Lesion volumes were similar between males and females at both time points. Increased blood brain barrier permeability at 1 day and glial reactivity at 7 days were observed in the infarct region. Interestingly, infarction of anterior lobules of the cerebellar cortex resulted in gait abnormality, motor coordination and ipsilateral limb impairments; whereas infarction of posterior lobules resulted in spatial memory deficits.
Conclusion: In conclusion, we have developed a reproducible model of cerebellar stroke that not only displays the pathophysiology of infarction but also presents motor and cognitive impairments previously evaluated in a clinical setting. This tool can help us understand the alterations that occur in the cerebellum as a result of cerebellar infarction and will become key to finding therapeutic targets to diminish the severity of behavioral outcomes.

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Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

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Program #/Poster #: 384.06/R1

Topic: C.09.Stroke

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Title: Targeting PTPσ enhances axonal regeneration, remyelination and functional recovery after intracerebral hemorrhage in mice

Authors: *H. SUN[1,4], M. YAO[1], H. LAI[1], S. RUXANDRA BADEA[2], Y. GAO[2], J. HUANG[1,3], G. K. LEUNG[1], W. WU[1]
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Abstract: Introduction:
One reason for limited recovery from intracerebral hemorrhage (ICH) may be the development of a glial scar at the border between normal and damaged tissue. The glial scar, which contains reactive astrocytes and associated extracellular matrix (ECM) proteins such as CSPGs, not only suppresses axon regeneration, but also results in demyelination due to oligodendrocyte death in both the immediate vicinity of the injury site, thereby interfering with long-term anatomical and functional recovery. CSPG inhibition of axonal regeneration,oligodendrocyte outgrowth and myelination are partially mediated by the protein tyrosine phosphatase sigma (PTPσ) receptor. Intracellular sigma peptide (ISP), a newly developed membrane permeable peptide which binds to PTPσ and relieves CSPG-mediated inhibition, has been used to promote regeneration and functional recovery after spinal cord injury and ischemic heart attack.
Aim: We hypothesize that treatment with ISP would lead to functional recovery by enhancing axon regeneration and remyelination after intracerebral hemorrhage.
Methods: Experimental ICH model in male mice was induced by intrastriatal injection of Collagenase IV. The functional recovery was evaluated weekly until 8 weeks post-ICH using rotarod test and Cylinder test following daily ISP (10μM) subcutaneous injection for the same period of time. Axon regeneration and remyelination weres assessed by histological staining, three-dimensional (3D) histological methods as well as immunoblot.
Results: Increased axonal sprouting and improved remyelination in the immediate vicinity of the injury site as well as functional recovery were observed in ICH mice treated with ISP.
Conclusions: These results suggest that modulation of PTPσ by ISP represents a potential therapeutic strategy for hemorrhagic stroke.

Disclosures: H. Sun: None. **M. Yao:** None. **H. Lai:** None. **S. Ruxandra Badea:** None. **Y. Gao:** None. **J. Huang:** None. **G.K. Leung:** None. **W. Wu:** None.

Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 384.07/R2

Topic: C.09.Stroke

Title: Nitrosonifedipine ameliorates neurological symptoms and prolongs the surival in malignant stroke-prone spontaneously hypertensive rats

Authors: *Y. IZAWA-ISHIZAWA, M. IMANISHI, Y. ZAMAMI, K. TAKECHI, T. TAMAKI, K. ISHIZAWA
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Abstract: A malignant stroke-prone spontaneously hypertensive rat (M-SHRSP) is a model animal showing high rate of cerebral stroke. The onset and progressions of stroke are known to be involved in oxidative stress. Nitrosonifedipine (NO-NIF) is photolytic metabolite of nifedipine, an anti-hypertensive drug. We have revealed that NO-NIF possesses potent radical scavenging activity and protective effects against several pathological conditions involving oxidative stress. Therefore, we investigated the effects of NO-NIF on the onset and progressions of stroke in M-SHRSP in the present study. M-SHRSPs were treated with NO-NIF (30 mg/kg/day, i.p.) or vehicle from the age of five weeks. Neurological symptoms were observed every day and scored by severity of general status and disordered motility of anterior/posterior limbs. The day of the stroke onset was determined by neurological symptoms and the change in body weight. Brain was harvested after death and histologically analyzed. For in vitro study, nerve growth factor (NGF)-induced neurite elongation and intracellular signaling pathway was investigated using PC12 cells. Contrary to our expectations, NO-NIF could suppress neither the incidence nor the timing of stroke onset compared to control group. However, NO-NIF prolonged the lifespan of M-SHRSP. The periods until 50% rats died from stroke were extended for 29 days in NO-NIF group compared to control. In control rats, the neurological score showed linear worsening after stroke onset. However, its exacerbation was significantly suppressed in NO-NIF treated group. Consistently, NO-NIF treated rats showed less pathological lesion, such as hemorrhage, thrombus, and liquefaction degeneration in brains. In PC12 cells, NGF-induced neurite elongation was enhanced by NO-NIF existence. Moreover, NO-NIF prolonged the co-localization of NGF receptor and flotillin, a lipid raft marker, and the activations of intracellular signaling molecules, such as Akt and ERK1/2. In our previous study, we have already showed that NO-NIF affects the fluidity of cellular membrane. Therefore, it was considered that NO-NIF enhanced NGF signaling by changing the cellular membrane fluidity and localization of NGF receptor. In the present study, we demonstrated that NO-NIF could prolong the lifespan after stroke induced by malignant hypertension. It was also suggested that NO-NIF elongated neurite by enhancing NGF-stimulated intracellular signaling activities and finally improved the stroke-related neurological symptoms in M-SHRSP.

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Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 384.08/R3

Topic: C.09.Stroke

Title: Effects of glibenclamide against early brain injury after subarachnoid hemorrhage

Authors: *R. KAJIMOTO[1], T. IGARASHI[2], N. MORO[3], H. OSHIMA[4], T. SUMA[4], A. YOSHINO[5]
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Abstract: Subarachnoid hemorrhage (SAH) is a devasting condition with high morbidity and mortality rate. Early brain injury (EBI), which is mainly caused by an increased intracranial pressure, occurring within 72 hours after SAH, has been considered as a prognostic factor and as a new therapeutic target. It has been reported that sulfonylurea receptor 1 (SUR1) is upregulated in neurons, glial cells and vascular endothelial cells in the early period after brain injury. It is known that the upregulation of SUR1 promotes influx of sodium and calcium into the cells through ion channel that will lead to cell swelling and causes cell death. It also has been reported that the excess upregulation of SUR1 causes inflammatory changes, although the mechanism in detail has not been elucidated yet. Glibenclamide is a therapeutic agent for diabetes mellitus that is used worldwide to decrease blood sugar level by acting to SUR1 on beta cell of the pancreas. Furthermore, it is reported that glibenclamide will reduce cerebral edema and decrease mortality rate in a middle cerebral artery occlusion model. However, the effects of glibenclamide against EBI after SAH has not been elucidated. In this study, we examined the effects of glibenclamide against EBI in a rat SAH model.
Male Sprague-Dawley rats were randomized into three groups, Sham group (n=3), SAH-control group (n=8) and SAH-glibenclamide group (n=8). Dimethyl sulfoxide (DMSO) was used as a control. Glibenclamide or DMSO was subcutaneously administrated via osmotic pump starting immediately after SAH. Brains were removed 24 hours after SAH. Brain edema was measured by dry-wet method. In the cortex, putamen and hippocampus, the expression of inflammatory cytokines was measured by polymerase chain reaction. In addition, 9 different rats (3 per group) were used to evaluate the expression of microglia by immunohistological method.
Brain edema in the SAH-glibenclamide group significantly decreased compared to the SAH-control group. SAH caused significant increase of interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNFα) and nuclear factor-kappa B in the cerebral cortex. In the SAH-glibenclamide group, IL-1β and TNFα levels were significantly suppressed compared to the SAH-control group. Activation of microglia was suppressed by glibenclamide administration.
Glibenclamide suppressed brain edema formation, activation of microglia and suppressed the release of inflammatory cytokines in a rat model of SAH.

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Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

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Program #/Poster #: 384.09/R4

Topic: C.09.Stroke

Support: NIH grant NS088084

Title: Treating cerebral ischemia and reperfusion induced neuronal injury with a cysteine precursor in mice

Authors: Y. LIU, J.-W. MIN, K. SUBEDI, *H. WANG
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Abstract: Oxidative stress aggravates brain injury following ischemia/reperfusion (I/R). We previously showed that ubiquilin-1 (Ubqln1) protects brains against oxidative stress and I/R induced brain injury. Here, we demonstrate that a small molecule compound, L-2-oxothiazolidine-4-carboxylate (OTC) that functions as a precursor of cysteine, upregulated Ubqln1 and is a potential therapeutic reagent in both the cell and mouse models of ischemic stroke. In cultured neuronal cells, OTC elevated Ubqln1 protein level and reduced oxygen glucose deprivation-caused cell death in a dose-dependent manner. In an ischemic stroke mouse model, OTC (100 mg/kg) administered via the tail vein at 1 or 3 hours after reperfusion, significantly attenuated brain infarct injury and improved behavioral outcomes. Administration of OTC in stroke mice also restored reduced-glutathione level and suppressed the elevation of oxidized-glutathione. OTC treatment in stroke mice also decreased both superoxide production and the oxidized protein levels in the penumbral cortex at 24 hours following ischemic stroke. Consistently, OTC also upregulated Ubqln1 protein level in in the penumbral cortex following stroke, which was associated with increased glutathione S-transferase (GST) level. Moreover, we demonstrated that Ubqln1 interacted with and stabilized GST in cell cultures. These results suggest that OTC upregulates Ublqn1 and other antioxidant molecules and is a potential therapeutic reagent for stroke.

Disclosures: Y. Liu: None. **J. Min:** None. **K. Subedi:** None. **H. Wang:** None.

Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 384.10/R5

Topic: C.09.Stroke

Support: Swedish Medical Center

Title: The AVP antagonist conivaptan prevents early inflammatory responses following stroke in mice

Authors: *S. M. JONES[1], E. ZEYNALOV[1], J. P. ELLIOTT[2]
[1]Neurotrauma Res., Swedish Med. Ctr., Englewood, CO; [2]Colorado Brain and Spine Inst., Englewood, CO

Abstract: Background: Stroke and traumatic brain injury are major causes of death and disability world-wide, and more research into mechanisms of cell death and treatments to improve recovery are necessary. Arginine vasopressin (AVP) exacerbates edema and promotes blood brain barrier breakdown and neural damage following ischemia or traumatic brain injury. Following brain injury, AVP is elevated in plasma and brain tissue.
We have previously shown that the V1/V2 receptor blocker conivaptan prevents edema and blood brain barrier (BBB) breakdown in a mouse model of stroke. Breakdown of the BBB following injury has been linked to increased neuro-inflammation. This study was designed to determine whether treatment with conivaptan reduces stroke-induced neuro-inflammation in mice.
Methods: Mice (C57/Bl6) were subjected to 60-minute focal middle cerebral artery occlusion (MCAO) followed by reperfusion and continuous infusion treatment with conivaptan or saline. Five hours after MCAO, qPCR analysis on harvested brain tissue (ipsilateral and contralateral) was performed to examine the induction of the AVPR1a receptor as well as inflammatory markers TNFα, CXCL1 and CCL2. In separate sets of mice, survival time was 24 hours and the extravasation of FITC-albumin and CD68 staining was examined.
Results: Our studies revealed increased ipsilateral expression of the V1a receptor (AVPR1a), TNFα and CXCL1 within 5 hours of occlusion, which were prevented by conivaptan treatment. After 24 hours, FITC extravasation was reduced and CD68 labelled cells were significantly less numerous in mice receiving conivaptan treatment following MCAO. In cultures of primary astrocytes, oxygen glucose deprivation (OGD) induced the expression of AVPR1a but had no effect on TNFα, CXCL1 or CCL2 expression.
Conclusion: The activation of these early cellular responses to injury initiate a cascade of processes leading to secondary inflammation and the infiltration of neutrophils and monocytes, which have been shown to contribute to edema, BBB disruption and cellular damage.

Conivaptan is currently FDA approved for the treatment of hyponatremia. Our data indicate that conivaptan may have additional beneficial effects by reducing the induction of an early inflammatory response following stroke.

Disclosures: S.M. Jones: None. **E. Zeynalov:** None. **J.P. Elliott:** None.

Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

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Program #/Poster #: 384.11/R6

Topic: C.09.Stroke

Support: NIH grant NS094896

Title: Limiting hematoma growth by red cell microparticles following intracerebral hemorrhage

Authors: *K. DAVE[1], A. K. REHNI[2], H. NAVARRO[4], C. BIDOT, Jr.[4], V. SHUKLA[4], S. KOCH[4], M. A. PEREZ-PINZON[3], Y. S. AHN[4], W. JY[4]
[1]Neurol., Univ. Miami Sch. Med., Miami, FL; [2]Neurol., [3]Univ. of Miami Sch. of Med., Miami, FL; [4]Univ. of Miami, Miami, FL

Abstract: Spontaneous intracerebral hemorrhage (sICH) is the deadliest stroke sub-type. No effective treatment is available so far. Preventing hematoma extension, and/or prevention of continued bleeding in sICH are attractive therapeutic targets. It has been shown *in vitro* that red blood cell-derived microparticles (RMP) enhance platelet function and accelerate coagulation, augmenting both primary and secondary hemostasis. In the present study, we determined the efficacy of RMP in reducing hematoma expansion in a rat model of sICH. RMPs were prepared from human RBCs using a high-pressure extrusion method. sICH was induced in male Sprague Dawley rats by injection of collagenase into the right striatum. Rats were randomly divided into various experimental groups. At ~24 hours post-collagenase injection, neurological scores were evaluated, rats were sacrificed, and the hematoma areas on images of brain sections were measured. Induction of sICH and evaluation of hematoma area and neurological score was carried out by an investigator blinded to the experimental conditions. To determine dose of collagenase required to create moderate hematoma, we performed a dose response curve. Mean hematoma volume for 0.12 U / rat (n = 5), 0.17 U (n = 4), and 0.22 U (n = 5) collagenase treated animals was 40.6 ± 3.44, 72.0 ± 3.40 ($p<0.001$ vs 0.12 U group), and 79.1 ± 7.13 mm^3, respectively. We chose 0.17 U collagenase for subsequent studies. Next, we determined optimal RMP dose required to lower hematoma growth following sICH. Placebo (vehicle-treated) and three RMP-treated groups (low dose, medium dose, and high dose received total of 7.19×10^9, 2.55×10^{10}, and 9.48×10^{10} particles / kg b.w., respectively) were included in the study. We observed that hematoma volume for placebo, low dose, medium dose, and high dose group was

71 ± 4 (n = 19), 59 ± 5 (n = 10), 43 ± 2 (n = 10, p<0.001 vs placebo group), and 67 ± 5 (n = 10), respectively. The hematoma volume was lower by 17, 40 (p<0.001 vs placebo group), and 6% in low dose, medium dose, and high dose treated groups when compared to placebo group, respectively. We observed that neurological score was lower in low dose (9.4 ± 0.4, n = 10, p < 0.005), medium dose (9.3 ± 0.4, n = 10, p < 0.001), and high dose (9.4 ± 0.5, n = 10, p < 0.005) groups as compared to placebo group (10.8 ± 0.2, n = 19). Our results indicate that a medium RMP dose had maximum effect on hematoma expansion. Our results also demonstrate that although low and high RMP dosages had no effect on hematoma volume, both of these dosages did lower neurological score when evaluated at 24 h post-collagenase injection. Our results indicate that RMPs have potential to lower hematoma growth in sICH patients.

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Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 384.12/R7

Topic: C.09.Stroke

Support: NIH Grant R01NS079691
UCLA fund for Innovation/Technology Development Group

Title: Dual-function injectable angiogenic biomaterial for the repair of brain tissue following stroke

Authors: *L. R. NIH[1], S. CARMICHAEL[2], T. SEGURA[3]
[1]Dept. of Neurology, David Geffen Sch. of Med., Univ. of Los Angeles, California, Los Angeles, CA; [2]Neurol., UCLA Sch. Med., Los Angeles, CA; [3]Duke Univ., Durham, NC

Abstract: Stroke is the primary cause of adult disability due to the brain's limited ability to regenerate damaged tissue. After stroke, an increased inflammatory response coupled with severely limited angiogenesis and neuronal growth result in a necrotic lesion that compartmentalizes the degraded tissue within a physical empty cavity. Although the vascular endothelial growth factor (VEGF) appears as the best candidate for therapeutic angiogenesis, its administration after stroke has been limited by poor penetration across the blood-brain barrier, a short half-life time, and severe side effects due to its ability to promote vascular permeability. Recent advances in biopolymer hydrogels have developed gels with extracellular matrix motifs that not only support cell infiltration when injected directly in the stroke cavity but also allows a time and space-controlled delivery of growth factors. We hypothesized that the brain administration of an engineered immuno-modulating angiogenic biomaterial directly to the stroke cavity can promote tissue formation de novo by modulating post-stroke inflammatory response and angiogenesis. For this, male adult mice were subjected to an ischemic stroke and injected with a hyaluronic acid-based hydrogel containing heparin nanoparticles and covalently bound VEGF. This biomaterial generates a vascularized network of regenerated functional neuronal connections within previously dead tissue and reduces the post-stroke inflammation in and around the stroke site. These results are lost with the absence or reduction of bound VEGF where only the immuno-modulator effect is observed, and with the absence or reduction of heparin particles where angiogenesis is no longer associated with axonal sprouting. This work lays the groundwork for the use of dual immuno-modulator and angiogenic materials to repair other neurologically diseased tissues.

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Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 384.13/R8

Topic: C.09.Stroke

Support: DST/INSPIRE Fellowship/2014/IF140742

Title: Liposomal formulation of novel dual cox-lox inhibitor protects brain from cerebral ischemia induced neuronal injury

Authors: *V. GUPTA, A. KUMAR
Pharmacol. Division, Univ. Inst. of Pharmaceut. Sci., Panjab Univ., Chandigarh, India

Abstract: Objective: To investigate the neuroprotective potential of liposomal formulation of licofelone against global cerebral ischemia induced brain injury in Wistar rats.
Methods: Bilateral common carotid artery occlusion was performed for 30 minutes in male wistar rats for the induction of global cerebral ischemia. Post treatment with liposomal formulation of licofelone (2.5 and 5mg/Kg; *i.p.*) was done at every 12 hour interval. After 48 hours of reperfusion period, behavioral assessments were done, animals were sacrificed and brains were harvested for biochemical, mitochondrial and histological assessments.
Results: Post treatment with licofelone restored the behavioral function (as assessed by locomotor activity, rota rod performance, hanging latency and neurological score), arrested oxido-nitrosative stress (as assessed by lipid peroxidation, reduced glutathione, superoxide dismutase, catalase and nitrite), decreased neuroinflammation (as assessed by TNF-α), decreased apoptosis (as assessed by caspase-3), lowered mitochondrial damage (as assessed by enzyme complexes I-IV), decreased infarct area (as seen by TTC staining) and recovered histological alterations (as seen by H&E staining).
Conclusion: Post treatment with liposomal formulation of licofelone protects the brain from neuronal damage after global cerebral ischemia in wistar rats.

Disclosures: V. Gupta: None. **A. Kumar:** None.

Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 384.14/R9

Topic: C.09.Stroke

Title: Robotic rehabilitation and chemogenetic activation of serotonergic neurons for post stroke functional recovery

Authors: *S. CONTI[1], C. SPALLETTI[2], N. GIORDANO[2], S. LAI[1], A. GIORGI[3], M. PASQUINI[1], M. PASQUALETTI[3], M. CALEO[2], S. MICERA[1,4]
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Abstract: Stroke is one of the leading causes of long-term motor disabilities. Re-acquisition of motor skills is a priority for stroke survivors, yet currently available rehabilitative therapies are not completely effective. Modern strategies aim to enhance motor rehabilitation by promoting neural plasticity, which is widely accepted to support functional recovery following brain damage. Serotonin, because of its neuroplastic properties, is a major candidate to drive the observed recovery. Here, we have investigated the role of serotonin and physical training in neuroplasticity and recovery after stroke. To this aim, we combined enhanced endogenous serotonin release with robotic rehabilitation and characterized motor recovery in a mouse model of stroke.We exploited a chemogenetic model for controlled serotonin release via systemic administration of clozapine-N-oxide (CNO) in transgenic mice expressing DREADD receptors specifically in serotonergic neurons. C-Fos expression in raphe neurons, together with increased serotonin transporter in peri-infarct cortex, demonstrated efficient and prolonged activation of the serotonergic system.We combined chemogenetic stimulation with robotic rehabilitation in stroke mice. Animals underwent unilateral photothrombotic lesion within the primary motor cortex (caudal forelimb area); 5 days after, they received daily doses of CNO and were rehabilitated using a robotic platform which allows quantitative assessment of forelimb flexion. Forelimb motor function was tested with two classical motor tests: Gridwalk and Schallert Cylinder Test. We also measured several kinematic parameters in a skilled reaching task.Results showed that the combined therapy promoted recovery of forelimb functions and that this motor improvement persisted beyond the treatment period. We next tested whether the recovery was associated to disinhibition of perilesional areas; to this aim, we measured GABAergic inhibition in the peri-infarct cortex. We found a reduction in somatostatin+ and parvalbumin+ interneurons as well as GABAergic terminals impinging on the soma of pyramidal neurons. These data supported the clinical evidence that a reduction in GABAergic inhibition promotes functional recovery in stroke patients.In conclusion, our study demonstrated that combining robotic rehabilitation with activation of the serotonergic system has a synergic effect and is beneficial for post stroke recovery.

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Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 384.15/R10

Topic: C.09.Stroke

Support: NIH Grant R01NS093057

Title: RNA sequencing analysis revealed a distinct motor cortex transcriptome in spontaneously-recovered mice after stroke

Authors: *M. Y. CHENG[1], M. ITO[6], M. ASWENDT[7], A. G. LEE[2], S. ISHIZAKA[3], Z. CAO[1], E. WANG[4], S. LEVY[1], D. L. SMERIN[3], J. A. MCNAB[8], M. M. ZEINEH[5], C. LEUZE[9], M. GOUBRAN[3], G. K. STEINBERG[3]
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Abstract: Background: Many restorative therapies have shown to promote recovery after stroke. These therapeutic-induced changes have revealed important insights on brain repair and recovery mechanisms, however, the intrinsic changes that occur in spontaneous recovery after stroke is less clear. In this study we used RNA sequencing to elucidate the intrinsic changes in spontaneous recovery after stroke, by directly investigating the transcriptome of primary motor cortex in mice that naturally recovered after stroke. Methods: Ischemic stroke was induced in C57BL/6J adult male mice by transient MCAO for 30 min. Neurological score, vertical pole, rotating horizontal beam test and body weight monitoring were performed at baseline and post-stroke days 4, 8, and 14 to evaluate their recovery. All mice were sacrificed at post-stroke day 15, and a subset of these mice was processed for immunostaining with antibodies for inflammatory mediators such as astroglial (GFAP) and microglial activation (CD68). Infarct size and locations were evaluated either using T2-weghted MRI or histology. Ipsilesional and contralesional primary motor cortices (iM1 and cM1) were dissected and processed for RNA sequencing (RNA-seq) transcriptome analysis. Results: Cluster analysis of the stroke mice behavior performance revealed two distinct recovery groups: a spontaneously-recovered and a non-recovered group. This cluster separation corresponded well to their rotating beam performance, as spontaneously-recovered mice showed good performance at post-stroke day14, whereas non-recovered group exhibited poor performance. Lesion mapping analysis showed that there was no difference in the lesion size and locations between the groups. RNA-seq transcriptome analysis revealed distinct biological pathways in the spontaneously-recovered stroke mice, in both iM1 and cM1. Correlation analysis revealed that 38 genes in the iM1 were significantly correlated with improved recovery, while 74 genes were correlated in the cM1. In

particular, ingenuity pathway analysis highlighted the cyclic adenosine monophosphate (cAMP) signaling in the cM1. Conclusions: Our RNA-seq data revealed a panel of recovery-related genes in the motor cortex of spontaneously-recovered stroke mice and highlighted the involvement of contralesional cortex in spontaneous recovery. Current studies include validation of key molecular candidates in the cAMP pathway and their role in recovery. Developing drugs targeting the cAMP pathway after stroke may provide beneficial recovery outcome.

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Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 384.16/R11

Topic: C.09.Stroke

Support: Swiss National Science Foundation
European Research Council (ERC) advanced grant (Nogorise)
Christopher and Dana Reeve Foundation

Title: Targeting antibodies to the CNS: A comparative study of intrathecal, intravenous and subcutaneous antibody treatment after stroke

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Abstract: With more and more antibody-based potential therapeutics emerging as promising treatments for central nervous system (CNS) disorders, the question of how to bring these high molecular compounds into the CNS parenchyma is of utmost importance. Here, we provide a comparison of antibody distribution in the CNS tissue, the cerebrospinal fluid and the serum following three routes of administration: intravenous (i.v), intrathecal (i.t), and subcutaneous (s.c) in intact rats or after stroke. For the stroke experiments, rats were subjected to a photothrombotic stroke destroying >95% of the sensorimotor cortex unilaterally. We infused full length IgG antibodies against the neurite growth inhibitory CNS membrane protein Nogo A by lumbar intrathecal pumps (for 2 weeks after stroke). Alternatively, intravenous antibodies dosed 10 times higher than the total amount given i.t. were injected twice, within the first week after stroke. Subcutaneous antibodies were injected at total amounts of 15 times higher than the total amount given via intrathecal pump in the first 3 days post insult followed by booster doses once

every day, over the next 7 days. We measured the plasma pharmacokinetics (PK), tissue concentrations in the brain, functional readouts including detailed characteristics of grasping behaviour during stroke recovery, and analysis of neuronal, corticospinal rewiring in the spinal cord. We find that the intrathecal application of anti-Nogo antibodies results in the highest recovery levels after stroke. Functional recovery and anatomical rewiring were much lower in the groups with intravenous or subcutaneous antibody application, in spite of the high plasma levels of the antibodies. Serum concentration of antibodies was higher within the first 7 days in the intravenous than the subcutaneous condition. This study shows that intrathecal lumbar infusion of anti-Nogo-A antibody achieves excellent CNS targeting, leading to a high level of functional recovery and enhanced anatomical plasticity, whereas even large amounts of peripherally administered antibodies failed to reach functionally effective levels in the CNS.

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Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

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Program #/Poster #: 384.17/R12

Topic: C.09.Stroke

Support: Canadian Foundation for Innovation
Heart and Stroke Foundation
Saskatchewan Health Research Foundation
Natural Sciences and Engineering Research Council

Title: The neuroprotective effects of non-competitive ampa receptor antagonist (perampanel) and a2a receptor antagonist (istradefylline) in a small vessel stroke model

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Abstract: Extracellular brain adenosine concentrations increase more than 100-fold after pathological trauma such as head injury, hypoxia and ischemia. The neuroprotective effect of adenosine through A1R is short lived as A1R desensitization occurs after 12-24 hour of chronic A1R stimulation. Therefore, ischemic stroke is associated with low expression of the neuroprotective inhibitory A1R and high expression of A2AR, which enhances neuronal excitability and glutamate-induced neurotoxicity. We hypothesize that induced neurodegeneration in our in vivo focal cortical stroke model, is mediated by action of elevated

adenosine and glutamate on the highly expressed A2AR and calcium-permeable AMPAR, respectively. This can be prevented by administration soon after a stroke of a clinically approved drug Perampanel, a non-competitive AMPAR blocker, or Istradefylline a selective A2AR antagonist. Propidium Iodide and Fluoro-Jade C staining showed increased neurodegeneration in hippocampus in PVD group compared to SHAM, Perampanel- and Istradefylline-treated groups. Western blots of SHAM and Perampanel or Istradefylline treated groups have lower expression of both nNOS and iNOS compared to PVD group. Finally, 200 nM Perampanel significantly inhibited the post-hypoxic synaptic potentiation in fEPSP that occurs after hypoxia/reperfusion and returned the potentiated fEPSP back to baseline levels. Moreover, perfusing 75 nM perampanel after 5 min. of hypoxia prevented the post-hypoxic synaptic potentiation in fEPSP during the washout. Taken together, Perampanel and Istradefylline exhibit a neuroprotective effect in PVD model, by inhibiting glutamate excitotoxicity mediated by the upregulated expression of calcium-permeable AMPARs and A2AR after ischemic stroke.

Disclosures: M. Zaki: None. **F.S. Cayabyab:** None.

Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 384.18/R13

Topic: C.09.Stroke

Support: NRF Grant 2016M3C7A1904391

Title: Quercetin prevents the ischemic brain injury-induced decrease of neuronal calcium sensor protein, hippocalcin

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Abstract: Intracellular calcium is involved in a variety of biological events such as metabolism, cell proliferation and apoptosis. Hippocalcin is a neuronal calcium buffering protein that regulates intracellular calcium concentration and prevents cells death against apoptotic stimuli. Quercetin has an excellent anti-oxidant property and exerts a neuroprotective effect. The aim of this study was to investigate whether quercetin is capable of modulating hippocalcin expression in cerebral ischemia and glutamate excitotoxicity-induced neuronal cell death. Focal cerebral ischemia was induced by middle cerebral artery occlusion (MCAO). Male Sprague-Dawley (n = 40) rats were treated with vehicle or quercetin (30 mg/kg) 1 h prior to MCAO, and cerebral cortical samples were obtained 24 h after MCAO. Protein and transcription levels of hippocalcin were measured by Western blot and RT-PCR and hippocalcin decreased in vehicle-treated

animals with MCAO, whereas quercetin attenuated the ischemic injury-induced decline of hippocalcin expression. Glutamate exposure induced intracellular calcium overload in cultured mouse hippocampal cells, whereas quercetin remarkably alleviated the intracellular calcium increase induced by glutamate exposure. Glutamate-induced excitotoxicity decreased hippocalcin level, while quercetin treatment prevented the glutamate exposure-induced decrease of hippocalcin. Taken together, these results suggest that quercetin exerts a neuroprotective effect through regulating hippocalcin expression and modulating intracellular calcium levels during cerebral ischemia and glutamate-induced neuronal cell damage.

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Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

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Program #/Poster #: 384.19/R14

Topic: C.09.Stroke

Support: NEST-2017R1D1A1B05036195
NEST-2017R1C1B5017801

Title: Enhancements of intracellular cGMP as a potential therapeutic effector following mild ischemic stroke

Authors: *J. KANG[1], Y. YU[1], K. LEE[1], D. YOO[1], I. HWANG[2], D.-K. PAKR[1], K.-H. PARK[1], M.-R. LEE[3], J.-S. OH[4], D.-S. KIM[1]
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Abstract: Ischemic stroke is one of the major diseases in the worldwide. Also, the one of most common causes of death in Korea is stroke and many people suffer from stroke as well. Especially, about 10% of stroke cases lead to epilepsy, and repeated strokes lead to vascular dementia. So, there is increasing interest in clearing the underlying pathological mechanisms and identifying possible treatment strategies. Sildenafil, as known as Viagra is a phosphodiesterase-5 inhibitor and effect the nitric oxide-cyclic guanosine monophosphate pathways, which are deeply involved in the pathogenesis of the neurological diseases by causing intracellular accumulation of cGMP. Thus, it may have a favorable therapeutic effect on the treatment of stroke, neurodegenerative disorders and vascular dementia by enhancing angiogenesis and neurogenesis. Therefore, in this study, we investigated whether its beneficial effects involve to the change of

stroke infarct size and the cognitive functions. The first stage of this study is to confirm the phenotype after mild MCA occlusion (MCAo). The focal cerebral ischemia was induced by 30 min of MCAo in adult rats. After establishing the mild stroke-induced MCAo animal model, we injected Sildenafil in mild stroke model and conducted a behavioral test to identify the cognitive function. In addition, mild stroke animal models were tested by using variety of electrophysiological parameter following MCAo. Moreover, to confirm the infarct volume of brain, histological staining was performed. As a result, we obtained significant results compared with the mild stroke animal model treated with and/or without Sildenafil, which means that improving the cognitive behavioral phenotypes and the potential role for reducing the symptomatic outcomes after mild MCAo. According to these results in this study, therefore, we suggested that it may involve to the novel therapeutic effects for progressing the diverse phenotypes of mild ischemic stroke following MCAo.

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Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

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Program #/Poster #: 384.20/R15

Topic: C.09.Stroke

Support: Swedish Research Council
Alborada Foundation
HGAT Wachtmeister Foundation

Title: Removing the brakes on brain recovery after stroke by metabotropic glutamate receptor 5 negative allosteric modulators

Authors: J. HAKON[1], J. QUATTROMANI[1], F. MASTROIACOVO[2], E. ENGLUND[1], C. SJÖLUND[1], L. DI MENNA[2], K. BEIRUP[1], S. MOYANOVA[2], K. RUSCHER[1], F. NICOLETTI[2,3], A. Q. BAUER[4], *T. W. WIELOCH[1]
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Abstract: Stroke is a main cause of life-long disability worldwide. Whilst task-specific rehabilitative training is an evidence based stroke treatment, there is no pharmacological therapy that enhances neurological recovery after stroke beyond the therapeutic time window of neuroprotection. In the experimental setting, housing rodents in an enriched environment (EE) accelerates behavioural recovery and enhances brain-wide functional connectivity in cortical regions local and distant from the injury (Hakon et al., NeuroImage: Clinical, 2018). We

investigated how modulation of mGluR5 receptors influences the recovery of neurological function after stroke in rodents. We employed the photothrombosis or endothelin-1 stroke models in rats or mice. Housing in an EE or treatment with mGluR5 modulators typically started 2 days after stroke and continued for 14 days. Neurological function was assessed by paw placement, grid, and grip tests. Resting-state functional connectivity (RS-FC) was assessed using optical intrinsic signal (OIS) imaging. Treatment with 5 structurally different mGluR5 negative allosteric modulator (NAM) molecules provided a robust improvement of neurological function compared to vehicle-treated animals 14 days after stroke. Improvement was attained even when treatment started 7 days after stroke, and the therapeutic effect remained at least 7 days after termination of treatment. In mGluR5 knock-out mice, function improved to the same degree as observed in wild type animals treated with the mGluR5 NAM 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP). Housing in an EE in combination with MTEP treatment provided a synergistic recovery enhancing effect. Daily pre-treatment with the mGluR5 positive allosteric modulator VU0360172 prevented the recovery enhancing action of MTEP. In addition, the recovery enhancing effect of EE was prevented by concomitant treatment with VU0360172. None of the treatments affected infarct size when assessed at 7 or 14 days of recovery. At the network-level, treatment with MTEP increased intra-hemispheric RS-FC in contralesional sensorimotor areas, and at the cellular level a time-dependent modulation of mGluR5 mediated cell signalling was found. The presence of mGluR5 was confirmed in both brain hemispheres of stroke patients. We conclude that post stroke mGluR5 activation hampers neurological recovery in rodents and that treatment with mGluR5 NAMs provide strong recovery of integrated sensorimotor function associated with contralesional cortical remodelling. Selective mGluR5 NAMs could become a future pharmacological therapy for promoting functional recovery after stroke.

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Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 384.21/R16

Topic: C.09.Stroke

Title: Coadministration of metformin and the dpp-4 inhibitor evogliptin counteracts stroke in diabetic rat brain

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Abstract: Presenting hyperglycemia and diabetes mellitus (DM) are predictors of a poor outcome in acute ischemic stroke. Beta cell dysfunction and insulin resistance are the two main underlying physiology of DM. The aim of the study was to determine the ant-ischemic effects of counteracting beta cell dysfunction and insulin resistance by pretreatment with evogliptin, a dipeptidyl peptidase (DPP-4) inhibitor, and metformin, respectively, and combined. Type 1 diabetes was induced by intraperitoneal injection of streptozotocin in rats aged 6~8 weeks. The rats were treated with vehicle, evogliptin, metformin, or evogliptin/metformin co-administration for 30 days. Stroke was induced by transient middle cerebral artery occlusion afterwards. Blood DPP-4 activity, GLP-1 levels, glucose, body weight, and food intake were evaluated. Blood levels of fasting insulin and glucose were measured after 30 days of medical treatment for assessment of homeostatic model assessment (HOMA) of insulin resistance and beta cell function in each groups. Ischemic damage was measured by determining infarct volume through T2 weighted magnetic resonance imaging. Immunohistochemistry for GLP-1 receptors in infarct tissue and penumbra were performed. Western blotting of AMPK, Akt/PI3K pathways, and markers of apoptosis were performed in ischemic core and penumbra. The results showed pronounced reduction of infarct volume in the co-administration group. Blood glucose lowering effects were significant in the metformin group and co-administration group, while glycated hemoglobin levels were significantly lower in the co-administration group. Basal insulin levels and HOMA-ß% was significantly higher in the co-administration group, while HOMA-IR did not differ. These results show that combined treatment of metformin and evogliptin strengthen the beta cell protective effects in streptozotocin induced diabetic mice, and this this may offer neuroprotective effects in addition to the direct effects of the antidiabetic drugs. This finding may provide guidance in selection of antidiabetic drugs to reduce neurovascular outcomes in diabetic patients, and give insight into the effects of prediabetic pathophysiology in stroke outcomes.

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Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

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Topic: C.09.Stroke

Support: NHRI-EX107-10412NC
MOST106-2813-C-039-066-B
MOST106-2813-C-039-067-B

Title: Hypothermia but not MK801 protects against brain infarction caused by distal middle cerebral arterial occlusion

Authors: *C. LIU[1,2], C. H. TSENG[1], K.-M. HUNG[2], T. W. LAI[1,2]
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Abstract: Stroke is a major cause of death and disability in Taiwan, USA, and other developed countries. Yet, there remains no effective neuroprotective treatment for stroke. NMDA receptor blockers like MK801 have been shown to be effective against the most popular animal model of stroke: the suture-insertion model, in which the middle cerebral artery is occluded by a suture inserted from the carotid artery. However, this model causes collateral damage to the hypothalamus, leading to hyperthermia that is widely believed to exacerbate brain damage. As a result, MK801-mediated protection of the hypothalamus, which in turn prevents hyperthermia, can contribute to neuroprotection in this model. To better evaluate whether MK801 is neuroprotective, we investigated the effect of hyper- and hypothermic treatment and MK801 administration in a stroke model that does not damage the hypothalamus nor cause hyperthermia. Specifically, we subjected mice to distal middle cerebral arterial occlusion (dMCAo), and 24 h later measured their infarct area/volume after staining by 2,3,5-triphenyltetrazolium chloride (TTC). As with the suture-insertion model, hypothermia strongly protected against brain infarction caused by dMCAo. Nevertheless, MK801 treatment had no appreciable effect on infarct volume. Our study supports the notion that hypothermia is neuroprotective against focal ischemia regardless of whether the stroke model predisposes animals to hyperthermia. Furthermore, and contrary to the results based on the suture-insertion model, MK801 failed to protect against stroke damage. Funding: C.L. and C.T. received undergraduate research scholarships from the Taiwan Ministry of Science and Technology (MOST106-2813-C-039-066-B; MOST106-2813-C-039-067-B). This work is supported by research funding from the National Health Research Institutes (NHRI-EX107-10412NC).

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Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

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Program #/Poster #: 384.23/R18

Topic: C.09.Stroke

Support: MOST Grant MOST-104-2320-B-001-006-MY3

Title: Identification and characterization of bioactive component which contributes to the beneficial extract of clinacanthus nutans against ischemia-reperfusion injury

Authors: *J.-S. WU, M.-H. KAO, H.-D. TSAI, W.-M. CHEUNG, T.-N. LIN
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Abstract: BACKGROUND AND OBJECTIVE:
Clinacanthus nutans Lindau (C. nutans), a popular traditional herb in many tropical Asian, is widely used for treating snake and insect bites, viral infections, due largely to its anti-inflammatory, anti-oxidative properties. Recently, we have reported that C. nutans (CN) protected cortical neuron from hypoxia/ischemic insult in experimental stroke study, however, biological active ingredients and the underlying molecular mechanisms remain largely unknown. In this study, we aimed to identified investigate whether this bioactive component protects against ischemic brain injury via C/EBPβ-dependent PPAR-γ signaling pathway.
METHODS:
Analysis of CN extract using HPLC and high resolution tandem MS/MS identified which component contributing to the neuroprotective effects. To investigate the protective effects of biological active ingredients of CN extract against stroke. Primary cortical neurons were subjected to oxygen/glucose deprivation-reoxygenation (H-R) *in vitro* hypoxic model. MTT assay was used to detect cell viability. Flowcytometry was used to monitor apoptosis. Reporter assay and ChIP were used to detect the transcriptional activity of PPAR-γ. For *in vivo* study, rats were subjected to 3-vessel occlusion (MCAO)/reperfusion. Neurological deficit scores and infarct volumes were used to evaluate functional and cellular damage.
RESULTS:
In vitro**:* With bioactivity-guided HPLC fractionation and high resolution tandem MS/MS, we identified Schaftoside, a C-glycosyl flavones, as the major component contributing to the neuroprotective effect of CN. Using pharmacological antagonist, siRNA knockdown, reporter and ChIP analyses, we further unraveled that Schaftoside selectively enhanced C/EBPβ binding to a unique C/EBPβ binding site (-332~-325) on the PPAR-γ promoter to drive its transcription and the subsequent activation of 14-3-3ε→p-Bad anti-apoptotic signaling. ***In vivo: Intraperitoneal injection of Schaftoside, either pre-or post-ischemic, significantly reduced infarct brain volumes and improved functional recovery up to 2 weeks of reperfusion. Notably, these

beneficial effects could still be obtained even Schaftoside administered at 24hr after 30-min MCA occlusion.

CONCLUSIONS:

It is the first report Schaftoside to exert neuroprotective effects through the C/EBPβ-PPAR-γ pathway not only provides novel therapeutic targets but also paves new ways for potential drug candidates for prevention and treatment of ischemic stroke and possibly other neurodegenerative diseases.

Disclosures: J. wu: None. **M. Kao:** None. **H. Tsai:** None. **W. Cheung:** None. **T. Lin:** None.

Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

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Program #/Poster #: 384.24/S1

Topic: C.09.Stroke

Support: NIH Grant NS074895

Title: Mir363 treatment improves depressive-like behavioral phenotype and rescues retrograde degeneration of meso-striatal projections in middle aged female rats

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Abstract: Introduction: Stroke survivors suffer from long-term physical, cognitive and affective disabilities. These disabilities lower the quality of life, contribute to social isolation and anhedonia, and clinical disorders such as post-stroke depression (PSD), which disproportionately affects women. Behavioral tests measuring sociability, helplessness/hopelessness and anhedonia can be used to assess depressive-like behavior in animal models. **Hypothesis**: Ischemia induced by MCAo leads to depressive-like behavior in middle aged female rats which is improved by mir363-3p treatment. The depressive-like behavior is associated with retrograde degeneration of the meso-striatal pathway in the ischemic hemisphere. **Methods:** Middle-aged Sprague-Dawley female rats (12 months old) were subjected to ischemic stroke using stereotaxic injection of a vasoconstrictor, Endothelin-1, and randomly assigned to two treatment groups: scrambled oligos or mir363-3p mimic. Effort-based Sucrose consumption, Social Interaction and Forced Swim Test were performed over the 100 days to assess depressive-like behavioral phenotype. Thereafter, rats were anesthetized and the retrograde tracer, Fluorogold (Flg), was injected into the left and right striatum. Four days later, animals were overdosed with anesthetic, perfused with saline and formaldehyde and the brain removed for cryosectioning.. In three sections per animal, Flg-labeled cells in the VTA and SNc were counted on both hemispheres, using fluorescent illumination. **Results:** After stroke, there was a 3 fold decrease of high-reward choice

(anhedonia) in the Effort-based Sucrose consumption test in the group that received scrambled oligos, as compared to the group that received mir363-3p by 100 days. Social interaction for scrambled group was 3 times lower as compared to the mir363-3p group in 100 days post stroke. The swim test showed significant increase in helplessness for scrambled group (by 32.7%)as compared to mir363-3p (by 6.4%) in 100 days. Flg labeled neurons in the VTA and SNc were significantly reduced in the ischemic hemisphere as compared to the non-ischemic hemisphere (p&IT0.05) by 100 days.Conclusions: Our previous work shows that mir363-3p treatment given 4h after stroke reduces infarct volume in the acute phase (5days). The current studies show that post-stroke mir363-3p treatment improves long-term stroke disability by improving depressive-like behavior. Mir363-3p also preserves striatal-projecting VTA and SNc neurons in the ischemic hemisphere, a pathway that is involved in reward behaviors.

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Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 384.25/S2

Topic: C.09.Stroke

Support: Stem Cell Network
Ontario Institute for Regenerative Medicine

Title: Identifying the cellular basis for early and delayed metformin-induced recovery in a model of neonatal stroke

Authors: *J. LIVINGSTON-THOMAS, J. ANUPOL, T. SAYED, R. RUDDY, C. MORSHEAD
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Abstract: Neonatal hypoxia-ischemia (H/I) is one of the leading causes of childhood brain injury, resulting in profound physical and cognitive deficits. Currently there are no established treatment options to improve deficits following H/I, and current rehabilitation strategies award only modest recovery. We have previously shown that administration of an anti-diabetic drug, metformin (Met) beginning 24 hours after H/I injury on post-natal day (P8) leads to attenuation of motor deficits at P22 and activation of endogenous neural precursor cells (NPCs) including both expansion of the NPC pool and increased migration and differentiation. More recently, we demonstrated that Met treatment also promotes recovery of cognitive dysfunction. Interestingly,

there exists a population of NG2 expressing oligodendrocyte precursor cells (OPCs) that comprise up to 5% of brain parenchymal cells. Given the demonstrated correlation between oligogenesis and functional recovery, we hypothesized that endogenous OPCs may play an important role in Met-induced functional recovery. Using a transgenic approach, we used NG2CreERT2 x TdTomato mice to perform lineage tracing on this population of OPCs following early Met treatment. Moreover, we proposed that delaying Met treatment for 1 week (a clinically relevant paradigm) would be equally effective at improving short- and long-term recovery. Male and female C57/BL6 mice were subjected to H/I (or a sham procedure) at P8, then received either early or delayed Met treatment. Motor and cognitive functions were assessed across time using various behavioural tests (cylinder, foot fault, puzzle box, water maze). Tissue was collected at P28 (short-term) or 63 (chronic) and analyzed for the presence of proliferative cells, immature neurons, and oligodendrocytes. Our findings to date reveal differential effects of treatment on functional recovery in males and females. We found no significant differences in the number of immature neurons in the subventricular zone (SVZ), dentate gyrus (DG), or motor cortex between groups, nor the number of proliferating cells in the SVZ or DG. However, we did observe a significant increase in the number of proliferating cells in the motor cortex of H/I mice that received delayed Met treatment. These results suggest that a clinically relevant Met treatment paradigm is able to rescue functional deficits and lead to neural repair in certain patient populations.

Disclosures: J. Livingston-Thomas: None. **J. Anupol:** None. **T. Sayed:** None. **R. Ruddy:** None. **C. Morshead:** None.

Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 384.26/S3

Topic: C.09.Stroke

Support: NIH NS088413, NS091585 and NS085568

Title: Gpr37 regulates proliferation of reactive astrocytes in peri-infarct region after ischemic stroke in adult mice

Authors: *C. QU[1,3], S. S. ZOU[1], S. OWINO[2], X. H. GU[1], Z. Z. WEI[1], L. WEI[1], R. A. HALL[2], S. P. YU[1]
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Abstract: GPR37 belongs to the superfamily of G protein-coupled receptors (GPCRs). In both human and rodents, GPR37 is enriched in the brain; it plays an important role in the pathogenesis

of Parkinson's disease and possibly in autism spectrum disorder. Previous investigations including ours demonstrated that GPR37 is cytoprotective after brain injuries including ischemic stroke. Our recent data reveals that the expression of GPR37 regulates astrocyte activation and gliogenesis in the peri-infarct region after stroke. The present investigation explored the possibility that GPR37 may play a regulatory role in promoting proliferation of reactive astrocytes. GPR37 knockout (KO) mice were subjected to focal ischemic stroke targeting the sensorimotor cortex. Immunohistochemical staining in brain sections compared the number of BrdU+/GFAP+ and Nestin/GFAP+ cells in the cortical peri-infarct area of wild type (WT) and GPR37 KO mice. Three days after stroke, the number of proliferating astrocytes was significantly decreased in GPR37 KO stroke brain. Meanwhile, Sox2+/GFAP+ cells in the subventricular zone (SVZ) were significantly less in GPR37 KO mice compared to WT mice. Interestingly, fewer Nestin/GFAP double positive cells were seen in the peri-infarct region of the KO brain, implying that GPR37 might affect neural progenitors in the post-stroke brain. Long-term study of functional assessments showed that GPR37 KO mice are more likely to exhibit behavioral deficits 21 days after stroke. The available data suggest that GPR37 may play an important regulatory role in astrocyte activation and proliferation. Moreover, the possibility that GPR37 might regulate SVZ neurogenesis and astrogliogenesis should be further investigated.

Disclosures: C. Qu: None. **S.S. Zou:** None. **S. Owino:** None. **X.H. Gu:** None. **Z.Z. Wei:** None. **L. Wei:** None. **R.A. Hall:** None. **S.P. Yu:** None.

Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 384.27/S4

Topic: C.09.Stroke

Support: KO8NS088563-01, NINDS/NIH

Title: Role of DNA repair gene in estrogen receptor alpha expression following neonatal hypoxic ischemic encephalopathy

Authors: V. CHANANA[1], D. ZAFER[1], D. HANALIOGLU[1], M. SEREBIN[1], K. M. AMBORN[1], M. FROBER[1], M. B. HACKETT[1], P. FERRAZZANO[2], A. P. AUGER[3], J. E. LEVINE[4], *P. CENGIZ[2]
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Abstract: Objective: Neonatal hypoxia ischemia (HI) related encephalopathy is one of the major causes of learning disabilities and memory deficits in children. Male neonatal brains are

two times more vulnerable to the effects of HI. We recently reported that HI increases hippocampal estrogen receptor α (ERα) expression leading to neuroprotection only in the female mice hippocampi through crosstalk with the neurotrophin receptor, tyrosine kinase B (TrkB). Phosphorylation of the TrkB via its agonist, 7,8-dihydroxyflavone (7,8-DHF) improves long-term neurological outcome only in female mice. In order to understand the underlying mechanisms of this upregulation, we investigated the panel of methylating and demethylating genes, and found that gadd45b is sexually upregulated in female hippocampi compared to males one day post-HI. We hypothesized that gadd45b upregulation is required for ERα upregulation and TrkB mediated neuroprotection in female hippocampi following neonatal HIE.
Methods: HI was induced in P9 B6/C57 male and female wild type (WT) and gadd45 knock out (KO) mice by using Vannucci's HI model. Hippocampi were extracted at 1 day and 3 days after HI for gadd45b and ERα mRNA expressions using qPCR, respectively.Recognition and object location memories were assessed at P60+ by novel object recognition (NOR) and location (NOL) tests in WT and gadd45b KO mice with and without 7,8-DHF(5mg/kg, i.p.) therapy following neonatal HI. The time spent exploring each object was recorded. Then the discrimination ratio (DR) (time spent with novel object divided by total time spent with both objects) was calculated for 5 min exploration time. ANOVA was used for analysis (mean ± SEM).
Results: The demethylating DNA repair enzyme, gadd45b, was significantly upregulated in the ipsilateral female hippocampi compared to male following in HI on day 1 ($p<0.05$)**.** Increased ERα expression in female hippocampi was ablated in gadd45b KO female mice ($p<0.05$) 3 days post-HI. HI decreased the DRs for both NOR and NOL tests in ERα WT male (% 28 ± 2 and %23 ± 3) and female (% 28 ± 9 and % 28 ± 4) mice compared to sham WT male (% 72 ± 5 and % 55 ± 6) and female (% 68 ± 4 and % 71 ± 12) mice ($p < 0.001$), respectively. HI induced decline in recognition and location memories were recovered by 7,8-DHF therapy only in ERα WT females for both NOR and NOL tests, respectively [% 64 ± 6 and % 67 ± 4, ($p < 0.001$)].
Conclusion: Our preliminary studies suggest that gadd45b is upregulated in female neonatal mice hippocampi and sexually differences seen in ERα mRNA expression is ablated in gadd45b KO female mice hippocampi. NOR and NOL tests in gadd45b KO mice are underway to explore the role of gadd45b in ERα dependent TrkB mediated neuroprotection.

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Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 384.28/S5

Topic: C.09.Stroke

Support: Heart and Lungfoundation

Title: Advanced theranostic nanocarrier-mediated delivery of NGF in a combination therapy stimulates recovery after stroke

Authors: *S. ANSAR[1], K. ARKELIUS[1], T. FECZKO[2], F. BLIXT[1], M. WACKER[3]
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Abstract: ***Background:*** The majority of people who survive the acute phase of stroke remain permanently disabled. So far no treatment to improve function exist, nerve growth factor (NGF) has emerged as excellent candidate to boost recovery processes. However, the approach to effective deliver NGF has not been possible since it does not pass the blood brain barrier. We have previously shown that mitogen activated protein kinase (MEK)1/2 inhibition promotes recovery after stroke.
Aim: The aim of this study was to use an advanced theranostic nanocarrier-mediated delivery of NGF to evaluate if combination therapy (U0126 in acute phase together with NGF in subacute phase) have more beneficial outcome after stroke compared to single treatment by U0126.
Method: Transient middle cerebral artery occlusion was induced in male rats for two hours followed by reperfusion. The specific MEK1/2 inhibitor U0126 was administered i.p at 6 and 24 hours and the NGF was given i.v at day 3 post-reperfusion Neurological functions were evaluated by 28-point tests and 9.4 T magnetic resonance imaging was used to monitor morphological infarct changes.
Results: The combination therapy with NGF and U0126 significantly improved neurological function and reduced infarct compared to vehicle and U0126 treatment alone, 4 weeks after treatment.
Conclusion: NGF delivered by advanced theranostic nanocarrier promotes recovery processes after stroke and most importantly our delivery approach has demonstrated to be successful. This will be the first step that will lead to novel treatment strategies for neurological disorders.

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Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 384.29/S6

Topic: C.09.Stroke

Support: CIHR Doctoral Research Award
Brain Canada

Title: Age- and sex-dependent effects of metformin on neural precursor cell activation and cognitive recovery in a model of neonatal stroke

Authors: *R. M. RUDDY[1], K. ADAMS[1], B. DONVILLE[2], C. M. MORSHEAD[3]
[1]Inst. of Med. Sci., [3]Div. of Anat., [2]Univ. of Toronto, Toronto, ON, Canada

Abstract: Resident neural stem and progenitor cells, collectively known as neural precursor cells (NPCs) contribute to ongoing neurogenesis in the adult mammalian brain throughout life. Recruiting these cells as a source for endogenous repair presents a promising opportunity to improve recovery following brain injury. NPCs in the subventricular zone lining the lateral ventricles reside in a well-characterized niche and factors such as age and sex lead to changes in the niche that can alter the behaviour of NPCs. Clinically, these factors play a decisive role in how patients respond to an injury as well as subsequent treatment. Therefore, it is important to investigate the effect of age and sex when examining potential therapeutics for brain injury. The commonly used anti-diabetes drug, metformin, has been shown to activate NPCs in the early postnatal brain, as well as enhance neuro- and oligogenesis. Most importantly, administration of metformin reverses motor impairments in a model of neonatal stroke. In this study, we investigated the age- and sex-dependent effects of metformin on the NPC population from the SVZ of both males and females, at different ages. In addition, we examined whether these age- and sex-dependent effects play a role in cognitive recovery following metformin treatment in a neonatal stroke model. Using the neurosphere assay, we determined that while metformin activates NPCs in the SVZ of both male and female early postnatal mice, the neural stem cell pool is unresponsive to metformin in juvenile mice. Interestingly, metformin activates the neural stem cell pool in adult females, but not males. We determined that the observed sex-differences are due to hormone-mediated effects on the niche as ovariectomized females lost their responsiveness to metformin, suggesting a potential permissive effect of the female niche. Furthermore, when we administered estradiol to previously unresponsive females, we were able to rescue the effect of metformin on NPCs. Finally, we administered metformin to male and female mice following a neonatal stroke injury and assessed cognition using the puzzle box task. Both male and female mice demonstrated impairments in the acquisition of a new task post-stroke; however, the deficit was only rescued by metformin in female mice, coincident with our finding that only female NPCs are responsive to metformin. This study demonstrates the importance of investigating the effects of age and sex when considering a new potential therapeutic for brain repair.

Disclosures: R.M. Ruddy: None. **K. Adams:** None. **B. Donville:** None. **C.M. Morshead:** None.

Poster

384. Stroke Recovery: Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

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Topic: C.09.Stroke

Support: Special Coordination Funds for Promoting Science and Technology
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Creation of Innovation Centers for Advanced Interdisciplinary Research Areas Program in the Project for Developing Innovation Systems (grant 42890001)

Title: Edonerpic maleate enhances motor function recovery from the internal capsule hemorrhage in primates

Authors: *W. NAKAJIMA[1], H. ABE[1], Y. MURATA[3], N. HIGO[4], T. OKUDA[5], T. TAKAHASHI[2]
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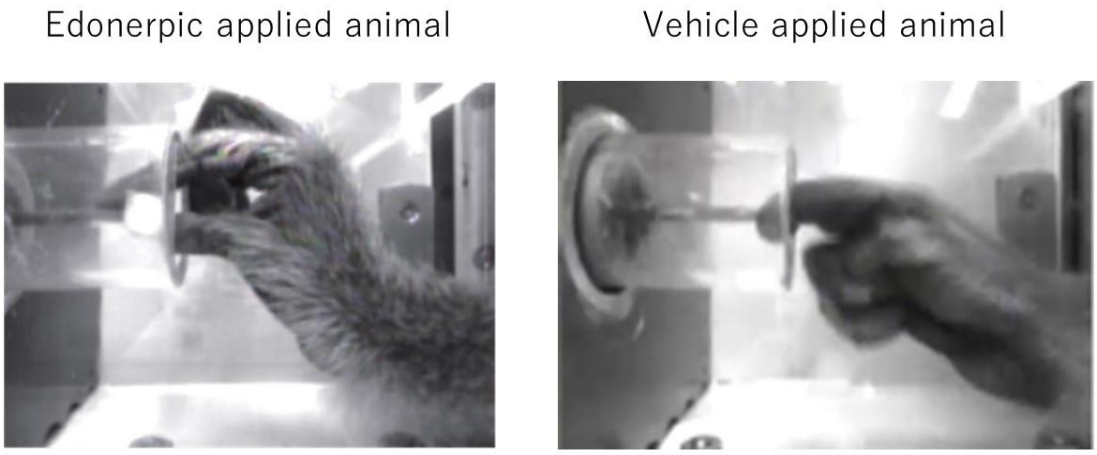
Abstract: The central nervous system injury such as stroke can severely limit the motor function. Despite the recent advances in the approach of the rehabilitative training, many patients still face the restrictions in their daily living after rehabilitation. Thereby, a small molecule compound with strong potential to accelerate motor function recovery with rehabilitation is an unmet medical needs. Here, we found a novel small molecule compound, edonerpic maleate, dramatically accelerated motor function recovery after brain damage in rodents and as a next step for clinical evaluation we established stroke model using macaque monkeys. The followings were conducted to evaluate the effects of edonerpic maleate in monkey stroke model: a focal hemorrhage of the internal capsule was induced by collagenase <typeIV> injection. This lesion initially caused flaccid paralysis in the contralateral hand and after initial reaching of affected hand, we started evaluation, drug administration and rehabilitative training for about 2 months. We evaluated two tasks, the simple reach-to-grasp task and the vertical-slit task, for assessment of forelimb function. In the simple reach-to-grasp task, we measured the time to retrieval of pellets. The rate of recovery in the edonerpic maleate-injected monkeys was significantly faster than that in the vehicle-injected monkeys. In the vertical-slit task, we measured the success rate (successful trial/total number of trials). The success rate in the edonerpic maleate-injected

monkeys was significantly improved than that in the vehicle-injected monkeys. These indicated that edonerpic maleate enhanced the upper limb function recovery of macaque monkeys with the internal capsule hemorrhage. Thus, edonerpic maleate is an effective accelerator of functional recovery after brain damage in primates as well as in rodents.



Disclosures: **W. Nakajima:** A. Employment/Salary (full or part-time):; Yokohama city university. **H. Abe:** None. **Y. Murata:** None. **N. Higo:** None. **T. Okuda:** None. **T. Takahashi:** None.

Poster

385. Stroke Rehabilitation Movement Manipulation, Diagnostics, and White Matter

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 385.01/S8

Topic: C.09.Stroke

Support: NIH NINDS 1U44NS104138
Feinstein Institute

Title: Non-invasive treatment of spasticity in patients with chronic stroke using trans-spinal direct current stimulation with peripheral nerve stimulation

Authors: A. PAGET-BLANC[1], J. CHANG[1], M. SAUL[1], R. LIN[1], N. YAGHOUBI[2], Z. AHMED[3], *B. T. VOLPE[1]

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Abstract: Post-stroke spasticity is a velocity-dependent increase in muscle tone that impedes motor performance and causes pain and discomfort. Preclinical studies have demonstrated that trans-spinal direct current stimulation (tsDCS) paired with peripheral direct current stimulation acts in a polarity-dependent manner to modulate muscle tone, so that anodal tsDCS decreases, and cathodal tsDCS increases muscle tone. In this clinical trial we tested whether DoubleStim™ treatment (non-invasive direct current stimulation via surface electrode at C6 (4mA), paired with simultaneous direct current stimulation of the median nerve (1mA)) delivered by a MyoRegulator™ device (PathMaker Neurosystems) would alter spasticity of the flexor carpi radialis (FCR) in patients with chronic stroke (>6months from ictus). A preliminary safety trial demonstrated that a single 20 minute session of DoubleStim™ caused no untoward effects in 17 patients and controls. Here, we examined whether 5 consecutive 20 minute sessions of anodal DoubleStim™ altered post-stroke spasticity of FCR, as measured by clinical and objective measures of muscle resistance. A single-blind, within-subject crossover design study of 14 patients used clinical assessments: Modified Tardieu Scale (MTS) 11 joints of the upper extremity; Fugl-Meyer Upper Extremity Scale (UE-FM), Wolf Motor Function Test (WMFT)) and objective force measures of passive muscle resistance collected with a calibrated torque sensor at three velocities that permitted analysis of the slope of muscle resistance. Clinical and objective measures were collected before treatment, immediately following Day 5 of treatment, and again weekly for 5 subsequent follow ups (FU) Results, expressed as a ratio (discharge-admission/admission), showed that after DoubleStim™ treatment, there was reduced MTS: FU2 ($p<.05$) and FU3 ($p<.05$), and significant improvements in UE-FM ($p<.05$) and WMFT ($p<.05$) observed immediately after DoubleStim™ treatment were maintained through the last FU. Objective measurements revealed significant reduction of muscle resistance that peaked at FU2 ($p<.05$). Overall, the results indicated that spasticity reduction in upper distal affected limb occurred after 5 consecutive sessions of anodal DoubleStim™ with optimal results observed 2 to 3 weeks after this regimen. Changes in objective measures were consistent with clinical measures, and the improvement in motor function as measured by UE-FM and WMFT suggest that the future details of the timing and dose of DoubleStim™ treatment may have important and enduring clinical effects on motor recovery after stroke.

Disclosures: A. Paget-Blanc: None. **J. Chang:** None. **M. Saul:** None. **R. Lin:** None. **N. Yaghoubi:** None. **Z. Ahmed:** None. **B.T. Volpe:** None.

Poster

385. Stroke Rehabilitation Movement Manipulation, Diagnostics, and White Matter

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 385.02/S9

Topic: C.09.Stroke

Support: Grant-in-Aid for Scientific Research 17K01493

Title: Time and use dependent effects of constraint induced movement therapy (CIMT) on functional recovery and neural network remodeling after severe stroke in rats

Authors: *N. OKABE, N. HIMI, E. NAKUMURA-MARUYAMA, N. HAYASHI, I. SAKAMOTO, O. MIYAMOTO
Kawasaki Med. Univ., Kurashiki, Japan

Abstract: Constraint induced movement therapy (CIMT) is a rehabilitative therapy which has been demonstrated to improve functional recovery after stroke in both animal models and stroke patients. Because the effect of rehabilitative training is influenced by training modification such as initiation time, duration and intensity of training, optimization of training is critical issue for post-stroke recovery. However, guideline for optimization of rehabilitation protocol has not been established due to lack of sufficient information about training modification. The present study investigated how the initiation time (Time-dependency) and intensity of affected forelimb use (Use-dependency) impact the effects of CIMT on the functional recovery. Time-dependency of CIMT was investigated by the comparison of rats treated with CIMT beginning immediately, 1day (acute) and 4days (subacute) after stroke. Use dependency of CIMT was investigated by the comparison of rats with affected forelimb restriction [CIMT (Non-use)], unaffected forelimb restriction [CIMT (Use)] and combination of CIMT (Use) and skilled forelimb training (SFT). Axonal remodeling in the corticospinal projections was assessed by double labelling of total corticospinal neurons (CSN) and forelimb CSN with two types of retrograde tracers (Fastblue and Retrobeads). In the time-dependency analysis, acute and subacute CIMT induced functional improvement in skilled forelimb reaching task although immediate CIMT did not. Furthermore, movement element analysis revealed that only subacute CIMT normalized compensative movement. In the use-dependency analysis, CIMT (Use) and CIMT (Use) + SFT induced functional improvement in skilled forelimb reaching task, whereas CIMT (Non-use) caused transient impairment of restrained forelimb use. In addition to skilled forelimb reaching task, CIMT (Use) + SFT also improved motor performance in staircase test. In the histological analysis, only CIMT (Use) + SFT increased the number of forelimb CSN without significant changes in the total CSN. These results indicate that additional skilled training is critical to enhance functional recovery induced by CIMT while too early initiation time may decrease the beneficial effects.

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Poster

385. Stroke Rehabilitation Movement Manipulation, Diagnostics, and White Matter

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 385.03/S10

Topic: C.09.Stroke

Title: Functional reorganization of the peri-infarct sensorimotor cortex to compensate for fine motor skill impairment

Authors: *A.-S. WAHL[1,2], C. VON ACHENBACH[2], A. SCHROETER[3], L. SUMANOVSKI[4], W. OMLOR[4], F. HELMCHEN[4]
[1]Central Inst. of Mental Hlth., Univ. of Heidelberg, Mannheim, Germany; [2]Brain Res. Inst., Univ. and ETH Zurich, Zurich, Switzerland; [3]Inst. for Biomed. Engin., ETH Zurich, Zurich, Switzerland; [4]Brain Res. Inst., Univ. of Zurich, Zurich, Switzerland

Abstract: Once a stroke occurs victims suffer from lifelong disabilities including the impairment of speech, vision and motor control. No pharmacological therapy is currently available to stimulate the restoration of function. However, even without a therapeutic intervention a form of spontaneous recovery exists while the underlying principles of increased plasticity promoting map-shifts and rewiring of neuronal circuitry are not well understood. The brain region adjacent to stroke damage - the penumbra zone - has been shown to be critical for stroke rehabilitation. Thus, understanding the reorganization of the peri-infarct tissue around small cortical strokes and its contribution to functional recovery may inaugurate the development of new treatments and improve rehabilitative strategies. Here we used Thy1-ChR2-YFP transgenic mice to initiate a small photothrombic stroke through a chronically implanted cranial window targeting the forelimb motor cortex. Optical mapping was performed to study the reorganization of the peri-infarct sensorimotor cortex in accordance with the level of regained forelimb function using detailed kinematic analysis. Animals showed constant improvement and complete recovery during 4 weeks after stroke in two grasping tasks, while optical mapping revealed a shift for the center of forelimb function towards the cortical area representing the hindlimb. Temporarily and reversibly shutting-off neurons in the peri-infarct area using a virus-based pharmacogenetic approach resulted in a decline of regained motor performance and the re-emergence of motor errors observed initially after stroke onset, indicating the functional relevance of the penumbra for the recovery and re-establishment of motor engrams involved in skilled forelimb function.

Disclosures: A. Wahl: None. **C. von Achenbach:** None. **A. Schroeter:** None. **L. Sumanovski:** None. **W. Omlor:** None. **F. Helmchen:** None.

Poster

385. Stroke Rehabilitation Movement Manipulation, Diagnostics, and White Matter

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 385.04/S11

Topic: C.09.Stroke

Support: Stroke Association Post-doctoral Fellowship 2015/02

Title: Can reaching training alter cortical connectivity from the affected and unaffected hemisphere early after stroke?

Authors: *U. HAMMERBECK[1], P. SAMRAJ[1,2], K. L. HOLLANDS[3], S. TYSON[1], J. C. ROTHWELL[4]
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Abstract: Backgrounds: Chronic stroke survivors present with greater ipsilateral connections from the unaffected hemisphere to proximal shoulder muscles. However, these connections are not correlated to function. We hypothesize that ipsilateral connections are strengthened early after stroke but don't become functionally relevant because of limited arm therapy in this period. We therefore investigated whether reaching training alters the prevalence and functional relevance of these connections.
Methods: 38 sub-acute (23.5 days post stroke) stroke survivors (mean age= 61.2, 14.9 SD) with significant arm weakness attended to measure presence, onset latency and size of contralateral and ipsilateral motor evoked potentials (cMEP & iMEP) at 3 and 6/52 after stroke. 20 TMS stimulations up to 100% stimulator output (cMEP mean 74.3 SD 21.6, iMEP mean 94.0 SD 8.9) were delivered over the affected and unaffected hemisphere and responses measured in the preactivated affected triceps muscle. A blinded assessor established clinical characteristics including Fugl-Meyer upper limb score (mean=30.5, SD=16.4). Individuals were randomized to reaching training attending 6 times, performing up to 420 (mean 371 reps SD 71.5) supported, 20cm reaching movements per session, or a control group. Chi-square test established connection, and repeated measures ANOVA, MEP latency and amplitude change. Spearman correlations assessed correlations of Fugl-Meyer scores with MEP measures on a subject-by-subject basis.
Results: At baseline cMEPs were evoked in 22 individuals. 10 of these individuals also showed iMEPs. iMEPs only occurred in isolation in one participant. 15 individuals did not demonstrate either connectivity. At follow-up this presentation did not change nor was their evidence of a difference between groups. The amplitude of cMEP and iMEP did not change but the onset latency for cMEPs became shorter (Time $F(1,18)=10.41$, $p=0.031$) without evidence of a

difference due to training. Fugl-Meyer scores increased over time (F(1,30)=31.84, p<=0.001) without evidence that training altered this. At baseline a correlation for cMEPs size and Fugl-Meyer score (rho=.491.p=0.028) was evident, not observed for iMEPs, without evidence that time or training altered this relationship.
Conclusion: We did not find evidence that reaching training early after stroke altered the presence or functional relevance of connections. For all participants latency of cMEPs reduced and the presence of cMEPs was associated with less impairment. We further observed that ipsilateral responses from the unaffected hemisphere were more common after stroke but were not correlated with function.

Disclosures: U. Hammerbeck: None. **P. Samraj:** None. **K.L. Hollands:** None. **S. Tyson:** None. **J.C. Rothwell:** None.

Poster

385. Stroke Rehabilitation Movement Manipulation, Diagnostics, and White Matter

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 385.05/S12

Topic: C.09.Stroke

Support: AHA grant 14BFSC17760005

Title: Exploring the mechanisms of motor rehabilitation-induced recovery after white matter stroke

Authors: *T. G. MENGISTU[1], M. A. MARIN[2], T. S. CARMICHAEL[2]
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Abstract: Subcortical white matter stroke (WMS) accounts for 25% all stroke subtypes. White matter is composed of axons that relay brain signals and oligodendrocytes, which produce myelin. Myelin is a multi-lamellar extension of oligodendrocyte membrane that wraps around the axon and provides it with both electrical insulation and metabolic support. WMS is characterized by the formation of white matter lesions, which results in oligodendrocyte death and myelin degeneration. Loss of myelin results in axon degeneration and functional impairment. Previous work in the Carmichael lab demonstrates that after WMS, there is a significant increase in proliferation of oligodendrocyte precursor cells (OPCs), a resident stem cell that functions in part to differentiate into mature oligodendrocytes during development and in adulthood. However, despite proliferation, these OPCs fail to mature into oligodendrocytes following WMS. Recent work in our lab suggests that subjecting mice to motor rehabilitation, such as skilled reach, enhances recovery by promoting both OPC proliferation and their mature into myelinating oligodendrocytes. The goal of this project is to build upon these initial findings by identifying molecular markers of myelin and oligodendrocyte recovery following motor rehabilitation.

Understanding the mechanisms that drive recovery and re-myelination in mice after WMS will enable us to better understand the pathology of WMS and develop novel therapeutic strategies for recovery.

Disclosures: T.G. Mengistu: None. **M.A. Marin:** None. **T.S. Carmichael:** None.

Poster

385. Stroke Rehabilitation Movement Manipulation, Diagnostics, and White Matter

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 385.06/S13

Topic: C.09.Stroke

Support: Grant-in-Aid for Young Scientists (B) 90737355
Grant-in-Aid for Young Scientists (B) 17K13069
Grant-in-Aid for Scientific Research (C) 17K01482

Title: Three-dimensional kinematic evaluation of motor deficit in a rat model of photochemically induced focal stroke

Authors: *A. YOSHIKAWA[1], S. MORISHITA[2,3], K. HOKAMURA[4], K. UMEMURA[2], M. IZUMIZAKI[1], T. KUMADA[5]
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Abstract: Rehabilitation such as exercise therapy is known to contribute for recovering the motor function in patients suffering from cerebral stroke. However, its mechanism remains unclear. Thus, we developed a rat model of focal motor cortex infarction by photochemically induced thrombosis (PIT) method and have investigated the role of neuronal reorganization with regard to motor functional recovery. After PIT operation, the rats with the left side of cortical infarction exhibited paralysis-like behavior in the right side of hindlimb. In addition, beam-walking test revealed that these rats significantly increased the slipping errors in the hindlimb compared with sham-operated rats. Therefore, we hypothesized that the rats with focal motor cortex infarction might have some considerable difficulties even in gaiting and these difficulties should be more unmasked by the quantitative evaluation methods with kinematical analysis. Thus we utilized three-dimensional motor analysis (Kinema Tracer, KISSEI COMTEC Co.,Ltd.) with some modifications to evaluate the gaiting movements of PIT operated stroke rats. Here, we analyzed and compared the rat's hindlimb movement between pre- and post 1 day PIT operation. There were no significant differences in gait parameters (gait cycle, stance phase, swing phase, step length and step width) between pre- and post-operated rats, which was similar to the results

of foot-printing tests. On the other hands, the kinematical motions of the ankle, knee and hip joint on each x, y and z plane revealed significant differences in some parameters in the PIT operated rats. Our results suggest that more precise evaluation of the motor behavior by three-dimensional motor analysis should unmask the motor deficits in the model animals of neurologic disorder with seemingly subtle impairments.

Disclosures: A. Yoshikawa: None. **S. Morishita:** None. **K. Hokamura:** None. **K. Umemura:** None. **M. Izumizaki:** None. **T. Kumada:** None.

Poster

385. Stroke Rehabilitation Movement Manipulation, Diagnostics, and White Matter

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 385.07/S14

Topic: C.08. Ischemia

Support: NS056839

Title: Non-paretic forelimb training does not interfere with recovery of paretic forelimb strength after experimental middle cerebral artery occlusion

Authors: *K. S. VALENZUELA, M. BLAKER, N. COHEN, B. COOK, D. BHANDARI, T. A. JONES, T. SCHALLERT
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Abstract: Humans often compensate with their unimpaired (non-paretic) limb after surviving a stroke. Research in rats shows that this can be maladaptive after focal motor cortical strokes. The middle cerebral artery is the vessel most commonly affected by cerebrovascular incident. This type of stroke often causes large strokes that affect both cortical and subcortical regions of the brain. It is unclear if behavioral experience with the non-paretic limb differentially affects paretic limb recovery depending on lesion locus. Long Evan rats were preoperatively trained with their preferred limb on the Isometric Pull Task (Vulintus), a skilled reaching task that sensitively assays forelimb weakness. Previous data from our lab suggests that rehabilitative training (RT) improves paretic limb function on the Isometric Pull Task after middle cerebral artery occlusion (MCAo). Transient focal ischemia (60 min) was induced in the hemisphere contralateral to the preferred limb by an intraluminal middle cerebral artery occlusion suture method. Rats received either non-paretic limb training (NPT) on the Isometric Pull Task or non-training control procedures for 14 days. All rats then received six weeks of RT with the paretic limb. Average paretic forelimb strength, as measured in grams, and the number of attempted trials per session were assessed weekly in all rats. At the completion of the behavioral study, the anterograde tract tracer biotinylated dextran amine (BDA) was pressure injected into the lesioned hemisphere. Brains were harvested 21 days later to examine axon sprouting induced by the behavioral

manipulations. NPT did not reduce paretic forelimb strength or trials per session during RT when compared with the control group. In fact, after six weeks of RT, NPT rats (150±12.4) had less forelimb weakness than control rats (115±29.5) when measuring grams of force. NPT rats (107±16.8) also completed more trials per session when compared with control rats (87±23.9). Data are mean±SEM. NPT on the Isometric Pull Task did not interfere with paretic limb recovery, suggesting that compensatory use of the non-paretic limb after strokes caused by occlusion of the middle cerebral artery may not be maladaptive. Understanding behavioral recovery after different types of strokes could influence clinical management of patients. Work to examine the influence of NPT on interhemispheric reinnervation patterns is ongoing.

Disclosures: K.S. Valenzuela: None. **M. Blaker:** None. **N. Cohen:** None. **B. Cook:** None. **D. Bhandari:** None. **T.A. Jones:** None. **T. Schallert:** None.

Poster

385. Stroke Rehabilitation Movement Manipulation, Diagnostics, and White Matter

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 385.08/S15

Topic: C.09.Stroke

Support: KAKENHI 15K16361
KAKENHI 18K10718

Title: Interaction between cortico-rubral tract and cortico-reticular tract in rehabilitation-induced functional recovery after capsular hemorrhage

Authors: *A. ISHIDA[1], K. KOBAYASHI[2], T. ISA[3], H. HIDA[1]
[1]Nagoya City Univ. Grad. Sch. of Med. Sci., Nagoya, Aichi, Japan; [2]Natl. Inst. For Physiological Sci., Okazaki, Japan; [3]Dept. of Neuroscience, Grad. Sch. of Med. & Fac. of Med., Kyoto Univ., Kyoto, Japan

Abstract: Stroke often disrupts cortico-spinal tract and causes severe sensorimotor deficit. Cortico-brainstem-spinal pathways can be the compensatory bypass. However, detailed role and interactions of these tracts in rehabilitation-induced functional recovery is still unclear. In this study, we investigated the rehabilitation-induced reorganization of the cortico-rubral tract (CRT) and the cortico-reticular tract (CReT) and its contribution to functional outcome. Intracerebral hemorrhage (ICH) model was made by collagenase injection into the internal capsule, and the rats were forced to use their impaired forelimb by fitting the one-sleeve cast for days 1-8 (D1-8) after ICH (forced-limb use: FLU). Skilled reaching task revealed that FLU promoted substantial recovery of forelimb function in ICH model at D12 and D28. Biotin dextran amine (BDA) injection into the ipsilesional motor cortex revealed the increase of abundant BDA-positive fibers in the ipsilateral red nucleus in ICH-FLU group at D12 and D51 although there were few

positive fibers in the reticular formation. To block the CRT and/or the CReT selectively, we used Tet-on system by injection of NeuRet-TRE-EGFP.eTeNT into the red nucleus and AAVdj-CaMKII-rtTAV16 into the motor cortex and DREADD system by injection of NeuRet-MSCV-Cre into the reticular formation and AAVdj-Flex-DIO-hM4D- mcherry into the motor cortex. Selective CRT blockade by doxycycline after FLU at D13-20 caused apparent impairment of the recovered forelimb function in ICH-FLU group. However, this selective CRT blockage was gradually disappearing in the later phase. Interestingly, additional CReT blockade by clozapine N-oxide administration at D21-28 under CRT blockade caused severe impairment of the forelimb function again. In contrast, only CReT blockade at D13-20 did not affect the FLU-induced recovery. These data suggest that CRT has causal link to FLU-induced recovery of forelimb function after ICH, indicating more dominant role in FLU-induced reorganization and recovery compared to CReT that is a potential compensational pathway in case of the CRT blockade in FLU after ICH.

Disclosures: A. Ishida: None. **K. Kobayashi:** None. **T. Isa:** None. **H. Hida:** None.

Poster

385. Stroke Rehabilitation Movement Manipulation, Diagnostics, and White Matter

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 385.09/S16

Topic: C.09.Stroke

Support: American Heart Association/Bugher Foundation (14BFSC17760005)
The Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Elucidating the mechanisms of activity dependent remyelination after stroke

Authors: *M. A. MARIN[1,1], K. NG[2], R. KAWAGUCHI[1], G. COPPOLA[1], S. T. CARMICHAEL[1]
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Abstract: Subcortical white matter stroke (WMS) damages neural connections via the formation of lesions along tracts of myelinated axons. These strokes kill oligodendroctyes resulting in demyelination of axons, and behavioral impairments, dementia, and death. Following WMS, there is a significant increase in oligodendrocyte precursor cell (OPC) proliferation, which if driven to maturity would repair the lesion as they do in the early stages of multiple sclerosis (MS). However, OPCs fail to mature into oligodendrocytes after WMS, the mechanisms of which are poorly understood. Here we demonstrate that motor activity releases this developmental block by driving the maturation of OPCs into myelinating oligodendrocytes and enhancing recovery after WMS. Furthermore, we have developed a transcriptional profile of OPCs using Ribotag mRNA precipitation after WMS and motor rehabilitation. Our findings will

be used to develop molecular strategies to target candidate genes and test their effect on repair and recovery after WMS (in the absence of motor rehabilitation) and identify the molecular systems that underlie remyelination.

Disclosures: M.A. Marin: None. **K. Ng:** None. **R. Kawaguchi:** None. **G. Coppola:** None. **S.T. Carmichael:** None.

Poster

385. Stroke Rehabilitation Movement Manipulation, Diagnostics, and White Matter

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 385.10/S17

Topic: C.09.Stroke

Title: Mental imagery as a tool to change muscle coordination patterns in chronic stroke survivors

Authors: *S. JAYASINGHE, R. RANGANATHAN
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Abstract: Mental imagery and visual feedback are widely used in real-world skill learning, and their utility in stroke rehabilitation has also received some attention. However, research on the use of mental imagery in stroke rehabilitation has not explored its effects on muscle coordination. This is a critical area of research since chronic stroke survivors exhibit incorrect muscle coordination patterns. This study focused on the effects of mental imagery and supplementary visual feedback on muscle coordination in healthy participants, with the goal of extending the approach to chronic stroke survivors.
We designed an isometric virtual reaching task where the electrical activity of six arm muscles was mapped on to the two-dimensional position of a cursor on the computer screen. Participants were asked to move this cursor into a target presented at six pseudorandom locations equidistant from the central start position. The muscle map was designed to be non-intuitive. We recruited 30 healthy college-aged students, and placed them in one of 3 groups: control i.e. no intervention, mental imagery intervention, and supplementary visual feedback intervention. Participants in the mental imagery group were given a specific kinesthetic imagery scenario to practice on. The supplementary visual feedback group was shown how the activity of each muscle individually contributed to the motion of the cursor on the screen by setting the gains for the other muscles to 0. This intervention was done to check for whether mental imagery was truly beneficial, or whether simply providing additional visual feedback would have been enough. Each intervention lasted approximately 2 minutes, and all participants completed 180 trials.
Results showed that both mental imagery and supplementary visual feedback reduce movement time at a faster rate than the control group. We also found that the mental imagery group was

able to reduce both task and null space variability more than both the other groups, with this reduction being significantly greater ($P < 0.05$) than the control group. These preliminary results suggest that mental imagery may be a potential tool for use in the clinical setting after testing for efficacy in stroke survivors.

Disclosures: S. Jayasinghe: None. **R. Ranganathan:** None.

Poster

385. Stroke Rehabilitation Movement Manipulation, Diagnostics, and White Matter

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 385.11/S18

Topic: C.09.Stroke

Support: FWO Grant

Title: Heterozygous deletion of ephrinA5 does not affect functional motor performance after experimental stroke in mice

Authors: A. DE BOER[1,2], A. STORM[1,2], L. RUÉ[1,2], W. ROBBERECHT[3,2], *R. LEMMENS[3,2]
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Abstract: Stroke is the main cause of adult disability, affecting about 15 million people each year worldwide. During an ischemic insult, different molecular mechanisms lead to neuronal and glial cell death causing impaired neurological functioning. Although stroke damage can be devastating, most patients survive the initial insult, resulting in large numbers of stroke survivors in long-term care facilities. After stroke, the brain is capable of some spontaneous recovery. However, this process is limited by inhibitory molecules, including various developmental guidance cues like members of the ephrin system.
The ephrin system is mainly known for its role in central nervous system (CNS) development but the majority of Eph receptors and ephrin ligands are still expressed during adult life. It has become clear that this system is important for neural repair during adulthood, as shown in various CNS injury models. After experimental stroke, ephrinA5 was shown to be upregulated in reactive astrocytes in the peri-infarct area and blocking ephrinA signaling improved remapping and behavioral outcome. In the present study, we aim to validate the role of ephrinA5 in stroke recovery using ephrinA5 heterozygous mice which are functionally assessed after experimental stroke introduced by the photothrombotic lesion model (PTL). Additionally, the effect of ephrinA5 reduction on astrocyte reactivity, glial scar formation and neural plasticity is determined.
Adult male mice were subjected to PTL and functionally tested to assess stroke recovery until four weeks post stroke. After 24 hours, a subset of mice was sacrificed to determine infarct size

and brain swelling and cortical brain tissue was collected at day 7 to identity the expression level of various astrocytic, glial scar and plasticity markers. Results show that heterozygous deletion of ephrinA5 did not affect infarct size and brain swelling after PTL. Using the single pellet reaching task and horizontal ladder task, no differences were observed in functional recovery between ephrinA5+/- and control mice. In accordance to this, ephrinA5 reduction did not affect astrocyte reactivity as assessed by GFAP and vimentin protein levels. In addition, the expression of various glial scar components were not affected in ephrinA5+/- mice. We determined NF200 levels as an indication of neurodegeneration/regeneration, however no differences were found between ephrinA5+/- and control animals.
In future experiments, we will determine the cell-type specific expression of ephrinA5 using in situ hybridization as well as the effect of ephrinA5 reduction on axonal sprouting into the denervated cervical spinal cord using BDA tracing.

Disclosures: A. de Boer: None. **A. Storm:** None. **L. Rué:** None. **W. Robberecht:** None. **R. Lemmens:** None.

Poster

385. Stroke Rehabilitation Movement Manipulation, Diagnostics, and White Matter

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 385.12/T1

Topic: C.08. Ischemia

Support: NSERC
HSFC
AIHS

Title: Exploring the temporal profile of myelination changes after stroke in relation to function remapping

Authors: *E. WENDLANDT[1,2], I. R. WINSHIP[3]
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Abstract: Functional recovery after stroke is driven by mechanisms of central nervous system plasticity - including the structural reorganization of surviving neuronal networks and, crucially, the re-establishment of a functional pattern of myelination across these newly created circuits. The time-course and extent of this type of re-patterning, however, are not well understood and their relation to cortical network impairment and recovery is not defined. Immediately after stroke, peri-infarct areas seem to undergo a period of severe de- and dysmyelination, followed by partial remyelination which appears to be temporally staggered depending on the region's distance from the stroke core. In this project, we longitudinally tracked and quantified in vivo

myelination changes in the peri-infarct cortex of mice for up to four weeks following targeted photothrombosis of the forelimb somatosensory cortex using Spectral COnfocal Reflectance microscopy - a label-free imaging technique that utilizes the high reflectivity of lipid-rich myelin and is able to resolve single cortical myelinated fibres. Functional changes were concurrently monitored via Optical Signal Imaging of the remapping responses in the fore- and hindlimb somatosensory representations and the time-course and spatial extent of the two measures compared to probe their putative relationship. We observed partial and prolonged demyelination of the peri-infarct cortex for up to 14-21 days post-stroke and a simultaneous loss of the forelimb representation. Eventual remyelination occurred in close temporal correlation to the re-emergence of the forelimb map. In our final experiment, we manipulated the time-course of the post-stroke myelin changes using an experimental compound called fluorosamine - a CSPG biosynthesis inhibitor previously demonstrated to enhance remyelination in a mouse model of Multiple Sclerosis - and found this resulted in a corresponding temporal shift in the cortical remapping response. These results help underscore the importance of myelination as a key post-stroke plasticity mechanism.

Disclosures: E. Wendlandt: None. **I.R. Winship:** None.

Poster

385. Stroke Rehabilitation Movement Manipulation, Diagnostics, and White Matter

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 385.13/T2

Topic: C.08. Ischemia

Title: Characterizing blood-brain barrier disruption in degenerative thalamus after cortical ischemic stroke

Authors: *S. HARVEY, Z. CAO, M. Y. CHENG, G. K. STEINBERG
Neurosurg., Stanford Univ., Stanford, CA

Abstract: Background: After an ischemic cortical stroke, a delayed secondary degenerative injury occurs in the ipsilesional thalamus. Anterograde and retrograde degeneration between thalamocortical circuits exacerbate neuronal cell death and hinder stroke recovery. Inflammatory responses from both resident glial cells and infiltrating monocytes are a hallmark of secondary thalamic injury. Blood-brain barrier (BBB) disruption and permeability in the thalamus may exacerbate this inflammatory response, thus worsening thalamic injury and stroke outcomes. We aim to investigate how BBB disruption contributes to secondary thalamic injury after stroke. **Material and Methods:** Cortical ischemic stroke was generated by permanent occlusion of left middle cerebral artery in male C57BL6 mice (12-15 weeks). Time course analysis of BBB permeability was assessed by immunostaining in brain sections collected on post-stroke day (PD) 3, 7, 14, 21 and 28. To assess BBB permeability, Alexa 488 dextran was injected via femoral

vein 90 minutes prior to sacrifice. BBB permeability was also assessed via immunostaining with antibodies specific for IgG and the blood vessel marker Isolectin-B4. Inflammatory cells were visualized with antibodies targeting microglia (Iba1) and astrocytes (GFAP). **Results:** After cortical ischemic stroke, Alexa 488 dextran showed strong signal in the ipsilesional somatosensory cortex (iS1) and persisted through day 28. Dextran signal in the ipsilesional thalamus (iTH) was delayed, detectable starting at PD7 and increasing through PD28. IgG immunostaining showed similar trend. The expression profile of inflammatory markers (GFAP and Iba1) mirrored the timeline of BBB permeability; iS1 showed robust astroglial and microglial activation beginning at PD3, and persisting through PD28. Inflammatory cell activation was delayed in iTH; microglia and astrocytes began exhibiting an activated morphology at PD7 and gradually increased through PD28. **Conclusion:** Our study suggests that BBB disruption in the thalamus contributes to neuroinflammation and secondary thalamic injury after ischemic stroke. This BBB permeability begins in the acute phases and persists through at least one month after stroke. Current studies investigate the cellular and molecular mechanism of BBB disruption after stroke. Identifying specific BBB-related proteins in endothelial, astrocyte, and pericyte populations may provide insight into how the BBB is maintained in the healthy brain, and reveal potential targets to alleviate secondary thalamic injury after stroke.

Disclosures: S. Harvey: None. **Z. Cao:** None. **M.Y. Cheng:** None. **G.K. Steinberg:** None.

Poster

385. Stroke Rehabilitation Movement Manipulation, Diagnostics, and White Matter

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 385.14/T3

Topic: C.09.Stroke

Title: Detection of altered network function after stroke is task-dependent

Authors: *S. M. SCHINDLER-IVENS[1], B. D. SCHMIT[2], K. VINEHOUT[3]
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Abstract: Recently, our group used fMRI to examine brain activation during pedaling and tapping in people with and without stroke (Promjunyakul et al., 2015). Brain activation volume in the stroke group tended to be higher than controls during paretic foot tapping and lower than controls during pedaling. These observations led us to consider that tapping and pedaling may be differentially sensitive to stroke-related changes in local and global network function of the brain. The work presented here sought to examine this issue by quantifying task-based functional connectivity during pedaling and tapping. We hypothesized that global network connectivity would be reduced and local network connectivity would be elevated in people with stroke, as compared to controls. We also predicted that the ability to detect these effects would be task

dependent. Pedaling would reveal between-group differences in global connectivity; tapping would reveal differences in local connectivity. 15 people with stroke and 8 controls performed pedaling and foot tapping during fMRI. ROIs were identified with independent component analysis. Correlations were computed among ROIs; values were used to quantify global connectivity. Correlations were also computed for all pairwise combinations of time series within each ROI; the mean of these values defined local connectivity. During pedaling, global connectivity among all ROIs was lower in the stroke than the control group ($p<0.001$). The largest effect was for connections to S2 on the lesioned side of the brain. During paretic tapping, global connectivity was not different between groups for any ROI ($p>0.057$). People with stroke displayed greater local connectivity than controls during paretic foot tapping ($p=0.002$). However, during pedaling, they displayed lower local connectivity than controls ($p=0.02$). Our data indicate that local and global networks of the brain are affected by stroke. The ability to detect these changes is affected by task. Pedaling is more sensitive to global changes; tapping is more likely to reveal changes in local network function. It may be important to examine functional connectivity of the brain across tasks to gain a full appreciation of the nature and extent of altered brain function post-stroke.

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Poster

386. Spinal Cord Injury and Plasticity: Training, Rehabilitation, and Repair: Human

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 386.01/T4

Topic: C.11. Spinal Cord Injury and Plasticity

Title: The effect of physical exercise participation on Brain-Derived neurotrophic factor among people with SCI

Authors: *A. A. ALGHAMDI[1,2], A. M. WILLIAMS[1,2], M.-S. POORMASJEDI-MEIBOD[1], G. EGINYAN[1,2], T. LAM[1,2]
[1]Intl. Collaboration On Repair Discoveries, Vancouver, BC, Canada; [2]Sch. of Kinesiology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Background**:** Beyond the benefits of physical activity on overall health, there has been increasing recognition of the positive effects of exercise on brain function (e.g. memory and learning). It is thought that the mechanisms behind this effect are related to increased blood flow to the brain and release of neurotrophins, especially brain-derived neurotrophic factor (BDNF). Participation in long-term physical activity programs has been associated with elevated levels of basal BDNF. In the spinal cord injury (SCI) literature, elite athletes with SCI showed a high basal BDNF concentration, even compared to the general able-bodied population. However, it is unknown whether the general SCI population similarly experiences an increase in basal BDNF

from regular physical activity participation. The aim of this study was to 1) compare basal BDNF levels in active vs. inactive persons and 2) characterize the effect of high vs. low levels of physical activity participation on other measures of health and fitness (e.g. VO2 peak, depression, fatigue, pain) among people with SCI.
Methods: Individuals with chronic SCI were enrolled in this study. A blood sample was taken after fasting for serum BDNF analysis. Participants also completed the Physical Activity Scale for Person with Physical Disability, Beck's Depression Inventory, Perceived Stress Scale, Fatigue Severity Scale, and Multidimensional Pain Inventory. Body composition was measured by whole-body Dual-energy X-Ray Absorptiometry. VO2 peak was tested by an incremental arm ergometer test.
Results**:** The majority of participants were males and more than half of them with incomplete SCI (iSCI). There were no significant differences in BDNF concentration, other physical outcomes, or questionnaire results between iSCI and cSCI participants. Active subjects had significantly lower scores in depression and stress, and higher VO2 peak ($p < 0.05$) when compared to inactive subjects. In addition, active participants tended to have higher lean body mass and basal BDNF concentration compared to inactive, but the difference was not significant.
Conclusion**:** Individuals with SCI classified as physically active exhibited expected benefits of exercise on health measures (e.g. depression and stress levels) and fitness (VO2 peak) compared to physically inactive individuals. However, the purported effect of physical activity participation on basal levels of circulating BDNF was not as apparent.

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Poster

386. Spinal Cord Injury and Plasticity: Training, Rehabilitation, and Repair: Human

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 386.02/T5

Topic: C.11. Spinal Cord Injury and Plasticity

Support: ICORD Spring 2017 Seed Grant
CIHR Canada Graduate Scholarship Master's Award
UBC School of Kinesiology Graduate Student Research Grant 2017

Title: Exploring pelvic floor muscle sparing in spinal cord injured individuals using transcranial magnetic stimulation, targeted exercises, and exoskeleton walking

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Abstract: Background: The pelvic floor muscles (PFM) are important for maintaining urinary continence. In able-bodied individuals, exercise programs intended to strengthen the core and PFM are commonly used to treat incontinence. However, limited research has explored applying these exercises to the spinal cord injury (SCI) population where 80% of individuals experience neurogenic bladder dysfunction. PFM training programs may not have been attempted in people with SCI because of a lack of understanding about how the PFM functions post-injury. Further, for those with high-thoracic motor-complete SCI (mc-SCI), it is often incorrectly assumed that they cannot engage core muscles based on standard neurological assessment. Previous work has shown that sparing in abdominal muscles can be detected using manual palpation, electromyography (EMG), and transcranial magnetic stimulation (TMS). Since the abdominal and PFM are part of the core, this raises questions as to the extent that PFM muscles may be similarly spared in this population. The purpose of this project was to a) evaluate corticospinal excitability to the PFM via TMS and b) characterize and compare activation patterns of the PFM and other core muscles during validated PFM training exercises and exoskeleton walking in individuals with mc-SCI. **Methods**: This study used a two-part cross-sectional design. EMG signals were recorded bilaterally from rectus abdominis, external oblique, erector spinae, levator ani, and gluteus maximus. In Part 1, TMS was delivered over the PFM region of the primary motor cortex in both supine and supported standing conditions. In Part 2, EMG signals were recorded while participants attempted validated PFM exercises (e.g. kegels) and performed overground walking using the Ekso robotic exoskeleton. **Results**: Motor-evoked potentials in the levator ani were elicited in response to TMS in able-bodied participants, but not as readily in individuals with mc-SCI. Participants with mc-SCI also had difficulty recruiting the PFM using the validated PFM exercises, however, activation could be elicited during Ekso-assisted walking. **Conclusion**: Our preliminary results suggest that PFM activity can be elicited in mc-SCI participants during Ekso-assisted walking, but conventional exercises may not be effective for this population. Furthermore, it is unclear if there is sparing in descending pathways to the PFM, similar to what we have previously observed in the abdominal muscles. However, the possibility of recruiting PFM activation by exoskeleton-assisted walking could lead to new training strategies to strengthen the PFM, potentially improving bladder function in individuals with SCI.

Disclosures: A.M.M. Williams: None. **G. Eginyan:** None. **A.E. Chisholm:** None. **T. Lam:** None.

Poster

386. Spinal Cord Injury and Plasticity: Training, Rehabilitation, and Repair: Human

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 386.03/T6

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH R01 HD081274

Title: Gains in overground walking performance following low oxygen therapy are not accompanied by reductions in intralimb kinematic variability in persons with iSCI

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Abstract: Mild breathing bouts of low oxygen (intermittent low oxygen therapy, iLOT) is a promising intervention that induces spinal plasticity, leading to improvements in both breathing and locomotion in rats with spinal cord injury (SCI). More recent work supports that iLOT induces profound motor recovery in persons with SCI, augmenting ankle strength and clinical measures of walking performance. Despite growing evidence of the translational potential of iLOT, it is unclear if gains in walking function are associated with shifts in neuromotor strategies. Recently, we found that excessive kinematic variability during overground walking post SCI predicts walking performance, suggesting that variability reliably reflects underlying motor control impairments. Characterizing changes in kinematic variability may therefore yield insight into the range and stability of motor patterns that accompany iLOT induced functional gains.

The purpose of this study is to characterize the effect of iLOT on the variability of endpoint foot trajectory (EPVAR) and hip-knee coordination (ACC) during overground walking. We hypothesize that improvements in walking performance following iLOT would be concurrent with reductions in both parameters of kinematic variability. To test our hypotheses, persons with chronic iSCI walked overground at cadence-matched self-selected speed before and after exposure to iLOT (15, 1.5 min episodes at 9% FiO_2 with 1 min intervals at 21% FiO_2 for 5 consecutive days). Kinematic walking features were captured using a motion analysis system at day 5 (T5), 1 week (F1), and 2 weeks (F2) following iLOT. EPVAR was computed from the spatial density profiles of 5^{th} MTP marker in the sagittal plane during the swing phase of gait. ACC was quantified from each gait cycles cyclogram.

Consistent with previous studies, we observed a significant reduction in 10-meter walk test time relative to baseline at T5 (-2.23s), F1 (-1.8s) and F2(-2.34s). Similarly, participants increased the distance walked in 6 min at T5 (+42.3m), F1(+38.2m), and F2 (+28.4m) relative to baseline. In contrast with our hypothesis, neither reductions in foot path variability (EPVAR: BL =2.84±0.68, F1=2.69±0.38, F2 =2.39±0.28) nor improvements in hip-knee coordination (ACC: BL = 0.83±0.03, F1 =0.84±0.02, F2 =0.83±0.03) accompanied increases in walking performance. These results suggest that the reversion of neuromotor strategies to able bodied patterns following iLOT do not sufficiently explain walking improvements. In addition to compensatory strategies, gains in interlimb performance must be considered when evaluating specific aspects of iLOT induced motor recovery.

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Poster

386. Spinal Cord Injury and Plasticity: Training, Rehabilitation, and Repair: Human

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 386.04/T7

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NICHD R01 HD079009-02 (EF-F)

Title: Dose-dependent effects of whole body vibration on spasticity in individuals with spinal cord injury

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Abstract: Background & Purpose: While muscle paralysis is the most obvious consequence of spinal cord injury (SCI), approximately 65% of individuals with SCI experience uncontrolled muscle contractions, stiffness, and decreased motor control associated with spasticity. Spasticity is a consequence of decreased presynaptic inhibition in the spinal reflex circuit caused by the disruption of descending signals from the brain to the spinal cord. While there is early evidence for the use of whole body vibration (WBV) as an anti-spasmodic treatment in people with SCI, there is limited understanding of the optimal dose to reduce spasticity. The purpose of this study was to compare the single-session effects of four different WBV frequency/duration dose conditions on spasticity. We also measured walking speed as a secondary outcome.
Methods: Thirty-five individuals with motor-incomplete SCI received 4 different dose conditions of WBV: high frequency (50Hz)/short duration (180 seconds), high frequency/long duration (360 seconds), low frequency (30Hz)/short duration, and low frequency/long duration, plus a sham-control condition. Quadriceps spasticity was measured using the pendulum test at 4 timepoints during each session: before and 3 timepoints after WBV. Walking speed was quantified using the 10-meter walk test at 3 timepoints during each session: before and 2 timepoints after WBV. The condition order was randomized across participants and sessions were separated by at least 1 week to minimize the potential for carry-over effects.
Results: In participants with more severe spasticity, the high frequency/long duration WBV condition was associated with a greater reduction in spasticity. The sham-control condition was also associated with reduced spasticity. There were no changes in walking speed from baseline to post-intervention in any of the WBV dose conditions compared to the sham-control.
Discussion & Conclusions: Our study suggests that inhibitory mechanisms are evoked in a frequency-dependent manner. Longer exposures to vibration resulted in greater reduction in

spasticity suggesting that duration is an important component of WBV dose. Finally, in our prior studies we have found that the immobility associated with extended sitting had a negative effect on spasticity. Conversely, in the current study the sham condition involved repetitions of sitting and standing, and this repeated activity appeared to have a beneficial influence on spasticity. WBV and other non-pharmacological approaches to management of spasticity warrant further study.

Disclosures: J. Hope: None. **S.P. Estes:** None. **J.A. Iddings:** None. **N.J. Kirk-Sanchez:** None. **E.C. Field-Fote:** None.

Poster

386. Spinal Cord Injury and Plasticity: Training, Rehabilitation, and Repair: Human

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 386.05/T8

Topic: C.11. Spinal Cord Injury and Plasticity

Support: SHAW Foundation

Title: Pediatric constraint induced movement therapy for children with brachial plexus palsy: Using the Shriners Hospital upper extremity evaluation tool to assess performance

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Abstract: In the past we reported upper and lower extremity function changes following a pediatric constraint induced therapy (pCI) camp for children with hemiplegia. We wondered if pCI can be used for other pediatric populations with upper extremity (UE) deficits. The objectives of this study were to a) assess if pCI can be used to treat children with brachial plexus (BP), b) determine the fesibility of using the Shriners Hospital Upper Extremity Evaluation (SHUEE) to assess functional changes due to treatment.
This is a randomized control study including 17 children with BP, 3-7 years of age, 9 of them randomly assigned in the experimental group (EG). No participant had history of other neuromusculoskeletal injury or pCI exposure. All subjects could use the affected arm as gross assist during play and self care tasks. Cognitively, they could follow two step commands. Treatment took place at a Children's Hospital.
We delivered 30 hours of treatment (3 hours of treatment specific training over 10 days). Activities focused on gross, fine motor and self feeding skills. Control group (CG) participants had traditional occupational therapy (OT). Outcomes were measured using the SHUEE. Participants in EG were tested pre post and six months post pCI. Participants in CG were tested

pre and post 30 hours of treatment (6 months). Discriminant analysis was used to determine if the SHUEE is appropriate for assessing UE function for BP and then results were explored for simplicity and reporting with t-tests ($a<0.05$).
Table 1 presents selected results based on the SHUEE. The discriminant analysis showed perfect discrimination between pre and post pCI, low correlations among the SHUEE components and different Eigen values.
Table 1. Means, standard deviations and p values for selected output.

Parameter	Pre-EG Mean*(SD)*	Post-EG Mean*(SD)*	p	6Post-EG Mean*(SD)*	p	Pre-CG Mean*(SD)*	Post-CG Mean*(SD)*	p
Spontaneous Functional Analysis	*71.6(9.9)*	*86.5(10.3)*	.00	73.4*(13.8)*	.07	68.5*(12.6)*	73.4*(16.7)*	.36
Dynamic Positional Analysis	*62.9(12)*	79.7*(9.1)*	.00	76.2*(8.6)*	.25	69.6*(9.3)*	74.2*(11.1)*	.15

This is, to our knowledge, the first randomized control study investigating the effects of pCI on UE function of children with BP. It is also the first study investigating the feasibility of the SHUEE to assess UE deficits of BP. The results demonstrate clear improvements and retention in UE function. They also suggest superiority of pCI over the traditional OT approach when treating the UE of children with BP. Furthermore, the SHUEE appears to be an appropriate tool for assessing UE function of children with BP.

Disclosures: S. Hsiang: None. **E.C. King:** None.

Poster

386. Spinal Cord Injury and Plasticity: Training, Rehabilitation, and Repair: Human

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 386.06/T9

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Wings for Life
International Foundation for Research in Paraplegia (IRP)
Michel-Adrien Voirol Foundation
Firmenich Foundation
Pictet Group Charitable Foundation
Panacée Foundation
Canton du Valais

Title: Spatiotemporal neuromodulation of the spinal cord combined with robot-assisted training in humans with spinal cord injury (STIMO): Long-term recovery of walking

Authors: *C. G. LE GOFF-MIGNARDOT[1,3], J.-B. MIGNARDOT[1,3], F. B. WAGNER[1,3], M. CAPOGROSSO[6], S. KOMI[1,3], R. DEMESMAEKER[1,3], I. SEÁÑEZ[1], M. VAT[4,7], L. A. MCCRACKEN[1,3], M. CABAN[7,2], A. WATRIN[7], A. ROWALD[1], K. VAN DEN KEYBUS[3], G. EBERLE[3], B. SCHURCH[5], S. CARDA[3], E. PRALONG[3], M. BOLLIGER[8], J. VON ZITZEWITZ[7], M. BAKX[9], R. BUSCHMAN[9], N. BUSE[9], V. DELATTRE[7], T. DENISON[9], H. LAMBERT[7], A. CURT[8], K. MINASSIAN[1], J. BLOCH[4,3], G. COURTINE[1,3,4]
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Abstract: We report preliminary results on the long-term effects of rehabilitation enabled by spatiotemporal neuromodulation of the lumbar spinal cord and a multidirectional gravity-assist on functional recovery in individuals with incomplete spinal cord injury. After surgical implantation of a spinal cord stimulator and personalization of the neuromodulation strategy, individuals followed a 5-month rehabilitation period. Functional improvements were tested using a range of advanced neurobiomechanical recordings, well-established electrophysiological measurements, and standard clinical tests that were repeated monthly. In all participants, gait training led to improvement of motor functions, even in the absence of stimulation. These improvements included improved kinematics and muscle activity during locomotion as well as increases in maximal and finely controlled forces, ASIA motor scores, Walking Index of Spinal Cord Injury and 6 Minute Walk Test. These preliminary results provide encouraging insights into the potential of this combined intervention to augment neural plasticity and functional recovery after SCI.

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drugs, supplies, equipment or other in-kind support); GTX medical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GTX medical. **G. Courtine:** 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.; GTX medical. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); GTX medical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GTX medical. F. Consulting Fees (e.g., advisory boards); GTX medical.

Poster

386. Spinal Cord Injury and Plasticity: Training, Rehabilitation, and Repair: Human

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: Wings for Life, International Foundation for Research in Paraplegia (IRP)
Michel-Adrien Voirol Foundation
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Panacée Foundation
Canton du Valais
Marie-Curie EPFL fellowship program

Title: Spatiotemporal neuromodulation of the spinal cord combined with robot-assisted training in humans with spinal cord injury (STIMO): Immediate recovery of walking

Authors: *F. B. WAGNER[1], J.-B. MIGNARDOT[1,2], C. G. LE GOFF-MIGNARDOT[1,2], M. CAPOGROSSO[3], S. KOMI[1,2], R. DEMESMAEKER[1,2], I. SEÁÑEZ[1], M. VAT[4,5], L. A. MCCRACKEN[1,2], M. CABAN[5,6], A. WATRIN[5], A. ROWALD[1], K. VAN DEN KEYBUS[2], G. EBERLE[2], B. SCHURCH[7], S. CARDA[2], E. PRALONG[4], M. BOLLIGER[8], J. VON ZITZEWITZ[5], M. BAKX[9], R. BUSCHMAN[9], N. BUSE[9], V. DELATTRE[5], T. DENISON[9], H. LAMBERT[5], A. CURT[8], K. MINASSIAN[1], J. BLOCH[4,2], G. COURTINE[1,2,4]
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Abstract: We report the immediate effects of spatiotemporal neuromodulation of the lumbar spinal cord on leg motor control in individuals with incomplete spinal cord injury. This spatiotemporal neuromodulation aims at activating or reinforcing the activity of muscle groups underlying lower limb movements during locomotion. Non-ambulatory Individuals with severe spinal cord injury were surgically implanted with an epidural electrode array. After recovery from the surgery, we personalized the spatial location, temporal structure and stimulation parameters of the electrical spinal cord stimulation strategy. First, we identified electrode configurations able to target specific regions of the spinal cord using personalized computational models and electrophysiological recordings. Second, we identified the optimal temporal structure to activate these spatially selective electrode configurations in order to reproduce the spatiotemporal maps of motoneuron activation underlying locomotion of healthy individuals. Application of these personalized spatiotemporal neuromodulation strategies during overground locomotion with robotic assistance immediately enabled overground walking in completely or partially paralyzed individuals who had suffered a spinal cord injury more than four years ago. These results establish the conceptual and engineering framework to develop neuroprosthetic systems that facilitate walking during activities of daily livingvin paraplegic individuals.

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Poster

386. Spinal Cord Injury and Plasticity: Training, Rehabilitation, and Repair: Human

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 386.08/T11

Topic: C.11. Spinal Cord Injury and Plasticity

Support: New York State Department of Health, Spinal Cord Injury Research Program, Contract C32095GG
New York State Department of Health, Spinal Cord Injury Research Program, Contract C32248GG.

Title: Locomotor training combined with transspinal and transcortical paired associative stimulation reorganizes spinal neural function after spinal cord injury

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Abstract: Spinal cord injury (SCI) disrupts descending supraspinal input to lower limb muscles and results in substantial pathological reorganization of spinal neuronal circuits affecting locomotor performance. It is well known that locomotor training normalizes the function of spinal neuronal circuits after SCI, and that paired associative stimulation (PAS), particularly transspinal-transcortical PAS, can enhance corticospinal excitability in non-injured individuals. However, the effectiveness of augmenting activity-based therapies with non-invasive transspinal-transcortical PAS on the recovery of sensorimotor function after SCI remains largely unexplored. In this study, we delivered paired transspinal and transcortical stimuli at an interstimulus interval in which TMS was delivered after transspinal stimulation in two people, one with chronic motor incomplete SCI (AIS D)and the other with complete SCI (AIS B) during Lokomat robotic gait training. Paired stimuli were delivered during the stance phase of the step cycle, triggered based on foot switches, throughout at least 20 sessions (1h/day, 5 days/week) of locomotor training. For transspinal stimulation, the cathode electrode was placed over T10-L1, and two anode electrodes were placed bilaterally on the abdominal muscles, while for transcortical stimulation a double cone coil was placed over M1. Both stimuli were delivered at intensities that evoked cortical or spinal motor responses in the soleus muscle. Soleus H-reflex excitability during Lokomat walking were assessed before and after intervention. Transspinal-transcortical paired stimulation and locomotor training produced a significant depression of the soleus H-reflex during the stance phase, and promoted soleus H-reflex inhibition during the swing phase of the step cycle. Despite being an ongoing project, the current findings clearly indicate that this novel intervention contributes to normalization of soleus H-reflex phase-dependent modulation in

people with motor incomplete and complete SCI. These findings provide evidence towards a novel rehabilitation paradigm to promote recovery of motor function after SCI.

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Poster

386. Spinal Cord Injury and Plasticity: Training, Rehabilitation, and Repair: Human

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 386.09/T12

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Neurotrauma Research Program of Western Australia project grant, 2016-2017

Title: Time to first urinary tract infection after traumatic spinal cord injury predicts subsequent infection incidence; A Western Australian inpatient cohort study

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Abstract: Introduction: Following traumatic spinal cord injury (SCI), urinary tract infection (UTI) results in high morbidity and is a major cause of re-hospitalisation. Bladder management differs at spinal units around Australia and the relationship between early management and subsequent UTI rates is largely unknown.
Methods: We undertook a retrospective audit of all new cases (n=70) of adult traumatic SCI managed at Royal Perth and Fiona Stanley Hospitals, Jan 2015-Feb 2017. Bladder management practices and UTI incidence were mapped using data from hospital electronic databases, medical records and nursing fluid balance charts. Modelling of infection rates and UTI during nursing-administered intermittent catheterisation (staff-IC) involved generalised linear models.
Results: Protocols for 6-hourly staff-IC were adhered to closely (85.7% of staff-ICs were performed within 6.5 hours, 96.1% within 8 hours; only 3.5% of bladder volumes were >800mL). The rate of symptomatic UTI was 1.1 starts/100 days and symptomatic UTI by multi-resistant organisms 0.1/100 days. Modelling of potential drivers of UTI showed longer duration of urethral indwelling catheterisation (IDC) was associated with shorter time to first infection (p-value 0.044), which in turn predicted a higher UTI rate during inpatient stay (p-value 0.039). During staff-IC periods, high volumes and interruptions requiring IDC led to an increased UTI rate in the following week (odds ratios (ORs)=1.59, 95% CI 1.12-2.27, p-value 0.009 and 2.93,

95% CI 2.58-5.92, p-value <0.001 respectively). Delays between staff-ICs (>8 hours) did not change the UTI rate. Subsequent analysis identified drivers of odds of UTI including: being female (OR=8.7, 95% CI 1.45-52.5, p-value 0.006) and complete injury (OR=12.3, 95% CI 1.28-117.4, p-value 0.008). Hospital stay was longer for those with complete compared to incomplete injuries (rate ratio (RR)=1.31, 95% CI 1.07-1.61, p-value 0.007) and longer for those with UTIs than those without (RR=1.39, 95% CI 1.16-1.69, p-value <0.001). Longer hospital stays were also associated with increased IDC days (p-value <0.001) and pre-existing neurological disorders (p-value 0.011).
Conclusions: Reducing initial IDC duration, preventing early UTIs, minimising high bladder volumes and IC interruptions and targeting specific patient sub-groups may optimise bladder management, reduce UTI rate and reduce hospital length of stay following SCI.

Disclosures: S.A. Dunlop: None. **G. Simpson:** None. **K. Murray:** None. **P. Boan:** None. **A. Watts:** None. **J. Bardsley:** None. **C. Hartshorn:** None. **J. Thavaseelan:** None. **A. Rea:** None. **L.M. Goodes:** None.

Poster

386. Spinal Cord Injury and Plasticity: Training, Rehabilitation, and Repair: Human

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 386.10/T13

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Eurostars

Title: Computational models steering the personalization of targeted spinal cord stimulation to restore motor function in humans with spinal cord injury

Authors: *A. ROWALD[1,2], E. NEUFELD[3], E. PAOLES[4], S. MANDIJA[5], M. FROELING[5], B. LLOYD[3], J. BAKKER[4], K. MINASSIAN[1], F. WAGNER[1], V. DELATTRE[4], C. A. T. VAN DEN BERG[5], N. KUSTER[3], J. BLOCH[6], G. COURTINE[1,6], M. CAPOGROSSO[2]
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Abstract: Epidural Electrical Stimulation (EES) applied over the lumbosacral spinal cord restored voluntary leg movements in animal models and humans with spinal cord injury (SCI). These studies have shown the importance of targeting the proprioceptive afferent fibers located in the individual dorsal roots in order to maximize the efficacy of the stimulation. Humans exhibit a broad range of anatomical features, emphasizing the importance of developing targeted surgical procedures and personalized stimulation protocols to deliver EES therapies in patients

with SCI. To this aim, we developed a computational platform supporting the semi-automatized creation of hybrid computational models of epidural electrical stimulation applied to the human spinal cord using the Sim4Life computational life sciences platform. These models combine personalized, geometrically realistic 3D finite element models of the lumbar and sacral spinal cord of individual patients with realistic neural dynamics models of proprioceptive feedback circuits. For this, we developed ad-hoc high-resolution MRI sequences of the spinal cord, which are reproducible across vendors, that allow the semi-automatic extraction of patient-specific anatomical features, including the 3D trajectories of the dorsal and ventral spinal roots. We established a computational pipeline to obtain (anisotropic) tissue properties maps, discretize the model, perform simulations using an electro-quasistatic solver, couple these simulations with NEURON-based electrophysiology models, determine stimulation selectivity for given electrode configurations, and map these configurations to task-specific motoneuron pool activation profiles. This platform enables the optimal surgical placement of electrode arrays, the personalization of stimulation protocols and even the tailoring of electrode arrays to the specific anatomical features of each patient. We validated this approach in three paraplegic patients with SCI.

Disclosures: A. Rowald: None. **E. Neufeld:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ZurichMedTech. **E. Paoles:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GTX Medical. **S. Mandija:** None. **M. Froeling:** None. **B. Lloyd:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ZurichMedTech. **J. Bakker:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GTX Medical. **K. Minassian:** None. **F. Wagner:** None. **V. Delattre:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GTX Medical. **C.A.T. van den Berg:** None. **N. Kuster:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ZurichMedTech. **J. Bloch:** None. **G. Courtine:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GTX Medical. **M. Capogrosso:** None.

Poster

386. Spinal Cord Injury and Plasticity: Training, Rehabilitation, and Repair: Human

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 386.11/T14

Topic: C.11. Spinal Cord Injury and Plasticity

Title: A novel task to measure lower limb coordination after spinal cord injury and its relation to proprioceptive sense

Authors: *R. N. MALIK[1,2], M. CHOW[1,2], G. EGINYAN[1,2], T. LAM[1,2]
[1]Intl. Collaboration on Repair Discoveries, Vancouver, BC, Canada; [2]Sch. of Kinesiology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Following motor-incomplete spinal cord injury (m-iSCI) community ambulation becomes limited, partly due to the difficulties of skilled walking in our everyday lives. During skilled walking tasks, appropriate modification of the gait pattern requires precise coordination between the joints of the lower limb (i.e. inter-joint coordination). Following SCI, inter-joint coordination can be impaired not only due to motor impairments, but also proprioceptive deficits. In this project, we developed a new task for examining inter-joint coordination in the legs with the aim of understanding the effect of proprioceptive impairments in people with SCI on lower limb coordination.
Both able-bodied individuals and individuals with SCI were recruited. Lower limb proprioceptive sense and clinical measures of skilled walking were determined in the subjects with SCI. All subjects then completed a lower limb pointing task where they aimed their toe from a "home position" to one of three possible targets. Each target required varying degrees of inter-joint coordination and the excursion of the leg to each target was normalized to every individual's active range of motion. The pointing task was completed under two visual conditions, full and obstructed vision. In the full vision condition, vision of the lower limbs was unobstructed, and participants received knowledge of performance after every trial. In the obstructed vision condition, vision of the lower limbs was blocked, and subjects did not receive knowledge of performance. Motion capture markers were used to determine foot trajectory, as well as hip, knee and ankle angles. From the kinematic data, end-point (toe) accuracy and inter-joint coordination, quantified by joint angle-angle plots, were extracted.
Individuals with SCI had deficits in lower limb proprioceptive sense and displayed poor end-point accuracy when pointing to the different targets, which was particularly exacerbated in the obstructed vision condition. Lower limb proprioceptive sense was related to lower limb coordination such that greater proprioceptive deficits was related to poor coordination. Able-bodied individuals on the other hand were able to accurately point towards all targets while displaying appropriate coordination patterns. There results show that our new task is well suited for measuring lower limb coordination and provides a new approach for future studies to examine how lower limb coordination affects community ambulation.

Disclosures: R.N. Malik: None. **M. Chow:** None. **G. Eginyan:** None. **T. Lam:** None.

Poster

386. Spinal Cord Injury and Plasticity: Training, Rehabilitation, and Repair: Human

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 386.12/T15

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Canadian Institute of Health Research
International Collaboration On Repair Discoveries

Title: Improvements in lower limb coordination following locomotor resistance training is related to improvements in skilled walking function, but not walking speed

Authors: *G. EGINYAN[1,2], R. N. MALIK[1,2], A. K. LYNN[1,2], T. LAM[1,2]
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Abstract: Introduction: Following motor-incomplete spinal cord injury (m-iSCI), functional ambulation is reduced partly due to the skilled walking requirements of everyday life. Our laboratory previously showed that body weight supported treadmill training (BWSTT) with Lokomat-applied resistance (Loko-R) led to greater improvements in skilled walking capacity compared to conventional Lokomat assisted (Loko-A) BWSTT. Following SCI, coordination can be impaired, which may contribute to the reduced ambulatory capacity among these individuals. The aim of this project was 1) to determine whether there were improvements in intra-limb coordination following Lokomat training, and 2) whether these improvements corresponded with improved functional walking capacity in individuals with m-iSCI. **Methods:** Nine individuals with chronic m-iSCI were randomly assigned to BWSTT with Loko-R (n=6) or Loko-A (n=3) groups and underwent a 3-month training intervention. Clinical measures of skilled walking and walking speed were conducted before and after training. Lower limb joint kinematics during treadmill walking with BWS was recorded at baseline and post-training. Motion capture markers were used to determine, hip, knee and ankle angles. Joint angle-angle plots were then created to quantify intra-limb coordination. Changes in intra-limb coordination before and after training for each subject was determined by comparing joint angle-angle plots against normative able-bodied data that was matched for speed and percentage of body weight supported.
Results and conclusion: Following training, both groups showed improvements in walking speed, but only individuals in the Loko-R group showed improvements in skilled walking. After Loko-R training most individuals showed improvements in intra-limb coordination which was related to skilled walking function, but not walking speed. We found that individuals with SCI whose coordination patterns became more similar to able-bodied controls after training had greater improvements in skilled walking function. A larger scale study needs to be conducted to further illustrate the differential effects of Loko-R and Loko-A training, and to determine the

effectiveness of Loko-R training to improve lower limb coordination and community ambulation following SCI.

Disclosures: G. Eginyan: None. **R.N. Malik:** None. **A.K. Lynn:** None. **T. Lam:** None.

Poster

387. Spinal Cord Injury and Plasticity: Neurophysiology II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 387.01/T16

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant R01NS076589-01
NIH Grant R01NS090622-01
VA Grant I01RX000815
VA Grant I01RX001807

Title: Corticospinal and reticulospinal contributions to spasticity after human spinal cord injury

Authors: *S. SANGARI[1,2], M. A. PEREZ[1,2]
[1]Dept. of Neurolog. Surgery, The Miami Project to Cure Paralysis, Univ. of Miami, Miami, FL; [2]Bruce W. Carter Dept. of Veterans Affairs Med. Ctr., Miami, FL

Abstract: Animal models of spinal cord injury (SCI) suggest that the corticospinal and reticulospinal tract contribute to the development of spasticity. The extent to which these descending motor pathways are involved in spasticity in humans with SCI remains poorly understood. We examined the contribution of the corticospinal pathway by measuring input-output motor evoked potential (MEP) recruitment curves in the rectus femoris muscle elicited by transcranial magnetic stimulation over the leg motor cortex. The contribution of the reticulospinal tract was explored by using the StartReact response measuring reaction time from the rectus femoris electromyographic activity during isometric knee extension in the presence of a startle acoustic stimuli. Experiments were performed in adults with chronic incomplete SCI with different degrees of spasticity and aged-matched controls. We found that SCI participants with severe spasticity exhibited smaller MEP-max and shorter reaction time during a startle stimuli compared with those with lesser spasticity and uninjured controls. SCI participants with severe spasticity also showed smaller maximal voluntary contractions compared with those with lesser spasticity and control subjects. Our results indicate balanced contributions of both corticospinal and reticulospinal pathways in incomplete SCI participants with spasticity. Thus, interactions between these residual supraspinal motor pathways might be critical for understanding the mechanisms of spasticity.

Disclosures: S. Sangari: None. **M.A. Perez:** None.

Poster

387. Spinal Cord Injury and Plasticity: Neurophysiology II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 387.02/T17

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Emory CMBC Interdisciplinary Neuroscience Pilot Research Fund

Title: Identifying cellular dynamics in mouse sympathetic neurons: A computational modeling approach

Authors: *K. TIAN[1], M. L. MCKINNON[2], S. HOCHMAN[2], A. A. PRINZ[3]
[2]Physiol., [3]Biol., [1]Emory Univ., Atlanta, GA

Abstract: Sympathetic postganglionic neurons (SPNs), located in the sympathetic ganglion chain, pass converged motor commands from the spinal cord to the downstream muscles and visceral organs. SPNs in the thoracic region of the sympathetic nervous system (tSPNs) regulate vasoculature and dysfunction of tSPNs is implicated in various autonomic disorders such as autonomic dysreflexia, yet because of experimental difficulties, little is known about the cellular mechanisms that control the excitability of tSPNs. By combining eletrophysiological data with computational modeling, we built the first physiologically-realistic single neuron model of tSPNs in mice, and elucidated several cellular mechanisms that govern tSPN dynamics. This model reproduced all the essential features of tSPNs *ex vivo* with eight types of ionic currents, which are a fast sodium current (I_{Na}), a delay-rectified potassium current (I_{Kd}), a slow and non-inactivating potassium current (I_M), a calcium-dependent potassium current (I_{KCa}), a fast transient potassium current (I_A), a persistent calcium current (I_{CaL}), a hyperpolarization-activated inward current (I_h), and a leak current (I_L). Among other results, we found that the post-inhibitory rebound that has been observed in tSPNs *ex vivo* was induced by I_{Na} and I_{Kd} rather than the T-type calcium current. We also found that both I_M and I_{KCa} were necessary and sufficient to replicate tSPN spike rate adaptation. To conduct a more comprehensive and rigorous examination of the range of tSPN cellular dynamics and responses to various synaptic inputs, we employed an ensemble modeling approach [1-2] to build a database of physiologically-realistic tSPN models. We further analyzed the pairwise correlations between ionic currents using both Pearson's correlation and mutual information. Simulation and analysis were written in Python 2.7.10 and executed on the Neuroscience Gateway Portal [3]. The database was parallelized with the SCOOP module. Overall, we built the first physiologcally-realistic single neuron model and database of tSPNs, which lays the foundation to examine both the recruitment principles of synaptic inputs at tSPNs and the role tSPNs play in autonomic disorders in the future.
Acknowledgement This work is supported by the CMBC Interdisciplinary Neuroscience Pilot Research Fund at Emory University.

Reference 1. Prinz AA (2010) Biological Sciences 365:2397-2405. 2. Gao P, Ganguli S (2015) Curr Opin Neurobiol 32:148-155. 3. S Sivagnanam, A Majumdar, K Yoshimoto, V Astakhov, A Bandrowski, M. E. Martone, and N. T. Carnevale. Introducing the Neuroscience Gateway, IWSG, volume 993 of CEUR Workshop Proceedings, CEUR-WS.org, 2013

Disclosures: K. Tian: None. **M.L. McKinnon:** None. **S. Hochman:** None. **A.A. Prinz:** None.

Poster

387. Spinal Cord Injury and Plasticity: Neurophysiology II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 387.03/T18

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH/NIBIB 1P41EB018783

Title: Raising the threshold: A perturbation protocol to improve non-stepping responses to rapid surface translations

Authors: *J. H. BARNES[1,2], A. EFTEKHAR[1], T. T. FAKE[1], C. S. CARMACK[1], J. R. CRENSHAW[2], J. R. WOLPAW[1,3]
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Abstract: The ability to maintain upright stance despite disturbances is a motor skill and as such should improve with practice. While training protocols that perturb standing balance by rapidly translating the support surface have been used to reduce the likelihood of falls in at-risk populations (e.g., older adults), their underlying mechanisms are not fully understood. By investigating motor learning in younger, unimpaired, individuals we can remove potential confounds of age and morbidity, however the degree to which this type of balance reaction is modifiable in high-functioning groups is unknown. To test this we developed a protocol that challenges younger healthy individuals to produce non-stepping responses to progressively larger disturbances. Each participant stands on a Bertec split-belt treadmill while perturbations (rapid surface translations) are triggered at random times by custom software. Each perturbation lasts ~600-700 ms and has a trapezoid-shaped velocity profile. Acceleration and deceleration phases are 200 ms each. Difficulty levels are defined by acceleration and deceleration rates that are equal in absolute value and increase in increments of 0.25 m/s^2. A tachometer is used to ensure perturbations reach target peak velocity. Limitations in treadmill instrumentation produce variability in time at peak velocity; this causes perturbation displacements to overlap across levels and challenges participants to respond to disturbances that vary differentially (i.e., those equal in acceleration may vary in displacement and vice versa). A successful response is defined as maintaining balance without stepping. Participants begin at a low level and move up one level

at a time until stepping occurs in 3 consecutive trials. This is defined as their stepping threshold. Then, over the course of 6 training sessions, each comprising 50-75 perturbations, participants are challenged to raise their threshold. They start one level below it, progress to the next higher level after 3 successful responses, and transition down a level after an observed step. A motion-capture system records the perturbation and the kinematics of the participant's response; and electromyography (EMG) is recorded from ankle flexor and extensor muscles bilaterally. Further, at the beginning of each session, right and left soleus H-reflexes are recorded during standing. Initial data suggest that healthy individuals can learn to respond to faster and larger perturbations without stepping. Ongoing studies seek to confirm this preliminary result, to define the associated kinematic and EMG changes, and to explore the possibility that reflex function is affected.

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Poster

387. Spinal Cord Injury and Plasticity: Neurophysiology II

Location: SDCC Halls B-H

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Program #/Poster #: 387.04/U1

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant R01NS076589-01
NIH Grant R01NS090622-01
VA Grant I01RX000815
VA Grant I01RX001807

Title: Bilateral asymmetries in spasticity following spinal cord injury

Authors: *B. CHEN[1,2], J. LORENTZEN[3], J. B. NIELSEN[3], M. A. PEREZ[1,2]
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Abstract: Spasticity is one of the most common symptoms present in humans with spinal cord injury (SCI). Although SCI mostly result in bilateral damage of neuronal pathways, the extent to which spasticity is affected on both sides of the body remains unknown. The aim of our study was to assess ankle plantar flexor stiffness in adults with and without chronic SCI bilaterally. We performed slow and fast dorsiflexion stretches of the ankle joint to measure the range of motion (ROM), passive stiffness, and reflex mediated stiffness bilaterally using a dynamometer. We found that passive ROM was reduced in both sides of SCI participants compared with control subjects. Passive stiffness between 30 to 50% of the dorsiflexion ROM was increased in one side

more than the other in SCI participants and remained similar in controls. The joint angle at which the stretch reflex was elicited was larger in controls compared with SCI participants. In addition, the total torque and reflex induced torque was larger in one side in SCI participants and remained similar in controls. Our results indicate that symptoms of spasticity in ankle plantarflexor muscles are asymmetrically present in humans with chronic SCI.

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Poster

387. Spinal Cord Injury and Plasticity: Neurophysiology II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 387.05/U2

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH (NIGMS) Institutional Development Award (IDeA) U54-GM104941 (Binder-MacLeod)
South Carolina Spinal Cord Injury Research Fund

Title: Corticospinal excitation and inhibition for the ankle dorsiflexor tibialis anterior: A mapping study

Authors: *R. COTE[1], A. K. THOMPSON[2]
[1]Col. of Hlth. Professions, [2]Dept. of Hlth. Sci. and Research, Col. of Hlth. Professions, Med. Univ. of South Carolina, Charleston, SC

Abstract: The corticospinal tract (CST) is important in motor control, motor skill learning, and re-learning after CNS injury. While it has become increasingly common to measure motor evoked potential (MEP) to transcranial magnetic stimulation (TMS) as a measure of corticospinal excitability and plasticity, its relation to the silent period (SP; suppression of ongoing EMG, reflecting corticospinal inhibition) after MEP or cortical map representation of the SP are less known. Thus, to better understand the corticospinal excitation and inhibition, we are currently examining cortical maps and recruitment curves of MEP and SP for the tibialis anterior (TA), using two different coils.
All TMS trials occur while the subject is seated in a chair with a leg fixed in a custom-made apparatus with ankle, knee, and hip joint angles fixed at ≈100, ≈120, and ≈110°, respectively. TMS is performed using Magstim 200^{-2} and a 110 mm double-cone coil or a custom-made bat-wing coil with radii of 9 cm, held over the scalp such that the induced current flows in the posterior–anterior direction in the brain. When the absolute TA EMG level is maintained at ≈15% MVC, an MEP is elicited at an interval of 5-6 seconds between stimuli. To determine the stimulus intensity to be used for mapping, an MEP recruitment curve is measured at a tentative optimum location typically identified near the vertex. Then, using the TMS intensity that

produces a half-maximal response at this tentative location, MEP mapping is performed over -3-to-+3 cm anterior and -1-to-+4 cm contralateral to the vertex. Four stimuli are applied at each location. After mapping, an MEP recruitment curve is obtained at the optimum location. For each stimulus location and intensity, the end of SP is determined as the recovery of background EMG activity in 50% of the responses.
Our initial results suggest that the double-cone coil and batwing coil do not produce the same MEP or SP maps. While the MEP hotspot for TA can be identified using either coil, the location differs between the coils by ≈1.69±0.78 cm. With the batwing coil the SP duration differs across stimulus locations and the SP hotspot is identifiable. In contrast, with the double-cone coil, the map distribution of SP duration is diffused, indicating a broader recruitment of inhibitory interneurons. Although the MEP and SP maps are not identical, there is a positive correlation between the MEP size and the SP duration across the map locations. This supports a possibility that inhibitory neurons responsible for producing the SP may be closely located to CST neurons for a given muscle in the cortex. To further understand the MEP- SP relation, additional analyses are currently underway.

Disclosures: A.K. Thompson: None.

Poster

387. Spinal Cord Injury and Plasticity: Neurophysiology II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 387.06/U3

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH (NIGMS) Institutional Development Award (IDeA) U54-GM104941 (Binder-MacLeod)
NS069551 (NINDS)
South Carolina Spinal Cord Injury Research Fund

Title: Chronic stability of soleus stretch reflexes during standing in neurologically normal adults

Authors: *A. OLIVIER[1], A. LUNDGAARD[5], K. ORITA[2], B. A. POULIOT[3], J. B. ANDERSEN[5], A. K. THOMPSON[4]
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Abstract: People can learn to change spinal reflexes through operant conditioning. Previous work shows that successful reflex operant conditioning changes not only the reflex pathway that is conditioned but also the activity of other spinal and supraspinal pathways. In order to further understand the mechanisms of this plasticity, we are currently investigating the chronic stability

of the stretch reflexes in the soleus during standing, as well as the tolerability of repeated ankle joint rotation. Stretch reflexes are affected by fusimotor control of muscle spindle sensitivity. Unlike electrical nerve stimulation that elicits the H-reflex, excitation of spindle afferents by muscle stretch is temporally dispersed and may result in activating motoneurons via several different spinal and supraspinal pathways. In humans, the soleus stretch reflexes usually appear in a series of three components: spinal short-latency "M1" (mainly Ia afferent origin), spinal long-latency "M2" (presumably mainly II afferent mediated), and the long latency "M3" (suggested to be transcortical or subcortical).
Healthy adults with no known neurological conditions are exposed to 30 stretch reflex sessions over 10 weeks (3/week). In each session, the subject completes an H-reflex/M-wave recruitment curve measurement, 1 block of 20 submaximal H-reflex trials, and 3 blocks of 75 stretch reflex trials while s/he stands on the custom ankle joint-rotation device (JAKraft, Aalborg University, Denmark). A reflex trial occurs when the subject has maintained a preset level (i.e., natural standing level) of background soleus EMG activity for at least 2 s and at least 5 s has passed since the last trial. To elicit stretch reflexes, a fixed amount (6 or 12°) of dorsiflexion rotation is applied at a fixed initial speed (125°/s or 170°/s). For a given subject, these stretch parameters are maintained throughout the study.
To date, four subjects have completed the 30 reflex sessions. In these subjects, amplitudes of the soleus maximum M-wave and H-reflex, submaximal H-reflex, soleus and tibialis anterior background EMG, M1 and M2 reflexes did not change systematically across sessions ($p>0.05$ for all, by repeated measures ANOVA), indicating the stability of the H- and stretch reflexes over an extended period of time (i.e., 10 weeks). All subjects have well tolerated either amount or speed of joint rotation, and preferred mechanical perturbation (stretch reflex elicitation) over electrical stimulation (H-reflex elicitation). These initial results support the feasibility of operantly conditioning the soleus stretch reflex during standing in people with and without neurological disorders.

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Poster

387. Spinal Cord Injury and Plasticity: Neurophysiology II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 387.07/U4

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant NS102871
DOD Grant SC130225

Title: Plasticity of thoracic sympathetic post-ganglionic neuron after spinal cord injury

Authors: *Y. LI, M. L. MCKINNON, M. HALDER, S. HOCHMAN
Emory Univ., Atlanta, GA

Abstract: Autonomic dysreflexia (AD) is a hypertensive crisis seen in high-thoracic spinal cord injured (SCI) patients normally triggered by noxious stimuli below injury. Previous animal research on mechanisms underlying AD have mostly focused on neural plasticity within the injured spinal cord. No studies have assessed whether there is also plasticity in the sympathetic post-ganglionic neurons - the final neural element responsible for vasoconstriction.
Thoracic sympathetic post-ganglionic neurons (tSPNs) are located in thoracic paravertebral ganglia that provides dominant sympathetic control of vasculature. Their cellular properties are understudied due to their anatomical difficulty. The few previous *ex vivo* studies all used sharp electrodes that likely leads to significant impalement injury-induced changes. Here, we undertook whole-cell patch clamp recordings in an adult mouse tSPN *ex vivo* preparation, in which the thoracic sympathetic post-ganglionic chain was isolated and perfused in artificial CSF at room temperature. Compared to earlier reports, we observed order of magnitude greater values for passive membrane properties (cell resistance and membrane time constant) and repetitive rather than phasic firing properties (n=39, 100% of recorded tSPNs). These observations support greater ability for synaptic integrative actions and stronger output than previously assumed.
Using the same methodology, we then studied whether these neurons undergo plastic changes after high-thoracic (T2) spinal cord transaction in the young adult mouse as a model of AD. Recordings were obtained from T3-T9 ganglia at different time points after SCI. Five days after SCI, resting membrane potential (RMP) was 10mV more hyperpolarized (n=6). Interestingly, in response to depolarizing current injection, 3/6 tSPNs were inexcitable, 2/6 fired phasically, with only one neuron capable of repetitive firing. At both 3 weeks (n=5) and 6 weeks (n=13) post-SCI, mean tSPN RMP and capacity for repetitive firing returned to that in naïve animals. Notably, at 3 weeks one tSPN showed spontaneously period of single channel activities and maintained bouts of spontaneous synaptic activity that lead to large shifts in membrane potential. By 6 weeks, these bouts of spontaneous activity and associated membrane potential shifts were seen 6/13 neurons. Together, these results highlight the temporal changes in tSPN properties that emerge after SCI.

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Poster

387. Spinal Cord Injury and Plasticity: Neurophysiology II

Location: SDCC Halls B-H

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Program #/Poster #: 387.08/U5

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH NIBIB EB018783

NYS SCIRB DOH01-C32236GG

Title: Towards operant conditioning of the flexor carpi radialis: Methods and initial results

Authors: *J. NORTON, A. EFTEKHAR[1], S. HECKMAN[1], J. H. BARNES[1], L. MCCANE[1], J. R. WOLPAW[1,2]
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Abstract: Operant conditioning of the H-reflex (electrical analog of the spinal-stretch reflex) is a promising new therapeutic intervention for those with motor dysfunction following stroke or spinal cord injury (e.g., Thompson et al. J Nsci, 2013). To extend H-reflex operant conditioning to the upper extremity, we are developing a methodology for operant conditioning of the H-reflex of the forearm muscle flexor carpi radialis (FCR). Because the conditioning protocol comprises 30 1-hr sessions over 10 weeks, signal quality (amplitude and signal-to-noise ratio [SNR]) and signal stability are critical. Here we compare the quality and stability of H-reflexes and M-waves (direct muscle responses) obtained with alternative forearm positions and electrode locations over multiple study sessions.
Data were recorded during 5 sessions over two weeks from 7 total participants. The participant sat in a comfortable chair with their right forearm supported on a table. We collected data with two different arm positions and two different reference electrode locations: condition one (CND1) (forearm fully supinated; an EMG electrode pair (3cm separation) over the muscle belly); and condition two (CND2) (forearm at neutral position with some external rotation of the upper arm; one EMG electrode over the muscle belly and the other over the tendon). FCR H-reflexes were elicited by digitally controlled 0.5-ms biphasic median-nerve stimulation (Digitimer DS5) while the participant maintained a defined level of FCR muscle activation (5-10% of M-wave$_{max}$) guided by feedback on a video screen. In the first session, stimulation and recording electrode locations were determined for each participant. During subsequent sessions, the same stimulation and recording sites were used to elicit and measure M-wave and H-reflex recruitment curves.
Across participants and sessions, H-reflex (~1100%) and M-wave (~375%) amplitudes were larger in CND2. The H-reflex SNR also appears (~260%) larger in CND2, while the coefficients of variation appear smaller (CND1 - 0.15 M-wave, 0.33 H-reflex; CND2 - 0.08 M-wave, 0.19 H-reflex).
Based on these results, we are now proceeding with FCR operant conditioning using the positioning and electrode locations of CND2. We expect that CND2 will provide H-reflex conditioning equal to, and perhaps better than, that previously obtained with CND1. If the new protocol is validated, we plan to assess the ability of FCR H-reflex conditioning to enhance recovery of hand/arm function after stroke.

Disclosures: J. Norton: None. **A. Eftekhar:** None. **S. Heckman:** None. **J.H. Barnes:** None. **L. McCane:** None. **J.R. Wolpaw:** None.

Poster

387. Spinal Cord Injury and Plasticity: Neurophysiology II

Location: SDCC Halls B-H

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Program #/Poster #: 387.09/U6

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH/NIBIB P41EB018783

Title: Molecular basis of H-reflex operant conditioning: Methods development

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Abstract: The spinal stretch reflex (or its electrical analog, the H-reflex) represents one of the simplest mammalian behaviors. When appropriately rewarded, animals and people can produce long-lasting increase or decrease in H-reflex size. Thus, operant conditioning of the H-reflex constitutes a novel model for studying the mechanisms of neuronal plasticity involved in the acquisition of a new motor skill. Previous studies from our laboratory have shown that plasticity involved in H-reflex conditioning requires hierarchical control at multiple levels in the brain and spinal cord that mediate learning of a new behavior while maintaining previously acquired skills. This work has implicated cortically controlled spinal GABAergic interneurons and their receptors on motoneurons in the induction and maintenance of H-reflex change after operant conditioning. However, the molecular mechanisms that mediate H-reflex conditioning remain largely uncharacterized. Recent advances in next-generation genomic technologies make novel approaches possible to determine the molecular factors that mediate adaptive plasticity associated with H-reflex conditioning. This study describes the development of RNA-Seq methods that will be used to determine genome-wide transcriptional changes due to H-reflex conditioning. This preliminary work has focused on laser-captured motoneurons and the surrounding grey matter in control animals to establish sample variance and best practices for tissue collection and signal amplification. We were successful in isolating highly purified motor neuron pools from soleus muscle that, when compared to distinct tissue sources such as white matter from the same spinal cord, show minimal expression of glial cell markers (e.g. GFAP) and enrichment for neuronal genes (e.g. FoxP1). Within-sample variance is currently being optimized to increase the sensitivity and overall reproducibility of this system. Our goal is to determine cell-level effects before, during, and after H-reflex conditioning using a combination of gene-set enrichment approaches and correlations with phenotype outcomes (e.g., H-reflex increase/decrease, successful conditioning). These data will provide the molecular basis for this

simple learning paradigm and generate new hypotheses to ultimately utilize this approach in clinical therapy.

Disclosures: B. Herron: None. **J.S.W. Carp:** None. **Y. Wang:** None. **X.Y. Chen:** None. **J.R. Wolpaw:** None.

Poster

387. Spinal Cord Injury and Plasticity: Neurophysiology II

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH/NIBIB 1P41EB018783
NYS SCIRB DOH01-C32236GG-3450000
NCNM4R Pilot Grant, NIH P2CHD086844

Title: Multi-electrode arrays for automatic selection of recording and stimulation sites in spinal reflex operant conditioning protocols

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Abstract: Operant conditioning protocols that target beneficial plasticity to spinal reflex pathways can improve walking in rats and people with incomplete spinal cord injury (SCI) (PMC38620 & PMC3579496). Clinical translation of these protocols requires that the current complex lab system for operant conditioning be converted into a simple robust system for clinical therapists. One of the most demanding aspects of the current system is selection of nerve stimulation and EMG recording sites that provide EMG from the targeted muscle and an M-wave/H-reflex recruitment curve that enables stimulation that elicits a small M-wave and an H-reflex on the rising phase of H-reflex recruitment. Furthermore, these sites must be reliably re-located in subsequent treatment sessions. At present, these tasks require a highly trained person and substantial time. We explored the use of a multi-electrode array and automatic site selection algorithms to reduce the training and time requirements. H-reflex/M-wave recruitment curves for flexor carpi radialis (FCR) (median nerve (cubital fossa) stim) and soleus (posterior tibial nerve stim) were elicited and recorded with electrode arrays (OT Bioelettronica). FCR participants sat with right arm supinated and supported at a 90̊ angle in the sagittal plane; soleus participants were tested in a natural standing posture. EPOCS, a BCI2000-based operant conditioning program (PMID: 15188875), and a Digitimer D188 multiplexer connected to a DS5 stimulator

automatically cycled through stim sites. One-ms (soleus) and 0.5-ms (FCR) bi-phasic square pulses occurred at ≥5-s intervals while the person maintained a given EMG level (~5-10% maximum voluntary contraction). Recruitment curves were constructed by averaging 4 pulses for each stim levels from below H-reflex threshold to Mmax (~5-30 mA). EMG was digitized at 3200 Hz (bandpass 1-1000 Hz). EMG data were automatically analyzed to find the stim site that gave a maximum H-reflex response with the least current. Initial results from 3 people were very similar to those obtained with the lab system by expert operators. Multi-electrode array data were also useful for identifying and reducing artifacts and for separating overlapping artifact, M-wave, and H-reflex responses. Multi-electrode arrays with automated algorithms should reduce the expertise and time needed to locate and re-locate electrode sites and build recruitment curves. These arrays and algorithms are an important step toward the robust clinical system essential for widespread dissemination of spinal reflex operant conditioning as a new therapy that can enhance functional recovery for people with SCI and other disorders.

Disclosures: A. Eftekhar: None. **J.J.S. Norton:** None. **S.M. Heckman:** None. **A.K. Thompson:** None. **J.R. Wolpaw:** None.

Poster

387. Spinal Cord Injury and Plasticity: Neurophysiology II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 387.11/U8

Topic: C.11. Spinal Cord Injury and Plasticity

Support: VA P01 HD32
NIH HD36020
NS22189
NS061823
HD032571
NIBIB/P41EB018783

Title: Soleus H-reflex up-conditioning promotes H-reflex recovery and improves locomotor kinematics in rats during or after regeneration of a transected sciatic nerve

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Abstract: Operant conditioning of the spinal stretch reflex or its electrical analog, the H-reflex (HR), changes the brain and spinal cord (Curr Opin Behav Sci 20:138-144, 2018 for review). In rats and humans with incomplete spinal cord injury, appropriate reflex conditioning improves locomotion (J Neurosci 26:12537-12543, 2006 & 33:2365-2375, 2013). Reflex conditioning can

help restore spinal reflexes after nerve injury and regeneration (J Neurosci 30:16128-16136, 2010). This study explores in rats with sciatic transection the impact of soleus H-reflex conditioning during or after sciatic regeneration on HR and locomotion.
Sprague-Dawley rats are implanted with EMG electrodes in the right soleus and a stimulating cuff on the right posterior tibial nerve. After control data collection, the right sciatic nerve is transected (TX) and repaired. Data collection continues for 20 (TC_{120}, TU_{120}, TD_{120} rats) or 120 (TC_{220}, TU_{220} rats) more days. The rat is then exposed for 100 days to: continued control data collection (TC_{120}, TC_{220}); soleus HR up-conditioning (TU_{120}, TU_{220}) or down-conditioning (TD_{120}). Locomotor EMG, HR, and kinematics are assessed before transection, and 120 (TU_{120},TC_{120},TD_{120}) or 220 (TC_{220},TU_{220}) days after transection.
In 6 TU_{120}, 6 TD_{120}, and 7 TC_{120} rats, the final soleus protocol HR (HR during conditioning) averaged 47(10 SEM)%, 24(7)%, 17(3)%, respectively, of pre-transection value (p=0.01, 0.06, 0.78: TU vs TC, TU vs TD, TD vs TC by ANOVA & Tukey Test). In 6 TU_{220} and 7 TC_{220} rats, final HR averaged 47(11)% and 9(3)% (p=0.0040).
In 6 TU_{120} rats, step symmetry (normal 100) before TX (preTX), and 20 days (TX20) and 120 days (TX120) after TX, averaged 100.9(1.1SEM), 69.1(2.9), 95.2(6.7), respectively, ($p<0.001$, =0.61, =0.002: PreTX vs TX20, PreTX vs TX120, TX120 vs TX20); in 6 TD_{120} rats, it averaged 100.4(±1.0), 72.4 (2.0), 86.3(5.3) ($p<0.001$, =0.02, =0.03: PreTX vs TX20, PreTX vs TX120, TX120 vs TX20); in 7 TC_{120} rats, it averaged 99.8(0.4), 70.5 (6.4), 76.2(6.6) (p=0.003, 0.02, 0.74: PreTX vs TX20, PreTX vs TX120, TX120 vs TX20). In 7 TU_{220} rats, preTX, TX120, and TX220, it averaged 99.8(0.4), 76.2 (6.6), 84.0(5.0) (p=0.007, 0.08, 0.50: PreTX vs. TX120, PreTX vs TX220, and TX220 vs TX120); in 7 TC_{220} rats, it averaged 100.5(0.5), 91.5 (1.1), 89.4 (1.7) ($p<0.001$, <0.001, =0.44; PreTX vs TX120, PreTX vs TX220, TX220 vs TX120).
HR up-conditioning during or after sciatic regeneration improves HR recovery and locomotor symmetry. HR conditioning is a novel therapy that might complement standard rehabilitation methods and enhance functional recovery after peripheral nerve injury and regeneration.

Disclosures: L. Chen: None. **Y. Chen:** None. **X. Yang:** None. **Y. Wang:** None. **J.R. Wolpaw:** None. **X.Y. Chen:** None.

Poster

387. Spinal Cord Injury and Plasticity: Neurophysiology II

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: VA P01 HD32
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NS22189
NS061823

HD032571
NIBIB/P41EB018783

Title: Combining H-reflex conditioning and locomotor training appears to enhance locomotor recovery in rats with incomplete spinal cord injury: Initial results

Authors: *X. Y. CHEN[1], L. CHEN[1], X. YANG[1], Y. WANG[1], Y. CHEN[1], J. R. WOLPAW[1,2]
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Abstract: Operant conditioning of the spinal stretch reflex or its electrical analog, the H-reflex (HR), changes the brain and spinal cord (Curr Opin Behav Sci 20:138-144, 2018 for review). In rats and humans with incomplete spinal cord injury, appropriate reflex conditioning improves locomotion (J Neurosci 26:12537-12543, 2006 & 33:2365-2375, 2013). We are exploring the impact of combining H-reflex conditioning with locomotor training (an established therapy) in rats after spinal cord injury.

Under anesthesia, female Sprague-Dawley rats are implanted with EMG recording and nerve stimulating electrodes and receive a right spinal cord lateral column (LC) transection at T9. Twenty days later, each rat is exposed to a 60-day intervention of either right soleus H-reflex (HR) up-conditioning combined with 5 days/wk locomotor training (CB rats) or locomotor training alone (LT rats). Locomotor EMG, HR, and kinematics are assessed (during treadmill walking and horizontal ladder crossing) before and after LC transection, and at the end of the 60-day treatment.

To date, we have completed studies in 3 CB and 3 LT rats. Final soleus protocol H-reflex, locomotor H-reflex, and locomotor burst area after treatment in the CB rats averaged 203(±39 SEM)%, 220(±90SE)%, and 128(±10SE)%, respectively, of their pre-exposure values; in the LT rats, they averaged 101(±8)%, 105(±7)%, and 129(±17)%, respectively. Before LC transection, step symmetry (normal 100) averaged 101.9(±0.78SE) in CB rats and 99.6(±0.26) in LT rats ($p=0.83$ by ANOVA followed by Tukey test); after LC transection and before treatment, it averaged 90.8(±2.6) in CB rats and 88.4(±1.1) in LT rats ($p=0.81$); After treatment, it averaged 99.1(±1.6) in CB rats and 91.1(±0.54) in LT rats ($p=0.018$). Before LC transection, footfalls during horizontal ladder crossing averaged 0.33(±0.19SE)/crossing in both CB and LT rats; after LC transection and before treatment, it averaged 8.44(±0.19)/crossing for CB rats and 6.00(±1.33)/crossing for LT rats ($p=0.36$ by t-test); after treatment, it averaged 3.67(±0.19)/crossing in CB rats and 6.50(±0.09)/crossing in LT rats ($p=0.014$ by t-test).

In addition, in CB rats, up-conditioning increased the H-reflex more quickly and more than it did in 77 uninjured normal rats and 16 rats with spinal cord LC injury up-conditioned in all previous studies to date.

These initial results suggest that, in rats with unilateral LC transection, the combination of H-reflex up-conditioning and locomotor training is more effective than locomotor training alone (and may be more efficient that H-reflex conditioning alone). The additional studies needed to confirm these early results are underway.

Disclosures: L. Chen: None. **X. Yang:** None. **Y. Wang:** None. **Y. Chen:** None. **J.R. Wolpaw:** None.

Poster

387. Spinal Cord Injury and Plasticity: Neurophysiology II

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Program #/Poster #: 387.13/U10

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NIGMS Institutional Development Award (IDeA) U54-GM104941 (Binder-MacLeod)
NIGMS P20GMI09040-02 (Kautz) NIBIB 1P41EB018783 (Wolpaw).

Title: Operant conditioning of the soleus H-reflex in people with and without chronic CNS injury

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Abstract: People with chronic CNS injuries often suffer disabilities associated with spasticity and weak voluntary muscle contraction, even after conventional therapy. Abnormal spinal reflexes commonly contributes to these problems. In rats and people, an operant conditioning protocol can restore more normal reflexes and can thereby trigger wider plasticity that improves locomotion; benefits persist after conditioning ends (Neuroscientist 2014:21:203-215). To further understand the conditioning-induced plasticity, we are comparing the time courses of H-reflex change among people with no CNS injury, with chronic incomplete spinal cord injury (SCI), and with stroke.
The conditioning protocol comprises 6 baseline and 24 (in people without CNS injury) or 30 (in people with SCI or stroke) conditioning sessions (3/wk). In each baseline session, 225 control H-reflexes are elicited without any feedback on H-reflex size. In each conditioning session, 20 within-session control H-reflexes are elicited first, and then 225 conditioned H-reflexes are elicited while the subject is encouraged to change H-reflex size guided by immediate visual feedback. Background EMG activity and M-wave size are kept stable throughout data collection. Conditioning was successful for 29/39 (74%) people without CNS injury, 6/9 (67%) people with SCI, and 6/12 (50%) people with stroke. (Subsequent data are those of the successful

individuals.) For people without injury who were down-conditioned (N=18), final H-reflex size was 69±6(SE)% of baseline (-17% within-session task-dependent adaptation (TDA); -14% across-session long-term (control reflex) change (LTC)). For people without injury who were up-conditioned (N=11), final H-reflex size was 144±9% (+16% TDA; +28% LTC). For people with SCI who were down-conditioned (N=6), final H-reflex size was 69±11% (-7% TDA; -24% LTC). For people with stroke who were down-conditioned (N=6), final H-reflex size was 69±9% (-15% TDA; -16% LTC).
While final reflex sizes after down-conditioning were identical for the groups, they differed in the relative magnitudes of TDA and LTC, and in the time of onset of LTC (≈6 sessions later in people with SCI and ≈12 sessions later in people with stroke than in people without CNS injury). These differences are likely to reflect: inter-group differences in the characteristics and capabilities of the descending influence that is thought to be directly responsible for TDA and to eventually produce LTC; and inter-group differences in the functional impact of TDA vs. LTC (explicable in terms of the negotiated equilibrium model of spinal cord function (J Physiol in press)).

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Poster

387. Spinal Cord Injury and Plasticity: Neurophysiology II

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH NICHD P2C HD086844-01 (Kautz)
NIGMS Institutional Development Award (IDeA) U54-GM104941 (Binder-MacLeod)NIGM

Title: Soleus H-reflex modulation during a double-legged drop landing task

Authors: *M. A. LYLE[1], M. MCLEOD[2], B. A. POULIOT[3], A. K. THOMPSON[4]
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Abstract: Sensory feedback from muscle spindles is known to influence muscle activity during locomotor activities such as walking and running. Potential functional role of muscle spindle feedback may be reflected in the H-reflex amplitude during dynamic motion. For example, during walking the soleus H-reflex peaks during mid to late stance when muscle spindle feedback can reinforce and assist ankle extensor activation and is low during the swing phase

when feedback could interfere with ankle dorsiflexion. Tasks such as landing from a jump involve rapid joint flexion and thus muscle spindle feedback could have an important role in controlling muscle and joint stiffnesses. Several studies have evaluated soleus H-reflex amplitudes during landing, but a thorough understanding of H-reflex modulation is lacking and whether H-reflex magnitude is related to landing mechanics is unknown.
Here, we evaluate soleus H-reflex modulation and landing mechanics in healthy adults without a history of neurological injury. After collecting a standing H/M recruitment curve, each participant performed 100-120 double-legged drop landing trials from a 30.5 cm platform, with ≈10 s between trials. For ≈80% of landing trials, the soleus H-reflex is elicited unilaterally by tibial nerve stimulation just above M-wave threshold before, at, and after ground contact (-150, -100, -75, -50, -25, 0= ground contact, +25-50 ms). The test leg is randomly assigned. Bilateral surface EMG (soleus, medial gastrocnemius, tibialis anterior, vastus lateralis), vertical ground reaction force (GRF), and knee and ankle joint motion are measured. For comparison across different phases of landing, H-reflexes with the M-wave amplitude of ≈2-10% Mmax are included for analysis.
In the initial group of participants, knee and ankle joint angles at ground contact (10-18° and 30-35°) and the maximum GRF on the test leg (150-250% full body weight) remained relatively constant between stimulated landing (of various stimulus timings) and unstimulated landing within each participant. Thus, any systematic changes in H-reflex amplitude across different phases of landing would likely reflect phase-dependent modulation. H-reflex amplitude (expressed as %Mmax) steadily decreased during the flight phase from 58±13 (mean±SD) %Mmax at -150 ms to 9±7% at 0 ms, to 10±9 at +25-50 ms post ground contact. Our preliminary findings suggest the H-reflex modulation during drop landing differs from that observed for walking, running and drop jump. To elucidate the functional role of muscle spindle feedback during landing, further analysis to evaluate relations between H-reflex amplitude and landing mechanics is currently underway.

Disclosures: M.A. Lyle: None. **M. McLeod:** None. **B.A. Pouliot:** None. **A.K. Thompson:** None.

Poster

387. Spinal Cord Injury and Plasticity: Neurophysiology II

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH (NIGMS) Institutional Development Award (IDeA) U54-GM104941 (Binder-MacLeod)
NIBB 1P41EB018783 (Wolpaw)
NINDS NS069551 (Thompson)

NIChd/NCMRR Medical Rehabilitation Research Resource Award P2C-HD086844 (Kautz)

Title: Lower leg EMG and H-reflex modulation during two-leg hopping

Authors: W. HAUG[1], *A. K. THOMPSON[2]
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Abstract: Hopping is a series of jumps in which landing from a jump seamlessly transitions to the subsequent jump. While it is a common form of jump, motor control during generation of two-leg hopping is not well understood. The aim of this study was to examine generation and modulation of the lower leg EMG and H-reflex activity during two-leg hopping, in relation to jump height and joint kinematics. We also compared the EMG and H-reflex activity during maximal height hopping and landing after the last maximal height hop.
Healthy adults with various athletic backgrounds participated in this study. Each subject performed 14-20 sets of 2 build up hops + 4 maximal hops + 1 landing, with a 90 s interval. Surface EMG from the soleus, medial (MG) and lateral gastrocnemius (LG), and tibialis anterior, vertical ground reaction force, and knee and ankle joint motion were measured. Triceps surae H-reflexes were elicited across different phases of hopping cycle.
In 19 (10 men and 9 women) subjects studied, the flight time (reflecting jump height) increased from the lower buildup (349±47(SD)ms) to the higher buildup (420±44ms) to the maximal height hop (497±53ms), and the flight time was positively correlated with triceps surae EMG amplitudes for -100 to 0 ms and 0 to 100 ms after ground contact (GC) ($r > 0.57$ for all). Correspondingly, the rate of ankle planterflexion and knee extension increased with increasing flight time: from -525 to -586 to -673 °/s for the ankle and -297 to -367 to -484 °/s for the knee. H-reflex size in -100 to 100 ms post GC also increased from the lower buildup to the maximal height hop. During maximal height hopping, the soleus H-reflex was phase-dependently modulated in each individual (i.e., modulation index: 79±16%), with no consistent pattern across subjects. The MG H-reflex was phase-dependently modulated individually (modulation index: 77±16%) and systematically across subjects ($p=0.02$, ANOVA), with the largest H-reflex elicited from -100 to 200 ms post GC.
During landing, the triceps surae EMG became suppressed immediately after GC, and the H-reflexes were almost completely suppressed from 50 to 200 ms post GC, in clear contrast to hopping.
Our study shows that (1) the triceps surae muscle activity likely contributes to two-leg hopping performance (i.e., higher jumps), (2) H-reflex is phase-dependently modulated, but the modulation patterns may differ across subjects and muscles, and (3) from hopping to landing, the muscle activity and H-reflex are rapidly and dynamically modulated. These findings suggest that the maximal height two-leg hopping involves dynamic neural control of knee and ankle joint motion within the constraints of rapid motion.

Disclosures: W. Haug: None. **A.K. Thompson:** None.

Poster

387. Spinal Cord Injury and Plasticity: Neurophysiology II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: VA P01 HD32
NIH HD36020
NS22189
NS061823
HD032571
NIBIB/P41EB018783

Title: Soleus H-reflex down-conditioning affects the motoneuron axon initial segment (AIS)

Authors: *Y. CHEN[1], Y. WANG[1], L. CHEN[1], J. R. WOLPAW[1,2], X. Y. CHEN[1]
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Abstract: Operant conditioning of the spinal stretch reflex or its electrical analog, the H-reflex (HR), changes brain and spinal cord (Curr Opin Behav Sci 20:138-144, 2018 for review). Soleus (SOL) HR up-conditioning increases SOL motoneuron (MN) axon initial segment (AIS) length; the increase correlates with the HR increase (Chen et al. SFN Abs 780.07, 2017). We assessed the impact of SOL HR down-conditioning on the AIS.
Twelve successfully down-conditioned (DS, HR decrease ≥20%) rats, 8 down-conditioning failed (DF, HR change <20%) rats, and 13 naive control (NC) rats were studied. Each was injected in SOL with CTB-Fluor488 and perfused 3 days later. Serial 25-μm lumbar spinal cord coronal sections were immunohistochemically processed for CTB-Fluor488 (identifying SOL MNs) and anti-ankyrin G (labeling the AIS). SOL AIS length, proximal and middle widths, and location (distance from soma)), and ANK3-IF and Nav-IF on SOL MN soma and/or AIS, were measured in a blinded manner. ANK3-IF and Nav-IF were expressed in % of average NC rat values. Statistical analysis was by nested ANOVA followed by least mean contrast test.
A total of 433 SOL AIS (106 DS, 98 DF, 229 NC) were studied. SOL AIS lengths in DS, DF, and NC rats averaged 26.1±0.3SE μm, 25.1±0.4 μm, and 26.0±0.3 μm, respectively. The groups did not differ ($p>0.05$). SOL AIS locations in DS, DF, and NC rats averaged 7.49±0.41 μm, 8.48±0.45 μm, and 7.0±0.39 μm, respectively. AIS location was significantly increased in DF rats ($p<0.01$ vs. NC). AIS proximal width in DS, DF, and NC rats averaged 5.24±0.10 μm, 5.13±0.11 μm, and 5.44±0.08 μm, respectively; it was narrower in DF rats than in NC rats ($p<0.05$). AIS middle width in DS, DF, and NC rats averaged 3.45±0.06 μm, 3.44±0.06 μm, and 3.50±0.05 μm, respectively. The groups did not differ ($p>0.05$). Nav-IF on SOL MN soma in DS

and DF rats averaged 87(±3)% and 91(±3)% respectively, weaker than that in NC rats ($p<0.001$ and $p<0.05$, DS and DF vs. NC). Nav-IF on SOL AIS in DS and DF rats averaged 88(±3)% and 91(±3)%, respectively, weaker than in NC rats ($p<0.001$ and $p<0.01$, DS and DF vs. NC). ANK3-IF on SOL AIS_{MN} averaged 87(±2)% and 96(±2)%, respectively, in DS and DF rats; it was weaker in DS rats ($p<0.001$ DS vs. NC; $p>0.05$ DF vs. NC).
Successful HR down-conditioning is associated with decrease in AIS Nav-IF and ANK3-IF. This seems consistent with studies showing that down-conditioning is due to a positive shift in MN firing threshold (J Neurophysiol 72:431-442, 1994, 74:867-871, 1995). Together with the finding that successful up-conditioning is associated with increased AIS length, these results suggest that AIS plasticity, previously reported to occur during development, may also contribute to motor learning.

Disclosures: Y. Chen: None. **Y. Wang:** None. **L. Chen:** None. **J.R. Wolpaw:** None. **X.Y. Chen:** None.

Poster

387. Spinal Cord Injury and Plasticity: Neurophysiology II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 387.17/V2

Topic: C.11. Spinal Cord Injury and Plasticity

Support: VA P01 HD32
NIH HD36020
NS22189
NS061823
HD032571
NIBIB/P41EB018783

Title: Soleus H-reflex up-conditioning increases soleus motoneuron ionotropic glutamate 1 (GluR1) receptor labeling

Authors: *Y. WANG[1], L. CHEN[1], Y. CHEN[1], J. R. WOLPAW[1,2], X. Y. CHEN[1]
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Abstract: Successful up-conditioning of soleus (SOL) H-reflex (HR) in rats increases SOL motoneuron (MN) group-1 metabotropic glutamate receptor 1 immunoreactivity (mGluR1-IR) and the length of the SOL MN axon initial segment (AIS) (Wang et al., SFN Abs 917.11, 2011; Chen et al., SFN Abs 780.07, 2017). Furthermore, both increases correlate with the magnitude of HR increase produced by up-conditioning. To further explore the mechanisms of HR up-conditioning, we studied the effect of SOL HR up-conditioning on SOL MN surface ionotropic

glutamate 1 receptor (GluR1), phosphorylated GluR1 (pGluR1), and phosphrylated extracellular signal-regulated kinase (pERK).
Rats were implanted with EMG electrodes in the right SOL and a stimulating cuff on the posterior tibial nerve. Electrodes connected to a head-mounted tether. When EMG activity remained in a specified range for 2.3-2.7 s, nerve stimulation just above the M-response threshold elicited the HR. In the control mode (first 10-20 days), no reward occurred. In the HR up-conditioning mode (next 50 days), reward occurred when the HR size was greater than a criterion.
Seven SOL HR up-conditioning successful (i.e., HR increase ≥20%, US rats), 4 SOL HR up-conditioning failed (HR change <20%, UF rats), and 8 body-weight-matched naïve control (NC) rats were injected in the right SOL with CTB-Fluor 647 (to identify SOL MNs), and perfused 3 days later. The lumbar 4-5 spinal cord was blocked and cut coronally at 16 μm. One of every 3 consecutive sections was processed for anti-GluR1, anti-pGluR1 (Ser831), or anti-pERK (Thr202/Tyr204) immunofluorescent (IF) labeling of SOL MNs. Three-D image stacks were photographed, coded, and quantified with the Fiji-image J program in a blinded manner. Data were expressed in % of the average NC rat values, and analyzed with one-way ANOVA and tested by least mean contrast.
In the 7 US rats, SOL MN GluR1-IF, pGluR1-IF, and pERK-IF averaged 116(±3SE)%, 133(±4)%, and112(±2)%, respectively, of that in NC rats ($p=0.0004$, $p<0.0001$, and $p<0.001$ vs. NC, respectively). In the 4 UF rats, GluR1-IF, pGluR1-IF, and pERK-IF averaged 109(±4)%, 102(±6)%, and 108(±3)%, respectively, of that in NC rats ($p=0.11$, $p=0.76$, and $p=0.032$, vs. NC rats, respectively). These initial results suggest that increases in SOL MN GluR1 and phosph-GluR1 (which appeared to occur only in US rats) may contribute to operantly conditioned HR increase, while increase in pERK (which appeared to occur in both US and UF rats) may not.

Disclosures: Y. Wang: None. **L. Chen:** None. **Y. Chen:** None. **J.R. Wolpaw:** None. **X.Y. Chen:** None.

Poster

388. Molecular and Cellular Mechanisms of Itch and Touch Sensation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 388.01/V3

Topic: D.02. Somatosensation

Support: NIH Grant R01GM101218
NIH Grant R01DK103901
NIH Grant R01AR070116
NIH Grant P30 DK052574
NIH Grant K08AR065577

Title: Piezo2 channel-Merkel cell signaling modulates the conversion of touch to itch

Authors: *J. FENG[1], J. LUO[1], P. YANG[1], J. DU[2], B. S. KIM[1], H. HU[1]
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Abstract: The somatosensory system relays many signals ranging from light touch to pain and itch. Touch is critical to spatial awareness and communication. However, in disease states, innocuous mechanical stimuli can provoke pathologic sensations such as mechanical itch (alloknesis). The molecular and cellular mechanisms that govern this conversion remain unknown. We found that alloknesis in the setting of aging and dry skin is associated with a loss of Merkel cells, the touch receptors in the skin. Targeted genetic deletion of Merkel cells and associated mechanosensitive Piezo2 channels in the skin was sufficient to produce alloknesis. Chemogenetic activation of Merkel cells protected against alloknesis in dry skin. This study reveals an unknown function of the cutaneous touch receptors and may provide insight into the development of alloknesis.

Disclosures: J. Feng: None. **J. Luo:** None. **P. Yang:** None. **J. Du:** None. **B.S. Kim:** None. **H. Hu:** None.

Poster

388. Molecular and Cellular Mechanisms of Itch and Touch Sensation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 388.02/V4

Topic: D.02. Somatosensation

Support: Ragnar Söderberg Fellow

Title: Local input connections to GRPR expressing dorsal horn interneurons

Authors: *F. B. FREITAG, A. AHEMAITI, J. JAKOBSSON, M. LAGERSTRÖM
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Abstract: Somatosensory information from different modalities are detected by specific receptors in the peripheral primary afferent terminals but sensations as pain and itch can share common pathways, bringing this information to the dorsal horn. Early evidences suggested a dedicated circuit to process itch-related information through the use of the peptide Gastrin releasing peptide (GRP) and its receptor GRPR. Furthermore, itch sensation has also been shown to be negatively modulated by an inhibitory tone in the dorsal horn as an increase in scratch behavior could be induced by disinhibition. In order to investigate the GRP/GRPR system and its local connectivity, the calcium indicator GCaMP6 was delivered through viral injection in the spinal cord of Grpr-Cre animals. To retrogradely trace dorsal horn GRPR neurons and

characterize its local inputs, rabies virus was injected in the spinal cord of Grpr-Cre mice. Finally, whole cell patch clamp recordings were performed to confirm the prevalence of spontaneous inhibitory/excitatory input to this subpopulation of local dorsal horn interneurons. The results from these experiments bring important conclusions about local connectivity of GRPR dorsal horn interneurons and contributes to our increasing knowledge of how itch is processed and regulated in the spinal cord level.

Disclosures: F.B. Freitag: None. **A. Ahemaiti:** None. **J. Jakobsson:** None. **M. Lagerström:** None.

Poster

388. Molecular and Cellular Mechanisms of Itch and Touch Sensation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 388.03/V5

Topic: D.02. Somatosensation

Support: JP16K15337

Title: Characteristics of primary afferent nerve fibers elongated into the epidermis in dry skin with itch

Authors: *T. ANDOH, Y. ASAKAWA, Y. KURAISHI
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Abstract: Itch/pruritus is the most common symptom of cutaneous diseases (e.g. atopic dermatitis and xerosis) characterized by dry skin. Pathohistological analyses of chronic skin diseases with severe itch and dry skin have shown that the density of peripheral nerve fibers is increased in the epidermis. However, the characteristics of the elongated nerve fibers remain unclear. Therefore, we investigated the characteristics of the elongated nerve fibers using a dry skin mouse model with itch. In this study, we used male ICR mice. The dry skin mouse model was prepared via repetitive treatments with an acetone/ether mixture and water for 5 days. In this mouse model, the stratum corneum water content was decreased, whereas spontaneous scratching and epidermal hyperplasia were increased. In the epidermis of the dry skin, PGP9.5-immunoreactive nerve fibers were increased. In addition, the number of substance P (SP)- and calcitonin gene-related peptide (CGRP)-immunoreactive nerve fibers (C-fibers) was also increased in the epidermis of treated mice compared to that in non-treated control mice. However, neurofilament 200-immunoreactive nerve fibers (A-fibers) were not detected in the epidermis of treated mice. These results suggest that the elongated epidermal peripheral nerve fibers comprise SP/CGRP-containing C-fibers but not A-fibers. Thus, these fibers may be involved in the induction of dry skin pruritus.

Disclosures: T. Andoh: None. **Y. Asakawa:** None. **Y. Kuraishi:** None.

Poster

388. Molecular and Cellular Mechanisms of Itch and Touch Sensation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 388.04/V6

Topic: D.02. Somatosensation

Support: Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2016R1C1B2009938)

Title: Proenkephalin-derivged pruritogen, BAM8-22, and increased activity of MRGPRX1 are responsible for pruritus in cholestasis

Authors: *B. SANJEL, H.-J. MAENG, W.-S. SHIM
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Abstract: Pruritus is an unexpected symptom observed in various liver diseases, which is caused by impaired bile flow or cholestasis. However, the mechanism via which cholestasis induces the intractable itch is still unclear. Here, we show that bovine adrenal medulla (BAM) 8-22, an endogenous itch-inducing peptide, could be involved in cholestatic pruritus. In this study, mice with bile duct ligation (BDL) were used as an obstructive cholestasis model to investigate cholestatic pruritus. We observed that BDL mice showed increased spontaneous scratching behavior than sham-operated mice. Importantly, the mRNA level of proenkephalin, a precursor polypeptide of BAM8-22, was significantly increased in the skin of BDL mice. Moreover, there was a significant increase in the mRNA level of *Mrgprx1*, which encodes a receptor for BAM8-22, in the dorsal root ganglia (DRG) of BDL mice. This was confirmed by calcium imaging, which showed that the primary culture of DRG neurons exhibited higher calcium influx upon BAM8-22 treatment than those observed in sham-operated mice. In addition, BDL mice showed more potentiated scratching behavior when BAM8-22 was injected, indicating increased activity of MRGPRX1. Finally, among the various bile acids, chenodeoxycholic acid significantly increased proenkephalin transcription in a human keratinocyte cell line (HaCaT). In conclusion, the intractable itch associated with cholestasis could be attributed to enhanced production of BAM8-22 and its receptor MRGPRX1 in sensory neurons.

Disclosures: B. Sanjel: None. **H. Maeng:** None. **W. Shim:** None.

Poster

388. Molecular and Cellular Mechanisms of Itch and Touch Sensation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 388.05/V7

Topic: D.02. Somatosensation

Support: National Natural Science Foundation of China (Grant No. 31371122, 31771158)

Title: Cortical representation of itch sensation

Authors: *X. CHEN, Y. SUN
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Abstract: Human functional imaging studies have shown that primary somatosensory cortex (S1) is activated by itch stimuli or itch-associated scratching. However, whether S1 receives peripheral itch information, and how S1 neurons encode itch information remain poorly understood. Here, we report that S1 pyramidal neurons exhibited itch-related response. We manipulated the spinal itch circuit by introducing ChR2 into the spinal neurons expressing gastrin-releasing peptide receptors (GRPR). By *in vivo* two-photon calcium imaging, we found that a large proportion of S1 pyramidal neurons were activated by optogenetic activation of spinal $GRPR^+$ neurons. Importantly, some of the S1 neurons responding to $GRPR^+$ neuron activation were activated by intradermal injection of pruritogens. Furthermore, a subset of S1 neurons displayed differential responses to spinal $GRPR^+$ neuron stimulation at different intensity, thus were capable of encoding itch intensity. Pharmacological inactivation experiments confirmed that contralateral S1 activity was necessary for pruritogen induced scratching behavior. Our results provide a comprehensive examination of the itch signal processing in the somatosensory cortex, and suggest that S1 plays a key role in encoding itch sensation.

Disclosures: X. Chen: None. **Y. Sun:** None.

Poster

388. Molecular and Cellular Mechanisms of Itch and Touch Sensation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 388.06/V8

Topic: D.02. Somatosensation

Support: NIH Grant AR057194

Title: Serotonin receptor subtypes involved in scratching behavior in the rat

Authors: *D. T. DOMOCOS[1], T. SELESCU[1], E. E. CARSTENS[2], M. IODI CARSTENS[2], A. BABES[1]
[1]Fac. of Biology, Univ. of Bucharest, Bucharest, Romania; [2]Neurobiology, Physiol. & Behavior, Univ. of California Davis, Davis, CA

Abstract: Serotonin (5-HT) is an important neuromodulator of both pain and itch. 5-HT elicited 3 types of calcium responses in rat dorsal root ganglion (DRG) cells: transient, sustained, and spiky. The transient responses were elicited by agonists and blocked by antagonists of 5-HT3, while sustained and spiky responses were elicited by agonists and blocked by an antagonist of the 5-HT1a receptor. We presently investigated roles for the 5-HT3 and 5-HT1a receptors in itch and pain in Wistar rats.
Male and female Wistar rats were used. Vehicle, or the 5-HT1a antagonist WAY100,635 (10 mM/ 10 μL) or the 5-HT3 antagonist granisetron (10 μg/ 10 μL) was injected id in the cheek, followed 30 min later by injection of 5-HT (1%; 10 μL) at the same site. The animal was videotaped, and hindlimb scratch bouts (indicative of itch) and forelimb wipes (indicative of pain) directed to the injection site were scored offline. The 5-HT1a agonists 5-CT (50 mM/ 10 μL) and DPAT (10 mM/ 10 μL), and the 5-HT3 agonist SR57227 (1 mM/ 10 μL), were injected id in the cheek as above.
5-HT elicited robust hindlimb scratching and little forelimb wiping. Males scratched more than females but the difference was not significant. Preinjection of WAY100,635 significantly attenuated 5-HT-evoked scratching behavior in males (54%), and non-significantly reduced scratching in females (28%). Granisetron did not reduce 5-HT-evoked scratching in males or females. In females the combination of WAY100,635 and granisetron significantly attenuated 5-HT-evoked scratching (63%).
Both the 5-HT1a and 5-HT3 antagonists significantly attenuated 5-HT-evoked wiping behavior in males and females. 5-CT elicited a significant increase in hindlimb scratching above control levels, although less that that elicited by 5-HT. DPAT and SR57227 did not elicit any significant increase in scratching or wiping.
The results support a role for the 5-HT1a receptor in 5-HT-evoked scratching behavior, at least in males. Wiping was also reduced, suggesting that 5-HT1a may have a role in pain as well as itch. Sustained and spiky 5-HT-evoked responses of DRG cells were reduced by 5-HT1a antagonists, and evoked by 5-HT1a agonists, suggesting that they reflect pruritic and possibly also algogenic input. Our results do not strongly support a role for the 5-HT3 receptor subtype in 5-HT-evoked itch. However, wiping and transient responses of DRG cells were suppressed by the 5-HT3 antagonist, suggesting that the transient response of sensory neurons may contribute to 5-HT-evoked pain. 5-HT-evoked scratching in females was attenuated by a combination of 5-HT1a and 5-HT3 antagonists, suggesting a role for both receptor subtypes in itch in females.

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Poster

388. Molecular and Cellular Mechanisms of Itch and Touch Sensation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 388.07/V9

Topic: D.02. Somatosensation

Support: Pfizer Grant WI203521

Title: Effects of tofacitinib on psoriatic itch in mice

Authors: *K. M. SANDERS, T. HASHIMOTO, K. SAKAI, G. YOSIPOVITCH, T. AKIYAMA
Dept. of Dermatol. & Cutaneous Surgery, Univ. of Miami, Miami, FL

Abstract: The Janus kinase 1/3 inhibitor tofacitinib has demonstrated a significant anti-pruritic effect in two phase III studies of patients with moderate to severe plaque psoriasis. However, the mechanisms behind this antipruritic effect are still largely unknown. We presently investigated whether tofacitinib affects spontaneous itch as well as expression of itch-related cytokines and epidermal nerve fiber density (ENFD) in the imiquimod-induced mouse model of psoriasis. Psoriasis-like skin lesions were produced by daily topical application of imiquimod to the back skin of adult male C57BL/6 mice (n = 8 per group). Alzet osmotic mini-pumps were subcutaneously implanted in the mice to deliver either tofacitinib or vehicle control (50% DMSO, 10% PEG 300, and 40% distilled water) at 15 mg/kg/day. On Day 7, animals were videotaped to assess spontaneous scratching. Following behavior testing, mice were perfused, and skin was collected. RT-PCR was used to quantify mRNA expression of itch-related cytokines. To investigate ENFD, skin was immunostained with antibodies against CGRP, a marker for peptidergic nerves, or P2X3, a marker for nonpeptidergic nerves.Imiquimod treatment resulted in increased spontaneous scratching, which was significantly inhibited by tofacitinib treatment. Imiquimod treatment significantly increased mRNA expression of IL-22, IL-23, and IL-31 compared to naive mice. Tofacitinib significantly decreased the expression of these cytokines. Finally, imiquimod treatment significantly reduced peptidergic ENFD and increased nonpeptidergic ENFD compared to naive mice. Tofacitinib significantly increased peptidergic ENFD, recovering it to naive skin levels, and further increased nonpeptidergic ENFD. These results indicate that tofacitinib may inhibit psoriatic itch through inhibition of cytokine expressions as well as modulation of epidermal innervation.

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Poster

388. Molecular and Cellular Mechanisms of Itch and Touch Sensation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 388.08/V10

Topic: D.02. Somatosensation

Title: Vesicular glutamate transporter 2-independent itch transmission from primary afferent neurons to spinal cord

Authors: *L. CUI[1], W. OLSON[1], M. MA[1], Q. LIU[2], W. LUO[1]
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Abstract: Itch is detected by pruriceptors in dorsal root ganglion (DRG) or trigeminal ganglion (TG), and transmitted to spinal cord. Interestingly, vesicular glutamate transporter (vglut2) knock out in primary afferent neurons in mouse showed deficit in pain response, but increased itch behavior. It is remains unclear what is the itch transmitter from vglut2 knock primary afferent neurons to spinal cord. Here, we used itch-specific Mas-related G-protein coupled receptor A3 (MrgprA3) Cre mice to knockout vglut2 from MrgprA3 DRG neurons and express ChR2. We found that Vglut2 null MrgprA3 afferents still release glutamate and display no deficit in itch behavior. Since no obvious expression of vglut1/3 is detected in these neurons, our results suggest that there is novel vglut2-independent glutamate release from A3+ primary afferent neurons to spinal cord for itch transmission.

Disclosures: L. Cui: None. **W. Olson:** None. **M. Ma:** None. **Q. Liu:** None. **W. Luo:** None.

Poster

388. Molecular and Cellular Mechanisms of Itch and Touch Sensation

Location: SDCC Halls B-H

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Program #/Poster #: 388.09/V11

Topic: D.02. Somatosensation

Support: Instituto de Salud Carlos III: FIS PI14/00141, FIS PI1700296, RETIC RD16/0008/0014
Generalitat de Catalunya: 2017SGR737

Title: Loss of TRESK enhances acute and chronic itch in mice

Authors: *A. ANDRES-BILBE[1,2,3], A. CASTELLANOS[1,2,3], L. CUENCA[1], A. PUJOL[1], N. COMES[1,2,3], D. SOTO[1,2,3], X. GASULL[1,2,3]
[1]Univ. De Barcelona, Barcelona, Spain; [2]Inst. of Neurosciences, Univ. de Barcelona, Barcelona, Spain; [3]IDIBAPS, Barcelona, Spain

Abstract: TRESK (K2P18.1) is a background K^+ channel expressed in sensory neurons, where it modulates the resting membrane potential, action potential firing and neuronal excitability. A subset of these sensory neurons, which express specific TRPs and Mas-related G protein-coupled receptors (Mrgprs), are activated by pruritogens and mediate itch sensations. Because TRESK is involved in somatosensitivity and pain perception, we evaluated the contribution of this channel to pruritic sensitivity and its potential as a target for the treatment of chronic itch pathologies including renal or liver failure, Hodgkin's lymphoma and different types of dermatitis. By combining calcium imaging experiments and behavioral approaches, we found that TRESK is involved in the modulation of non-histaminergic itch. Different populations of primary cultured sensory neurons from both wild-type and TRESK knockout mice were activated by chloroquine, β-alanine, BAM8-22 or histamine in calcium imaging experiments. At the behavioral level, subcutaneous injection of chloroquine in the cheek model produced an acute scratching response, which was significantly enhanced in mice lacking TRESK. Interestingly, TRESK ko mice also showed alterations in mice models of chronic itch. Induction of Allergic Contact Dermatitis showed a significantly higher scratching response in mice lacking TRESK compared to their wild-type counterparts. In the mouse model of imiquimod-induced psoriatic itch, the absence of TRESK produced a significantly enhanced scratching behavior, which developed earlier and was more robust. In summary, our data indicate that TRESK is involved in regulating the excitability of a subset of sensory neurons that mediate histaminergic-independent itch. Given the prominent role of this neuronal subpopulation in chronic itch diseases, TRESK appears as a new potential candidate for therapeutic intervention.

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Poster

388. Molecular and Cellular Mechanisms of Itch and Touch Sensation

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Program #/Poster #: 388.10/V12

Topic: D.02. Somatosensation

Support: Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2016R1C1B2009938)

Title: Glucosylsphingosine activates a member of TRP ion channel

Authors: *B. KIM, W.-J. LEE, W.-S. SHIM
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Abstract: Glucosylsphingosine (GS) is generally recognized as an accumulated by-product resulted from deficiency of glucocerebrosidases, mostly found in Gaucher disease. However, recent reports claimed that GS is also highly accumulated and specifically evokes itch-scratch responses in the skins of atopic dermatitis patients and in mice. Previously, we have reported that GS is able to activate mouse serotonin receptor 2a (Htr2a) and 2b (Htr2b), but not 2c. The GS-induced intracellular calcium increase was dose-dependent, and antagonists such as ketanserin (Htr2a antagonist) and RS-127445 (Htr2b antagonist) significantly blocked the GS-induced responses.Meanwhile, another recent study has revealed that scratching behavior was diminished in KO mice of a certain member of transient receptor potential (TRP) ion channel (TRPV4 hereafter), implying that the TRPV4 ion channel could be a downstream molecular entity in GS-induced itch signaling pathway. Along with this finding and our previous results, we hypothesized that GS might also act on TRPV4. To investigate the possibility, Surprisingly, GS was able to activate TRPV4 alone by dose-dependent manner, which was verified by calcium imaging technique. Moreover, mouse scratching behaviors induced by GS subcutaneous injection were significantly decreased when an antagonist of TRPV4 was pre-treated. In addition, a PLC inhibitor U73122 and a Gβγ inhibitor gallein also inhibited the response evoked by GS respectively, indicating that it follows PLC and Gβγ signaling pathway to transmit itch signals.Taken these together, we found for the first time that GS is able to specifically activate TRPV4, a member of TRP ion channel, which in turn may relay itch signals in the sensory neurons.

Disclosures: B. Kim: None. **W. Lee:** None. **W. Shim:** None.

Poster

388. Molecular and Cellular Mechanisms of Itch and Touch Sensation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 388.11/V13

Topic: D.02. Somatosensation

Support: JP15K08667
JP25860431
S1511031

Title: Electrophysiological and behavioral analysis of a mouse model of atopic dermatitis

Authors: *D. UTA[1], T. ANDOH[2]
[1]Dept. Applied Pharm, Univ. Toyama, Toyama, Japan; [2]Univ. Toyama, Toyama, Japan

Abstract: Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by severe difficult-to-treat pruritus. Clinically, itch in AD patients is resistant to conventional treatments, and such itch is called intractable itch. The itch induces scratching behavior, resulting in greater damage to the skin barrier. This disruption of skin barrier leads to further itch, causing a vicious cycle called the itch-scratch cycle. During this cycle, even painful stimuli evoke itch in AD patients. This intractable itch lowers the quality of life in AD patients. Determining the fundamental mechanisms underlying intractable itch is therefore important in developing antipruritic treatments. *Nevertheless*, its underlying mechanisms are poorly understood. The present study was designed to analyze pruritic synaptic responses in spinal dorsal horn neurons of a mouse model of AD (NC/Nga mice) by using an in vivo extracellular recording. Spontaneous scratching behavior was AD NC/Nga mice significantly higher than that of healthy. Next we investigated spontaneous neuronal activity of spinal dorsal horn (SDH) was recorded using in vivo electrophysiological techniques. In AD mice, the frequency of spontaneous firing in SDH neurons significantly increased compared with healthy mice. Spontaneous firing was blocked by either AMPA/kainate antagonist CNQX (10 μM) or voltage-gated Na+ channel blocker TTX (1 μM) applied to the surface of the spinal cord. Spontaneous firing neurons in AD mice were located in the superficial layer in SDH. This result consisted with Fos-like immunoreactivity analysis. These observations suggest that a subpopulation of superficial SDH neurons convey pruritic excitatory information to provoke scratching behaviors.

Disclosures: D. Uta: None. **T. Andoh:** None.

Poster

388. Molecular and Cellular Mechanisms of Itch and Touch Sensation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 388.12/V14

Topic: D.02. Somatosensation

Support: NIH grant AR063228

Title: Anxiety-like behavior and Fos expression in amygdala elicited by itch mediators in mice

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Abstract: Chronic itch is typically accompanied by negative emotional states. Itch intensity is linked to anxiety, and in turn anxiety and stress can exacerbate itch, leading to a vicious cycle that impacts the quality of life for chronic itch patients. However, the central mechanisms underlying this cycle are poorly understood. We aimed to measure anxiety-related behavior and neuronal activity following acute intradermal injection of three pruritogens: histamine, the major inflammatory pruritogen released by mast cells; chloroquine, a nonhistaminergic pruritogen that

induces itch through MrgprA3 receptors; and serotonin, a nonhistaminergic pruritogen that induces itch through multiple 5-HT receptor subtypes. Adult male C57BL/6 mice received an intradermal injection of histamine, chloroquine, serotonin, or phosphate-buffered saline (PBS) vehicle into the rostral back skin and were recorded on the elevated plus maze (EPM) or open field test (OFT) for 10 min. Open arm time on the EPM and the percent of center square entries on the OFT were used as measures of anxiety-like behavior. Mice displayed significantly reduced open arm time on the EPM following histamine, chloroquine, or serotonin injection compared to PBS control. Similarly, mice displayed significantly reduced percent center square entries in the OFT following histamine or chloroquine injection compared to PBS control. Next, we used immunohistochemistry to label c-Fos, a marker of neuronal activity, in several anxiety-related brain regions. Mice received an intradermal injection of histamine, chloroquine, serotonin, or PBS to the rostral back, and after 2 hr, they were perfused and dissected. c-Fos was labeled and quantified in the amygdala, parabrachial nucleus, and midcingulate cortex. Pruritogens evoked significantly a greater number of c-Fos+ neurons in these areas compared to PBS control. These results suggest that the amygdala, parabrachial nucleus, and midcingulate cortex may be part of a brain circuit that processes the affective component of itch and contributes to itch-induced anxiety-like behavior.

Disclosures: T. Akiyama: None. **K.M. Sanders:** None.

Poster

388. Molecular and Cellular Mechanisms of Itch and Touch Sensation

Location: SDCC Halls B-H

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Program #/Poster #: 388.13/V15

Topic: D.02. Somatosensation

Support: National Institutes of Health (R01DE23730) to Ajay Dhaka
Mary Gates (Undergraduate Research Research Award) to Logan Condon
Levinson Emerging Scholars Award (Undergraduate Research Award) to Logan Condon

Title: Scratching the surface of itch in fish: A cross-species model for selective pruritus via activation of TRPA1

Authors: *K. ESANCY, L. CONDON[1], J. FENG[2], C. KIMBALL[1], A. CURTRIGHT[1], A. DHAKA[1]
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Abstract: Little is known about the capacity of lower vertebrates to experience itch. In mammals, itch is thought to ensue when transient receptor potential (TRP) ion channels on

peripheral somatosensory neurons are activated downstream of G-protein coupled receptors (GPCRs) that bind to pruritic (itch-inducing) stimuli. By contrast, chemically- and thermally-induced pain is elicited when these TRP ion channels are directly activated by algogens. Through a combination of behavioral and neuronal activity assays in the zebrafish, we found that the TRPA1 ion channel can be directly activated to elicit both pain and itch behaviors. Pruritogens evoked robust lip-rubbing behaviors (analogous to scratching in mammals), while algogens elicited nocifensive behaviors such as freezing and reduced swimming velocity. These differential behaviors appear to result from the differential activation of distinct subsets of TRPA1-expressing neurons. One neuronal subpopulation is activated by the same low-intensity stimuli that evoke itch behaviors, such as low concentrations of the TRPA1 agonists imiquimod (IMQ) and allyl isothiocyanate (AITC). Neurons within this subpopulation also respond more robustly to other noxious stimuli, indicating that they likely comprise a more sensitive subset of neurons. Conversely, the remaining TRPA1-expressing neurons are less sensitive to noxious stimuli, and are only activated by high-intensity stimuli (i.e., high concentrations of TRPA1 agonists) associated with nocifensive behaviors. We replicated these findings in the mouse, suggesting that our observations are not unique to fish. Together, these results suggest a conserved model for selective itch via activation of a specialized subpopulation of somatosensory neurons with a heightened sensitivity to noxious stimuli. However, the molecular factors that distinguish these neurons remain unknown. Additional evidence from our studies suggests that multiple subpopulations with intermediate sensitivities exist between the two discrete populations we initially observed, and that this phenomenon may be generalizable to other nociceptive subtypes. We are investigating the factors that underlie the different sensitivities of nociceptors to a common agonist and what role neurons with different thresholds for noxious stimuli play in itch and pain sensation in both acute and pathological conditions.

Disclosures: K. Esancy: None. **L. Condon:** None. **J. Feng:** None. **C. Kimball:** None. **A. Curtright:** None. **A. Dhaka:** None.

Poster

388. Molecular and Cellular Mechanisms of Itch and Touch Sensation

Location: SDCC Halls B-H

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Program #/Poster #: 388.14/V16

Topic: D.02. Somatosensation

Support: SKM Start up Funds, North Carolina State Universiry

Title: Skin innervation patterns of itch neurons

Authors: *J. WHEELER, S. K. MISHRA
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Abstract: Decoding somatosensory information begins at the skin. One sensory input, itch, has only recently begun to be understood. Typically, one of two methods are widely used to study itch: intradermal injection into the nape of the neck and cheek injections. Despite giving the same basic information, the number of scratching bouts for a given compound in cheek assays is usually about one-fifth of the number of scratching bouts from nape injections. Additionally, itch compounds injected into the cheek also cause wiping, which is assumed to be a nocifensive response. In this study, we explored the discrepancy of itch response in the nape of the neck and in the cheek by examining the skin tissues derived from the mice, which express tdTomato in somatostatin (*SST)* neurons. In the dorsal root ganglia, *SST* is a marker for itch sensory neurons. These mice with labeled *SST* neurons were perfused and skin from the cheek and the nape of the neck were sectioned and imaged with confocal microscopy. These images were used to determine the density of *SST* positive neurons (in neurons/μm^2) for the nape and cheek regions. Additionally, it has been reported that female mice injected in the nape of the neck with chloroquine have a higher number of scratching bouts than males injected with chloroquine in the nape of the neck. Therefore, we used these mice to compare the skin innervation density of somatostatin neurons for the cheek and nape between male and female mice. Finally, we compared the changes in innervation patterns of somatostatin neurons by developing an atopic dermatitis mouse-model using compound MC903 (a vitamin D analog). Overall, our findings will help to understand the discrepancy in itch behavior in mice due to distribution of primary afferent innervation in the skin.

Disclosures: J. Wheeler: None. **S.K. Mishra:** None.

Poster

388. Molecular and Cellular Mechanisms of Itch and Touch Sensation

Location: SDCC Halls B-H

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Program #/Poster #: 388.15/W1

Topic: D.02. Somatosensation

Support: NIAMS 1R21AR068012

Title: Investigation of a mechanism for pruritic priming

Authors: *Z. K. FORD, S. DAVIDSON
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Abstract: Pain and itch are closely related, and are thought to share central and peripheral neural pathways. A well-established mechanism to explain the transition from acute to chronic pain is hyperalgesic priming. Hyperalgesic priming is characterized by extended hypersensitivity to an inflammatory mediator after recovery from a previous inflammatory injury. It is currently unknown whether a similar mechanism exists for itch, which could lead to chronic pruritus.

We hypothesized that an early pruritic event would cause latent sensitization, resulting in increased hypersensitivity to a pruritic stimulus in the future. To test this, we used the AEW (acetone/ether-water) model to mimic a chronic itchy, dry skin condition in mice. Previously, it was shown that this model increased the number of neurons responsive to chloroquine (CQ); however, no study has tested behavioral responses to pruritogens after AEW treatment. Additionally, while the time to initiate scratching with the AEW model has been well-documented, it has not been determined how long scratching behavior persists once the treatment has ended. In order to gain a greater understanding of the physiological changes taking place in the skin after AEW treatment, we explored whether the previously described hyper-innervation of fibers into the epidermis associated with the AEW model correlates with the timeline of scratching behavior, as well as if these changes were taking place in the CGRP-positive peptidergic or the P2X3-positive non-peptidergic population. We investigated the possibility of cross-modality priming, by using the traditional model of hyperalgesic priming in the cheek (carrageenan followed by PGE2) to determine whether this would elicit painful or pruritic behavior. To ascertain whether a pruritic secondary stimulus could cause hyperknesis after giving a subcutaneous carrageenan injection to the cheek, chloroquine, histamine, or AEW treatment was delivered and scratching behavior measured.
We found that 6 hours after the final AEW treatment scratching behavior increased from 8.12±2.4 bouts/hr at baseline to 36.75±8.12. A full day after AEW, scratching significantly increased to 127.5±37.59 bouts/hr, but then steadily returned to baseline levels (5.62±2.76 bouts/hr) by five days post treatment. Contrary to our hypothesis, mice that received the AEW treatment did not increase scratching following either acute pruritogen (CQ or histamine), nor did wiping behavior, an indicator of pain, change. These results suggest that AEW may not be capable of triggering pruritic priming, or inducing hyperalgesia.

Disclosures: Z.K. Ford: None. **S. Davidson:** None.

Poster

388. Molecular and Cellular Mechanisms of Itch and Touch Sensation

Location: SDCC Halls B-H

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Topic: D.03. Somatosensation: Pain

Support: DFG Grant FOR2149/P03
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DFG Grant KI1460/4-1

Title: The adhesion-GPCR CIRL promotes mechanosensory signal discrimination

Authors: *R. J. KITTEL, S. DANNHÄUSER, H. HOFMANN, N. EHMANN
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Abstract: Research on the sense of touch has long been a center stage for receptors that directly convert mechanical force into electrical signals, and the function of such mechanosensing ion channels remains a topical research focus. In contrast, evidence for mechano-metabotropic signal transduction and compelling models of force conversion into an intracellular second messenger response are limited, despite the vital role of metabotropic modulation in all corners of physiology. Adhesion-type G protein-coupled receptors (aGPCRs), a large molecule family with over 30 members in humans, operate in a vast range of physiological processes. Correspondingly, these receptors are associated with diverse human diseases, such as developmental disorders, defects of the nervous system, allergies and cancer. Several aGPCRs have recently been linked to mechanosensitive functions suggesting that processing of mechanical stimuli may be a common feature of this receptor family, not only in classical mechanosensory structures (Langenhan et al., 2016).
Drosophila Latrophilin/CIRL, one of the oldest members of the aGPCR family, modulates mechanosensory signal transduction. In addition to shaping sensory responses to gentle touch and sound (Scholz et al., 2015; 2017), we show here that CIRL also influences mechanical nociception *in vivo*. *Cirl* is expressed in peripheral larval nociceptors where it adjusts nocifensive behaviour under physiological conditions and in a chemical neuropathy model. By combining behavioural analyses with optogenetic manipulation of cyclic AMP levels *in vivo*, we find that CIRL exerts opposing modulatory effects in low-threshold mechanosensory neurons and high-threshold nociceptors. This bipolar action likely facilitates the differentiation of mechanosensory signals carrying different physiological information.
References
Langenhan T, Piao X, and Monk KR. Adhesion G protein-coupled receptors in nervous system development and disease. *Nat Rev Neurosci.* 2016;17(9):550-61.
Scholz N, Guan C, Nieberler M, Grotemeyer A, Maiellaro I, Gao S, Beck S, Pawlak M, Sauer M, Asan E, Rothemund S, Winkler J, Prömel S, Nagel G, Langenhan T, Kittel RJ. Mechano-dependent signaling by Latrophilin/CIRL quenches cAMP in proprioceptive neurons. *eLife.* 2017;6:e28360.
Scholz N, Gehring J, Guan C, Ljaschenko D, Fischer R, Lakshmanan V, Kittel RJ, Langenhan T. The Adhesion GPCR Latrophilin/CIRL shapes mechanosensation. *Cell Rep*. 2015;11(6):866-874.

Disclosures: R.J. Kittel: None. **S. Dannhäuser:** None. **H. Hofmann:** None. **N. Ehmann:** None.

Poster

388. Molecular and Cellular Mechanisms of Itch and Touch Sensation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 388.17/W3

Topic: D.03. Somatosensation: Pain

Title: Pre exposure of noxious heat and painful sensation in mice induce antipruritic effect

Authors: *R. KOPPARAJU, C.-C. CHEN
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Abstract: Itch is an unpleasant sensation caused by pruritogen transmitted by sensory neurons to the spinal dorsal horn and then to higher brain center, which eventually induces the desire to scratch. The act of scratching and vairable noxious counter stimuli like noxious heat and painful sensations relieve the itch sensation. However, it is not clear whether the noxious stimuli prior to injection of pruritogen could effectively reduce the itch sensation. Here we observed antipruritic effect produced by brief noxious stimuli applied on mouse skin prior to pruritogen. We demonstrated that application of noxious stimuli (e.g., passive scratching) on the nape skin of mild anaesthetized prior to prutrinogen application could significantly reduce itch response. Similarly itch reduction was observed in the nape and cheek skin models, when intradermal injection of capsaicin was given prior to prutinogen. The antipruritic effect produced by brief noxious stimuli lasted for more than 20 minutes. We further demonstrated that passive scratching of mice led to phosphorylation of ERK in cervical DRG neurons and enhanced c-Fos expression in lamina II of cervical spinal dorsal horn. Taken together, noxious stimuli prior to pruritogen challenges are effective to diminish itch responses by neural modulation at the spinal cord level, although possible involvement of supraspinal control cannot be excluded.

Disclosures: R. Kopparaju: None. **C. Chen:** None.

Poster

389. Central Mechanisms of Pain

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 389.01/W4

Topic: D.03. Somatosensation: Pain

Support: Asahi Kasei Pharma
Boston Scientific

Title: An EEG method for measuring spontaneous pain in awake, freely behaving rodents

Authors: *C. Y. SAAB[1], S. KOYAMA[2], J. W. GU[3], S. R. JONES[4], J. LEVITT[5]
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Abstract: Pain across evolutionary spectra is measured according to subjective criteria and behavioral observations. We developed an objective method for quantifying pain in rodents based on the fundamental principle that pain is a manifestation of brain activity, which in turn

engenders pain 'biosignatures'. We previously reported that theta (4-8 Hz) power of the resting state electroencephalography (EEG) over primary somatosensory cortex is elevated in rat models of pain (Leblanc et al. *Pain* 2014, 2016a,b). Pain-induced theta is phasic in rats with acute pain and tonic in those with chronic pain. We present new data showing that pain-induced theta is attenuated in rats with chronic neuropathic pain following dose-dependent administration of the effective analgesic EMA 401 (a peripherally-acting angiotensin II receptor inhibitor) and pregabalin (a centrally-acting calcium channel inhibitor), but remains elevated upon administration of minocycline (a glial inhibitor). Hence, EEG theta power detected false positive and false negative outcomes of the commonly-used thermal hyperalgesia test. Pharmacokinetic studies were performed to determine optimal doses for each drug. Moreover, neuromodulation by spinal cord stimulation at sub-paresthesia current intensity, which leads to modest analgesic effects in humans, attenuated EEG power in the 3-4 Hz range adjacent to the theta band (Koyama et al. *Sci Rep* 2018). Related to our EEG method, we developed a support vector machine (SVM) algorithm to automatically detect EEG artifacts in awake human, canine and rodent subjects. The levels of SVM accuracy for artifact classification in humans, Sprague Dawley rats and beagle dogs were 94%, 83%, and 85%, respectively with each result being significantly higher than chance. These data fully validate our empirical, real-time and cost effective method for the rapid and automated measurement of spontaneous pain in rodents and potentially other species.

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Poster

389. Central Mechanisms of Pain

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 389.02/W5

Topic: D.03. Somatosensation: Pain

Support: Brown University Seed project funding

Title: A multi-modal approach to elucidate the spinal circuits mediating tactile allodynia

Authors: *C. BLACK[1], A. ALLAWALA[1], R. THORPE[1], C. Y. SAAB[4], S. R. JONES[2], D. A. BORTON[3]
[2]Dept. of Neurosci., [3]Neuroengineering, [1]Brown Univ., Providence, RI; [4]Neurosurg. & Neurosci., Brown/RIH, Providence, RI

Abstract: Tactile allodynia (TA) is a symptom of neuropathic pain whereby innocuous tactile stimuli cause painful sensations. While TA is attributed in part to spinal disinhibition, a failure of the normal process of spinal inhibition by which dorsal horn interneurons silence feed-forward excitation of pain projection neurons, there is a lack of understanding regarding the emergence of such maladaptive plasticity. In an on-going multi-modal effort to dissect neural circuits mediating pain, we have implemented acute spinal electrophysiology in transgenic mice parallel to biophysically realistic computational modeling of the dorsal horn to elucidate the mechanisms of TA in more detail. Using a transgenic mouse line that co-expresses channelrhodopsin-2 (ChR2) with transient receptor potential vanilloid 1 (TRPV1) neurons, we selectively activate Aδ and C-fiber afferents through non-invasive optogenetic stimulation of the skin. Thus, differential activation of primary afferents has allowed us to isolate nociceptive responses in local field potentials (LFP) and single/multi-unit activity in the superficial dorsal horn from tactile responses during acute *in vivo* recordings. We are using this model to test if tactile evoked potentials are enhanced by optogenetic nociceptive stimulation. To better define the cellular circuits mediating the results observed in our acute electrophysiology studies, we have developed a computational model of the dorsal horn that contains peripheral inputs, inhibitory and excitatory interneurons, and pain projection neurons implicated in TA. We show that C-fiber volleys can drive feed-forward inhibition of laminae II-III interneurons to generate a 'window of opportunity' that facilitates the emergence of TA. In total, our results in vivo and in silico suggest that rampant nociceptive activity associated with chronic pain states could be responsible for causing the sustained and irreversible spinal disinhibition that leads to TA.

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Poster

389. Central Mechanisms of Pain

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Topic: D.03. Somatosensation: Pain

Support: NIBIB R01 EB022889-02
NIMH R01 MH106147
Carney Institute for Brain Science
Providence VA CfNN

Title: Characterization of circuit mechanisms underlying discriminatory EEG neural markers for pain perception in somatosensory cortex

Authors: *R. THORPE[1], C. J. BLACK[1], S. A. NEYMOTIN[2,3], C. Y. SAAB[2,4], D. A. BORTON[1,5,3], S. R. JONES[2,5,3]

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Abstract: Pain has long been characterized as a qualitative feature providing scale to the level of sensory discomfort. While behavioral states can be identified that indicate a painful percept (e.g. an evasive movement or a patient's descriptive words), interpretable biophysical markers in the central nervous system (CNS) have yet to be established that clearly discriminate between normal sensory perception and the feeling of pain. Additionally, mechanisms of non-invasive treatments such as transcranial direct current stimulation (tDCS) for pain are not well understood. Here, we investigate the circuit level origin of electroencephalography (EEG) measured markers of nociceptive pain using a new software tool whose foundation is a biophysically principled computational neural model that simulates the primary electrical currents underlying EEG: Human Neocortical Neurosolver (HNN, hnn.brown.edu). Specifically, we apply the neural model to interpret circuit mechanisms in primary somatosensory cortex (S1) that underlie differences in event related potentials (ERPs) from nociceptive laser stimulation as compared to non-nociciptive vibrotactile and median nerve stimulation, as identified in Lenoir et al., *J. Neurophysiology* (2017). Furthermore, we explore mechanisms of observed changes in ERP latency and amplitude components correlated with reduced pain perception in the nociceptive laser stimulation case due to tDCS. Initial results suggest involvement of higher-order feedback projections into the distal dendrites of layers 2/3 and 5 of S1 in the early-latency component of the median nerve stimulation evoked response. This work aims to provide a foundation for future quantitative classification of circuit mechanisms underlying pain-perceiving versus non-pain-perceiving signatures of EEG signals.

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Poster

389. Central Mechanisms of Pain

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Topic: D.03. Somatosensation: Pain

Support: NIH Grant R01NS45954-12 and R01DA37621-3 to BKT

Title: Nerve injury increases excitability of a subpopulation of Y1R-eGFP expressing dorsal horn neurons that exhibit Kv4.2 channel subunit mediated delayed firing

Authors: *G. P. SINHA[1,2], S. SCHMITT[2], L. ZHANG[2], B. K. TAYLOR[1,2]
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Abstract: Activation of NPY Y1-receptors (Y1R) on excitatory interneurons in the dorsal horn inhibits nociception. To understand the cellular mechanisms underlying Y1R-mediated analgesia, we first characterized the electrical properties of spinal cord lamina II Y1R-eGFP expressing neurons from lumbar L3/L4 segments in naïve, sham-injured, and spared nerve-injured (SNI) mice. Firing patterns of Y1R-eGFP neurons using patch-clamp electrophysiology included delayed firing in both naïve (74%) and SNI mice (90%). Kv4.2-mediated A-type currents underlie delayed firing. We found A-type currents with fast (~30ms) and slow (>100 ms) decay constants in the following populations: Fast - 50% of neurons in naïve animals and 67% from SNI animals; Slow - 24% in naïve and 23% in SNI. From immunohistochemical (IHC) studies we observed that 70% of Y1-eGFP expressing neurons co-localized with Kv4.2 immunoreactivity. 4-aminopyridine (4-AP), a Kv4.2 channel blocker, inhibited both fast and slow A-type currents. These data indicate that delayed-firing Y1R neurons express Kv4.2 - mediate A-type currents. As suggested by Gereau and colleagues phosphorylation and internalization of Kv4.2 channels can lead to a reduction in A-type potassium currents. By using a phospho-Kv4.2 (S616) antibody, we found that the percentage of phosphorylated Kv4.2 expression in Y1R-expressing neurons was increased in SNI-injured mice (67%) as compared to sham-injured (56%) mice. We next compared electrophysiological properties of neurons that express Y1R-eGFP and exhibit delayed firing in slices from SNI and sham mice. We found that compared to naive mice, SNI mice exhibited a resting membrane potential that was depolarized by ~2 mV, a latency to first spike that was shorter, and a decreased amplitude of A-type currents in the sub-type of neurons exhibiting fast A-type currents. This suggests that SNI increases excitability in this sub-population of Y1R-eGFP neurons. We suggest that nerve injury increases phosphorylation and possibly internalization of Kv4.2 subunits; this in turn might decrease fast A-type potassium conductance (reflected as shortened latency to stimulus) in delayed firing neurons. We conclude that: 1. A large population of Y1R expressing neurons exhibit delayed firing due to an enrichment of Kv4.2 channels that mediate fast A-type current; and 2. Peripheral nerve injury reducing the available of Kv4.2 channels, leading to an increase in the excitability of Y1R expressing neurons.

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Poster

389. Central Mechanisms of Pain

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Topic: D.03. Somatosensation: Pain

Support: JSPS KAKENHI Grant 17K11535

Title: Possible involvement of spinal cannabinoid receptors in trigeminal nerve injury-induced mechanical hypersensitivity of rats

Authors: *K. NAKAI[1], A. NAKAE[2], Y. MINEGISHI[1], R. URABE[1], M. MIYAMAE[1], T. KUBO[3], K. HOSOKAWA[3]
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Abstract: Background: Cannabinoids (CBs) produced analgesic effects via CB1 and CB2 receptors. CB1 receptors are distributed on neurons in the dorsal horn of the spinal cord. CB2 receptors are mainly expressed on peripheral immune cells, including macrophages. Recent studies have been shown the up-regulation of the CB2 receptors in the spinal cord following peripheral nerve injury. The contribution of spinal CB receptor subtypes in the trigeminal neuropathic pain remains unclear. Chronic constriction injury to the infraorbital nerve (ION-CCI) has proven to be a useful model for trigeminal neuropathic pain. The present study evaluates the possible role of spinal CB1 and CB2 receptors in ION-CCI rat model. Material and Methods: Male Sprague Dawley rats underwent unilateral CCI to the right ION by two nylon (5-0) ligatures. A series of von Frey filaments were used to determine pain hypersensitivity to mechanical stimulation on day 14 after surgery. A polyethylene (PE-10) catheter was implanted for upper cervical spinal injection of drugs. The time course of an antiallodynic effects of intrathecally administered a CB1 receptor agonist R(+)-Methanandamide, a CB1 receptor antagonist SR141716A, a CB2 receptor agonist HU308, and a CB2 receptor antagonist AM630 were examined. If the CB1 receptor agonist or the CB2 receptor agonist produced antiallodynic effects, an additional experiment was performed to evaluate the antagonizing effect of intrathecally pretreatment with the CB1 receptor antagonist or the CB2 receptor antagonist, on the antiallodynic action of the CB1 receptor agonist or the CB2 receptor agonist. The time course data for the dose-response effects were analyzed by two-way analysis of variance and Tukey-Kramer multiple-comparison test. Results: Intrathecal administration of SR141716A and HU308 significantly increased mechanical thresholds in a dose dependent manner. Intrathecal administration of R(+)-Methanandamide and AM630 did not alter mechanical thresholds. AM630 significantly reduced the antiallodynic effect of HU308. Conclusions: The inhibition of spinal CB1 receptors or activation of spinal CB2 receptors reduced the pain-related behavior. The results indicated that spinal CB1 and CB2 receptors may play an important role in a trigeminal neuropathic pain.

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Poster

389. Central Mechanisms of Pain

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 389.06/W9

Topic: D.03. Somatosensation: Pain

Title: Chiropractic spinal manipulation alters nociceptive processing at spinal and supraspinal levels

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Abstract: Objective: This study aimed to investigate how chiropractic spinal manipulation affects spinal and supraspinal nociceptive processing.
Methods: Fifteen volunteers attended two sessions (full-spine chiropractic care and passive movement control) in random order, in a crossover design randomized controlled trial. As a proxy of spinal pain transmission, nociceptive withdrawal reflexes (NWRs) to electrical stimulation on the sole of the foot were recorded at tibialis anterior before and after either intervention. For the supraspinal activity, 61-channel electroencephalogram evoked potentials (EPs) were simultaneously recorded. Areas under the curve of the NWR signals were analyzed. Latencies and amplitudes were computed for the major EP peaks. Brain source analysis of the EPs was performed.
Results: The NWR decreased due to the chiropractic intervention (P=0.01). The EPs had two main peaks at 116±19ms and 258±22ms. The amplitudes at both peaks were decreased due to chiropractic intervention at the frontal electrode (P=0.03). Brain source localization revealed no differences in location of brain activity but the strength of anterior cingulate activity decreased following the chiropractic intervention (P=0.04).
Conclusion: Chiropractic care alters nociceptive processing at spinal and supraspinal levels. This study adds to our understanding of how chiropractic care influences nociceptive processing.

Disclosures: H. Haavik: None. **D. Lelic:** None. **I. Niazi:** None. **K. Holt:** None. **A. Drewes:** None.

Poster

389. Central Mechanisms of Pain

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Program #/Poster #: 389.07/W10

Topic: D.03. Somatosensation: Pain

Support: NIH Grant R01-DA033059
VA Grant 1 I01-RX001646A

Title: Adenylyl cyclase activation induces substance P release in the rat spinal cord through protein kinase A and Epac

Authors: *J. G. MARVIZON, W. CHEN
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Abstract: Substance P release from nociceptive afferents contributes to chronic pain and is mediated by calcium entry in their presynaptic terminals through NMDA receptors and voltage-gated calcium channels (Cav2). Previous studies found that substance P release is increased by activation of adenylyl cyclase by prostaglandins and decreased by its inhibition by μ-opioid receptors. Our objective was to explore the mechanisms by which cyclic-AMP (cAMP) induces substance P release, measured as neurokinin 1 receptor (NK1R) internalization. We found that the adenylyl cyclase activator forskolin added to rat spinal cord slices induced substance P release with an EC_{50} of 6.6 μM, similar to its K_D for adenylyl cyclase (9.8 μM), and a $t_{1/2}$ of 22 min. The NK1R antagonist L-732,136 decreased forskolin-induced NK1R internalization, showing that it represents substance P release. The adenylyl cyclase inhibitor SQ22536 also decreased the effect of forskolin. The cell-permeant cAMP analog 8-Br-cAMP induced robust substance P release with a biphasic concentration-response curve. We hypothesized that these two components correspond to the activation of protein kinase A (PKA) and guanine nucleotide exchange factors for the G protein Rap (Epac). The PKA inhibitors KT5729 and PKI 14-22 decreased the effects of forskolin and 8-Br-cAMP. The selective PKA activator 6-Bzn-cAMP induced substance P release with high potency (0.85 pM). The selective Epac activator 8-pCPT-2'-O-Me-cAMP (CPTOMe-cAMP) also induced substance P release with a lower potency (5.2 μM) consistent with its EC_{50} for Epac (2.2 μM). We then studied the mechanisms downstream of PKA and Epac. The sodium channel blocker lidocaine did not inhibit substance P release elicited by 6-Bzn-cAMP or CPTOMe-cAMP, showing that the effects of PKA and Epac do not involve firing of action potentials. NMDA receptors are downstream of both PKA and Epac, since the effects of forskolin and the three cAMP analogs were decreased by the NMDA receptor blocker MK-801 and the NR2B-selective antagonist ifenprodil. Antagonists of AMPA and kainate receptors decreased substance P release induced by CPTOMe-cAMP but not by 6-Bzn-cAMP. Cav2 channel opening is also downstream of PKA and Epac, since the Cav2 channel blocker ω-

conotoxin MVIIC and the μ-opioid receptor agonist DAMGO decreased the effect of forskolin and all three cAMP analogs. The TRPV1 antagonist capsazepine did not decrease the effects of 6-Bzn-cAMP or CPTOMe-cAMP, indicating that TRPV1 do not mediate the effects of PKA and Epac. Therefore, activation of PKA and Epac induces substance P release by activating NMDARs and opening Cav2 channels in the central terminals of primary afferents.

Disclosures: J.G. Marvizon: None. **W. Chen:** None.

Poster

389. Central Mechanisms of Pain

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 389.08/W11

Topic: D.03. Somatosensation: Pain

Title: The action of norepinephrine on lamina X in the spinal cord

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Abstract: Backgroud: Lamina X is localized in the spinal cord within the region surrounding the central canal, and the neurons in lamina X receive the descending projection from supraspinal nuclei. Norepinephine (NE) is one of the neurotransmitters in descending pathways emanating from brain stem and it is known that NE-containting fibers terminate in the spinal dorsal cord, particularly the substantia gelatinosa, and are involved in the transmission and modulation of nociception. However, several studies have also indicated that NE-containing fibers from the locus coeruleus terminate densely not only in the superficial layers of the spinal dorsal cord, but also in lamina X. Therefore, it is possible that NE also acts on the lamina X and contributes the modulating nociceptive information. However, there are no previous reports investigating how NE released form descending pathway acts on lamina X and modulate nociceptive information at the cellular level. We therefore hypothesized that NE acts on lamina X in the spinal dorsal horn directly and inhibits nociceptive transmission at the spinal level.
Methods: We used the *in vitro* whole-cell patch-clamp technique to assess the underlying mechanisms of synaptic modulation of NE on miniature excitatory and inhibitory postsynaptic currents (mEPSCs and mIPSCs) at the neurons in lamina X from adult rat spinal cord slices.
Results: Bath-applied NE (20 μM, 2 minutes) to the lamina X did not affect the mean mEPSCs frequency, nor amplitude. However, bath-applied NE (20 μM, 2 minutes) increased the mean mIPSCs frequency (Control, 1.9 ± 0.9 Hz; NE, 5.4 ± 2.5 Hz; 291.1 ± 90.2% of control; n = 7; $P < 0.01$, paired t-test). NE also induced an outward current of mIPSCs in lamina X, and the average peak amplitude of the NE-induced outward current in mIPSCs was 5.7 ± 5.4 pA. In the presence of the α1-receptor antagonist prazosin (0.5 μM), NE-induced increase in mIPSCs

frequency was inhibited, and in the presence of the α2-receptor antagonist yohimbine (1 μM), NE-induced an outward current of mIPSCs was also inhibited. **Conclusion:** These results suggest that NE acts at presynaptic terminals of GABAergic and glycinergic interneurons in lamina X to facilitate inhibitory transmitter release through the activation of α1-receptors, and also depolarizes inhibitory interneurons in lamina X through the activation of α2-receptors. These actions of NE on neurons in lamina X may contribute to inhibition of the nociceptive transmission at the spinal level directly.

Disclosures: N. Ohashi: None. **M. Ohashi:** None. **H. Baba:** None.

Poster

389. Central Mechanisms of Pain

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Program #/Poster #: 389.09/W12

Topic: D.03. Somatosensation: Pain

Support: NRF-2016M3C7A1905074
2016R1A2B4009409

Title: Evans blue reduces neuropathic pain behavior by inhibiting spinal ATP release

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Abstract: Evans blue (EB), an azo dye used to assess the permeability of the blood-brain barrier to macromolecules, is also known as a vesicular nucleotide transporter (VNUT) inhibitor. Because VNUT plays an essential role in the vesicular storage of ATP and because the activation of purinergic receptors in the spinal cord by extracellular ATP is essential for neuropathic hypersensitivity, we investigated whether EB could decrease neuropathic pain in a L5 spinal nerve ligation (SNL) rat model. Surprisingly, intrathecal injection of EB efficiently and dose-dependently attenuated pain behavior (mechanical allodynia) for 7 days (5-50 μg). EB co-localized with neurons, but not with astrocytes and microglia, in the dorsal horn. The ATP assay showed decreased ATP concentration in the cerebrospinal fluid (CSF). Additionally, we confirmed EB blocked ATP release from neurons but not from glia *in vitro*. EB injection also decreased microglial activity in the spinal dorsal horn. Moreover, reactive oxygen species (ROS) production and proinflammatory mediators, such as IL-1β and IL-6, were decreased in the EB-treated group. Taken together, these data demonstrate that EB inhibited ATP release from

terminals of primary afferent neurons but not from astrocytes or microglia in a SNL-induced rat model. Eventually, the EB-inhibited release of ATP suppressed the ATP/purinergic receptor signaling involved in the spinal microglial activation associated with the pathophysiology of neuropathic pain. The results show that EB has an analgesic effect on neuropathic pain in the SNL animal model and is a new potential therapeutic for the treatment of chronic pain (NRF-2016M3C7A1905074, 2016R1A2B4009409).

Disclosures: Y. Yin: None. **J. Hong:** None. **J. Shin:** None. **D. Gwon:** None. **H. Kwon:** None. **N. Shin:** None. **H. Shin:** None. **S. Lee:** None. **W. Lee:** None. **D. Kim:** None.

Poster

389. Central Mechanisms of Pain

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Program #/Poster #: 389.10/W13

Topic: D.03. Somatosensation: Pain

Support: Grants-in-Aid for Scientific Research(KAKEN) 17K16718

Title: A novel rat model of neuropathic pain established by clamping the sciatic nerve shows mechanical allodynia and microglial hyperplasia in the spinal cord

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Abstract: Background: Neuropathic pain is difficult to treat and remains a major public health problem. Current treatments provide only limited therapeutic effect, and regenerative medicine is expected to be a curative therapy. In previous research, traditional animal models were often used. Although these models are useful to elucidate the mechanisms of pharmacological therapies, they are not suitable for the evaluation of recent nerve regeneration therapies because of the presence of residual threads at the injury site. Thus, animal models characterized by prolonged neuropathic pain without residual threads are necessary. Here, a novel rat model of neuropathic pain established by clamping the sciatic nerve was assessed.
Materials and methods:
Eight-week-old male Wister rats were anesthetized, following which their left sciatic nerve was exposed and clamped with surgical clips that applied a force of 60 g. Animals were divided into 1, 5, and 10 minutes clamped and sham-operated groups.
The threshold responses to mechanical and thermal stimuli were tested on both sides of their hind paws with the von Frey filaments and Hargreaves tests. Mechanical and thermal thresholds were assessed weekly until 56 days after injury.
Immunohistochemical assessment was performed at 21 days after injury. The expression of Iba-

1, a microglial marker, in the L5 spinal cord segments and ATF3, a marker of axotomy, in the dorsal root ganglia (DRG) was evaluated with immunofluorescent staining.
Results: Mechanical thresholds decreased significantly in all three experimental groups after sciatic nerve clamp injury from days 4 to 21 compared with the sham group. Thermal thresholds were slightly decreased in clamped groups. Both allodynia and thermal hyperalgesia recovered to the baseline state before 35 days after injury.
In the immunohistochemical experiments, the Iba1-positive areas significantly increased in the experimental groups compared with the contralateral side. Some L5 DRG neurons were positive for ATF3 on the experimental side, with no expression found on the contralateral side.
Conclusions: A novel rat model of neuropathic pain, without any threads, was assessed. The model rats showed statistically significant mechanical allodynia,
microglial hyperplasia in the spinal dorsal horn, and axonopathy. These results confirmed that the model rats indeed exhibited characteristics of neuropathic pain. These symptoms recovered to the baseline state after a certain period. This model is easily producible, does not impede nerve regeneration, and consequently can be used to develop nerve regenerative therapies and further elucidate mechanisms of neuropathic pain.

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Poster

389. Central Mechanisms of Pain

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Topic: D.03. Somatosensation: Pain

Support: NRF-2016M3C7A1905074
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NRF-2017R1D1A1B03028839

Title: p38 siRNA-encapsulated PLGA nanoparticles alleviate neuropathic pain behavior in rats by inhibiting microglia activation

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Konyang Univ., Daejeon, Korea, Republic of; [6]Dept. of Anat., Brain Res. Institute, Chungnam Natl. Univ. Sch. of Med., Daejeon, Korea, Republic of

Abstract: Aim: To investigate whether p38 siRNA-loaded nanoparticles (p38 siRNA NPs) attenuate spinal nerve ligation (SNL)-induced neuropathic pain in rats by suppressing spinal microglia activation via p38 targeting. **Experimental:** After synthesizing p38 siRNA NPs with sonication, physical characteristics were measured for size and zeta potential. p38 siRNA NPs were then administrated intrathecally into SNL rats if they could reduce pain behavior excellently. **Results:** p38 siRNA NPs significantly reduced mechanical allodynia as well as microgliosis in the spinal dorsal horns of SNL rats, consistent with a downregulation of p38-related proinflammatory mediators. **Conclusion:** As p38 in the spinal microglia plays a critical role in neuropathic pain, we expect that p38 siRNA NPs could be a promising tool for the treatment of neuropathic pain (NRF-2016M3C7A1905074, 2016R1A2B4009409).

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Poster

389. Central Mechanisms of Pain

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Topic: D.03. Somatosensation: Pain

Support: Funding from the United States Army Medical Research and Material Command.

Title: Global transcriptomic analysis of the rat brain during chronic neuropathic pain development

Authors: *J. L. CLIFFORD[1], R. KUMAR[2], S. SRINIVASAN[2], G. DIMITROV[2], A. GAUTAM[1], R. YANG[3], E. WORKMAN[4], J. WILLIAMS[3], R. CHAVEZ[4], N. SOSANYA[4], B. CHEPPUDIRA[4], R. CHRISTY[4], R. HAMMAMIEH[1]
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Abstract: Chronic pain afflicts roughly 44% of Service Members returning from Iraq and Afghanistan, and it is estimated that 100 million Americans suffer from this condition. The underlying molecular mechanisms of chronic pain development have remained elusive. The objective of this study is to identify gene expression changes in key brain regions during the onset and progression of neuropathic pain in a rodent model. We collected tissue from five brain

regions (thalamus, amygdala, cingulate cortex, insular cortex, and somatosensory cortex), from rats (n = 6/group) subjected to the spared nerve injury (SNI) protocol, a model for chronic neuropathic pain. Tissue was collected from the side of the brain contralateral to the SNI hind limb at 1, 3, 7, 14 and 21 days post-injury and RNA was extracted for mRNA sequencing (TruSeq total RNA protocol and Illumina HISCAN-SQ platform). Sequence data was processed through a novel workflow (pipeline) customized to provide the full complement of mRNA data (transcriptome) for this multidimensional data set. We first focused on the assessment of the transcriptome of the thalamus, which is an important center of pain processing in the brain. In rats experiencing chronic pain relative to controls, we observed a decreased expression of several neurotransmitter receptor mRNAs, including cholinergic receptors (CHRM4, CHRNB3, CHRNB4), dopamine receptors (DRD1, DRD2), the cannabinoid receptor (CNR1), adenosine A2a receptor (ADORA2A), bradykinin receptor B2 (BDKRB2); and associated G proteins, such as GPR52, GPR88, GNB3, and GNG7; and neurotransmitters such as TAC1 (substance P). The majority of these changes occurred at day 21, but not sooner. Our data also indicates suppressed signaling through the Extracellular Signal-Regulated Kinase (ERK1/2) pathway, as well as inhibited dopamine signaling, in the day 21 post-injury contralateral thalamus. These findings suggest a global suppression of neural signaling in the thalamus (desensitization), much of which does not commence until after 14 days post injury. This apparent desensitization remains to be confirmed by electrophysiological methods and does not correlate with reduced mechanical allodynia. The gene expression changes in the other brain regions were distinct and the majority non-overlapping. An integrated model for gene expression changes in the different brain regions during the transition from acute to chronic neuropathic pain is presented.

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Poster

389. Central Mechanisms of Pain

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NIH Grant U01HL117684
NIH research Fellowship F31NS092310

Title: Disrupting interaction of PSD95 with nNOS attenuates hemorrhage induced thalamic pain

Authors: *W. CAI, S. WU, Z. PAN, J. XIAO, Y. TAO
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Abstract: Hemorrhages occurring within the thalamus lead to a pain syndrome. Clinical treatment of thalamic pain is ineffective, at least in part, due to the elusive mechanisms that underlie the induction and maintenance of thalamic pain. The present study investigated the possible contribution of a protein-protein interaction between postsynaptic density protein 95 (PSD-95) and neuronal nitric oxide synthase (nNOS) to thalamic pain in mice. Thalamic hemorrhage was induced by microinjection of type IV collagenase into unilateral ventral posterior medial/lateral nuclei of the thalamus. Pain hypersensitivities, including mechanical allodynia, heat hyperalgesia, and cold allodynia, appeared at day 1 post-microinjection, reached a peak 5-7 days post-microinjection, and persisted for at least 28 days post-microinjection on the contralateral side. Systemic pre-treatment (but not post-treatment) of ZL006, a small molecule that disrupts PSD-95-nNOS interaction, alleviated these pain hypersensitivities. This effect is dose-dependent. Mechanistically, ZL006 blocked hemorrhage-induced increase of binding of PSD-95 with nNOS and membrane translocation of nNOS in thalamic neurons. Our findings suggest that the protein-protein interaction between PSD-95 and nNOS in the thalamus plays a significant role in the induction of thalamic pain. This interaction may be a promising therapeutic target in the clinical management of hemorrhage-induced thalamic pain.

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Poster

389. Central Mechanisms of Pain

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NIH research Fellowship F31NS092310

Title: ETA receptors contribute to sickle cell disease-associated pain via the NF-κB-triggered increase in $Na_v1.8$ in primary sensory neurons

Authors: *S. WU[1], B. M. LUTZ[2], A. BEKKER[1], Y.-X. TAO[1]
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Abstract: Sickle Cell Disease (SCD) is a hemoglobinopathy that is associated with acute painful episodes and persistent/chronic pain. Our previous study in humanized Townes mouse (HbSS) model of SCD reproted that pharmacological inhibition or neuron-specific knockout of endothelin type A (ETA) receptors in the dorsal root ganglion (DRG) alleviated basal and post-hypoxia-induced pain hypersensitivitities. We also showed that the ETA receptor inhibition blocked an increase in Nav1.8 in the HbSS DRG. The evidence indicates the potential involvement of the increased DRG Nav1.8 in ETA-mediated SCD-associated pain. Here, we further revealed that phosphorylated p65 (an indicator of NF-κB activation) levels in the HbSS DRG were significantly elevated compared to that in the HbAA (control) DRG. Behavioral observation showed that intraperitoneal administration of ammonium pyrrolidinedithiocarbamate (PDTC), a specific inhibitor of NF-κB, alleviated mechanical allodynia in the HbSS mice, without affecting the basal responses in the HbAA mice. Our chromatin immunoprecipitation showed that a fragment within the Scn10a (encoding Nav1.8) promoter (including two adjacent p65 binding motifs) was amplified from a complex immunoprecipitated with an anti-p65 antibody. This binding activity was markedly increased in the HbSS DRG compared to that in the HbAA DRG. The dual luciferase assay using a luciferase reporter vector containing the Scn10a promoter displayed that phorbol 12-myristate 13-acetate (PMA), an PKC activator, increased the activity of the Scn10a gene promoter. This increase was prevented when PDTC or bisindolylmaleimide (a PKC inhibitor) was co-administered. Our findings suggest the participation of NF-κB in ETA receptor-triggered Nav1.8 upregulation in the HbSS DRG neurons, which may provide a novel strategy for the management of SCD pain.

Disclosures: S. Wu: None. **B.M. Lutz:** None. **A. Bekker:** None. **Y. Tao:** None.

Poster

389. Central Mechanisms of Pain

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Topic: D.03. Somatosensation: Pain

Support: NIH Grant NS094664
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Title: Octamer transcription factor 1 in dorsal root ganglion contributs to neuropathic pain after peripheral nerve injury

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Abstract: Neuropathic pain genesis is related to peripheral nerve injury-induced gene alterations in the dorsal root ganglion (DRG). Transcription factors control gene expression. In this study, we investigated whether octamer transcription factor 1 (OCT1), a transcription factor, contributed to neuropathic pain caused by chronic constriction injury (CCI) of the sciatic nerve. CCI produced a time-dependent increase in the level of OCT1 protein in the ipsilateral L4/5 DRG, but not in the spinal cord. Blocking this increase through microinjection of OCT1 siRNA into the ipsilateral L4/5 DRG attenuated the initiation and maintenance of CCI-induced mechanical allodynia, heat hyperalgesia, and cold allodynia and improved morphine analgesia after CCI, without affecting locomotor functions and basal responses to acute mechanical, heat, and cold stimuli. Mimicking this increase through microinjection of recombinant adeno-associated virus 5 expressing full-length OCT1 into the unilateral L4/5 DRG led to significant mechanical allodynia, heat hyperalgesia and cold allodynia in naive rats. Mechanistically, OCT1 participated in CCI-induced increases in *Dnmt3a* mRNA and its protein and DNMT3a-mediated decreases in *Oprm1* and *Kcna2* mRNAs and their proteins in the injured DRG. These findings indicate that OCT1 may participate in neuropathic pain at least in part by transcriptionally activating *Dnmt3a* and subsequently epigenetic silencing of *Oprm1* and *Kcan2* in the DRG. OCT1 may serve as a potential target for therapeutic treatments against neuropathic pain.

Disclosures: Y. Tao: None. **J. Yuan:** None. **S. Wu:** None. **A. Bekker:** None.

Poster

389. Central Mechanisms of Pain

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Topic: D.03. Somatosensation: Pain

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Title: Endothelin-type A receptors mediate pain in a mouse model of sickle cell disease

Authors: *J. XIAO[1], B. LUTZ[2], S. WU[3], A. BEKKER[2], Y. TAO[4]
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Abstract: Sickle cell disease (SCD), resulting from an amino acid substitution in the β globin chain of hemoglobin, is associated with acute painful episodes and chronic intractable pain. Endothelin-1 (ET-1) can induce pain in humans and rodents. Given that its level in the blood

plasma is elevated in the SCD patients and SCD mouse models, ET-1 may play a key role in the SCD-associated pain. Here we reported that the levels of ET-1 and its endothelin type A (ETA) receptor were increased in the dorsal root ganglia from humanized Townes mouse (HbSS) model of SCD. Pharmacologic inhibition or neuron-specific knockdown of ETA receptors in primary sensory neurons of dorsal root ganglion alleviated basal and post-hypoxia evoked pain hypersensitivities in the HBSS mice. Our findings suggest that ETA receptors are the potential targets for the management of SCD-associated pain, although this expectation needs to be further verified in clinic settings.

Disclosures: J. Xiao: None. **B. Lutz:** None. **S. Wu:** None. **A. Bekker:** None. **Y. Tao:** None.

Poster

389. Central Mechanisms of Pain

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National Natural Science Foundation of China 31300905, 31471308, 31671057

Title: The mechanistic target of rapamycin (mTOR) pathway in dorsal root ganglion (DRG) neurons and spinal cord microglia differentially contributes to neuropathic pain

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Abstract: mTOR mediated signaling plays an important role in a wide range of biological processes. Studies have indicated that activation of the mTOR pathway is implicated in the neuropathic pain. However, the cell type in which mTOR activation is involved to regulate pain remains unclear. Identifying the cell type in which mTOR pathway participates in is vital for specific and precise management of neuropathic pain. To identify where mTOR was activated to modulate pain onset and maintenance, we used the spared nerve injury (SNI) model, in combination with DRG and microglia-specific deletion of mTOR, and pharmacological blockade to determine the role of mTOR signaling in different cells for pain genesis. We found that Rapamycin can dramatically alleviated the pain induced by the peripheral nerve injury at early stages (Day 1 to Day3 after the injury). Interestingly, we found mTOR activation in both dorsal horn microglia and DRG neurons. In DRG neurons, mTOR activation peaks at day 1, whereas in the dorsal horn microglia, mTOR activation peaks at day 3 after the nerve injury. Specific deletion of mTOR in microglia substantially reduced microglia proliferation in the dorsal horn; however, no significant changes in the pain behavior, suggesting that

microglia mTOR pathway minimally contribute to the pain. We are carrying out more experiments to determine the role of DRG mTOR in neuropathic pain generation and development.

Disclosures: Y. Hu: None. **L. Chen:** None.

Poster

389. Central Mechanisms of Pain

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 389.18/X3

Topic: D.02. Somatosensation

Support: CONACyT Grant 255548

Title: Nociceptive stimulation produces non random (structured) changes in the timing and direction of the information flowing between the dorsal horn neurons and the brainstem nuclei

Authors: *N. PLAMENOV DONCHEV[1], L. MORENO[1], A. RAMIREZ[1], D. CHAVEZ[1], E. HERNÁNDEZ[1], B. ÁLVAREZ[1], S. GLUSMAN[1,2], P. RUDOMIN[1,3]
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Abstract: We have shown that the nociceptive stimulation induced by the intradermal injection of capsaicin produces a non-random increase in correlation between the dorsal horn (DH) neuronal ensembles involved in the generation of the spontaneous L4-L7 cord dorsum potentials (CDPs). These changes underlie the development of inflammatory-neuropathic pain, are supraspinally modulated and transiently reversed by low doses of i.v. lidocaine (Contreras-Hernández et al., J. Physiol 2018). We now studied the effects produced by capsaicin and lidocaine on the functional relationship between the concurrent activity of spinal & supraspinal structures. In 2 anesthetized, paralyzed and artificially ventilated cats, we continuously recorded spontaneous L4-L7 CDPs, L6 DH intraspinal fields as well as ongoing activity in the brainstem Reticular Formation (ReF) and N. Raphe Magnus (NRM). Using the Pearson's coefficient, we found that capsaicin produced a differential increase in the correlation between the spontaneous CDPs and supraspinal potentials during the first 30 min after the onset of nociception. Power Spectrum Density analysis of the ongoing activity further indicated that the main changes occurred within the theta range (3-8 Hz). All these effects were temporarily reduced by i.v. lidocaine. In addition, we used Granger's statistical causality to examine, by means of autoregressive forecastability, the extent to which a particular time series of potentials influenced the activity generated in the other recording sites (Ding and Chen, Handbook of time series analysis, Ch 17, 2006). This allowed appraisal of the changes induced by nociception on the information flowing between the spinal and supraspinal structures. The obtained results appear to

indicate that in control conditions, the information flow between DH and the brainstem was essentially bidirectional. By 10 min after the onset of nociception, there was an initial increase in the ascending information, mostly from the DH to the ReF. Later on, the direction of the information flow reversed and was mainly from the NRM to DH laminae III-V. Following i.v. lidocaine, the descending flow of information was gradually and reversibly reduced. In summary, our data disclose the dynamics of the changes induced by nociceptive stimulation on signal processing of the spinal-supraspinal loop, as well as on the direction of the information flowing between these structures and its reversal by systemic lidocaine. These observations provide a novel approach to examine the development of nociceptive-induced supraspinal control on the functional connectivity between the spinal neurons leading to neuropathic pain.

Disclosures: N. Plamenov Donchev: None. **L. Moreno:** None. **A. Ramirez:** None. **D. Chavez:** None. **E. Hernández:** None. **B. Álvarez:** None. **S. Glusman:** None. **P. Rudomin:** None.

Poster

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Topic: D.02. Somatosensation

Support: ERC Advanced Grant (PainCells 740491)
Wallenberg Scholar and Wallenberg project grant
Ragnar Söderberg Foundation
Brain Foundation

Title: Neuronal atlas of the dorsal horn defines its architecture and links sensory input to transcriptional cell types

Authors: *M. HAERING[1], A. ZEISEL[1], H. HOCHGERNER[1], P. RINWA[1], J. E. T. JAKOBSSON[2], P. LÖNNERBERG[1], G. LA MANNO[1], N. SHARMA[1], L. BORGIUS[1], O. KIEHN[1], M. LAGERSTRÖM[2], S. LINNARSSON[1], P. ERNFORS[1]
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Abstract: Being aware of and discriminating between different types of cues through the somatosensory system is a critical function for interpreting the internal and external world and for protection from tissue damage. Primary sensory neurons in the dorsal root ganglion (DRG) display distal nerve projections in the skin and deep tissues. Upon activation by a diverse set of chemical, thermal or mechanical stimuli, they relay this information through their central terminations onto neurons located in the dorsal horn of the spinal cord. The dorsal horn of the spinal cord plays a critical role for discriminating different stimuli as well as processing distinct modalities of innocuous and noxious sensation. However, little is known of the neuronal

subtypes involved, hampering efforts to explain principles governing somatic sensation. Here, we used single-cell RNA sequencing to classify sensory neurons in the mouse dorsal horn. We identified 15 inhibitory and 15 excitatory molecular subtypes of neurons, equaling the complexity in cerebral cortex. Validation of our classification scheme *in vivo*, and matching cell types to anatomy of the dorsal horn by spatial transcriptomics reveals laminae enrichment for all cell types. Neuron types, when combined, define a multilayered organization with like neurons layered together. Employing our scheme, we find different sensory stimuli to activate discrete sets of both excitatory and inhibitory neuron types. This work provides a systematic and comprehensive molecular classification of spinal cord sensory neurons enabling functional interrogation of sensory processing.

Disclosures: M. Haering: None. **A. Zeisel:** None. **H. Hochgerner:** None. **P. Rinwa:** None. **J.E.T. Jakobsson:** None. **P. Lönnerberg:** None. **G. La Manno:** None. **N. Sharma:** None. **L. Borgius:** None. **O. Kiehn:** None. **M. Lagerström:** None. **S. Linnarsson:** None. **P. Ernfors:** None.

Poster

389. Central Mechanisms of Pain

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Program #/Poster #: 389.20/X5

Topic: D.02. Somatosensation

Support: NHMRC Grant 631000 and 1043933

Title: An optogenetic dissection of parvalbumin^{+} interneuron mediated presynaptic and postsynaptic inhibition in spinal cord sensory circuits

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Abstract: The dorsal horn (DH) of the spinal cord is an essential region for the appropriate encoding of sensory signals. The role of inhibitory interneurons in processing sensory inputs at the spinal level is critical to this sensory encoding. Parvalbumin-positive interneurons (PVINs) represent one inhibitory subpopulation known to play an important role in the segregation of tactile and nociceptive signals (Petitjean, 2015 Cell rep 13:1246). The precise circuitry underlying this role, however, remains unclear. To better understand the connectivity underlying the role of PVINs in sensory encoding we have taken an optogenetic approach, using transgenic mice that express Channelrhodopsin-2 in PVINs. Adult mice (30 ± 3 wks, both sexes) were deeply anaesthetized with ketamine (100 mg/kg, i.p.) and decapitated. Parasagittal spinal cord slices were prepared from the lumbar cord. Whole cell patch clamp recordings were made from

unidentified neurons and slices photostimulated using brief whole-field illumination (488nm, 1ms). Synaptic responses were recorded using CsCl- (inhibitory responses), and K-gluc based internals (excitatory responses). During inhibitory recordings, photostimulation evoked short latency mixed GABA/Glycine inhibitory postsynaptic currents (ie, bicuculline and strychnine sensitive) in 79% and 30% of neurons within LIIi-III and LI-IIo, respectively. These recordings showed a broad range of postsynaptic targets, with IPSC amplitude in both putative inhibitory and excitatory interneuron populations. During excitatory recordings photostimulation evoked longer latency bicuculline and temperature sensitive EPSCs in 38% and 49% of neurons within LIIi-III and LI-IIo, respectively. We were also able to demonstrate powerful PVIN mediated inhibition of primary afferent evoked EPSCs (ie presynaptic inhibition). Together these data provide strong evidence for PVIN mediated presynaptic inhibition, which could be mapped using optogenetics. Unlike postsynaptic inhibition, PVINs provided powerful presynaptic inhibition to specific neural circuits, with the strongest connections observed on a population of inhibitory 'deep' islet cells. Together our findings indicate PVINs provide distributed inhibitory control over interneurons within LI-III of the dorsal horn. PVINs also provide powerful, and specific presynaptic inhibition onto myelinated afferent fibers, and our optogenetic approach provides a useful tool for the identification of downstream local interneuron circuits regulated by this form of inhibition.

Disclosures: M.A. Gradwell: None. **R.J. Callister:** None. **D.I. Hughes:** None. **B.A. Graham:** None.

Poster

389. Central Mechanisms of Pain

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Topic: D.02. Somatosensation

Support: NHMRC grant 631000 and 1043933

Title: Interconnectivity between parvalbumin^{+}interneurons in the spinal cord: Implications for dorsal horn oscillations

Authors: *B. A. GRAHAM[1], R. J. CALLISTER[1,2], M. A. GRADWELL[1], D. I. HUGHES[2]
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Abstract: The dorsal horn of the spinal cord is an essential region for the appropriate encoding of sensory signals. A number of studies have established Parvalbumin-positive interneurons (PVINs) as an important inhibitory subpopulation for the segregation of tactile and nociceptive signals within spinal circuits (Hughes, 2012 J Physiol 590:3927; Petitjean, 2015 Cell rep 13:1246). Given PVINs in other brain regions exhibit synaptic and electrical coupling and

generate oscillatory activity, our study has assessed the interconnectivity of spinal PVINs in the dorsal horn. This work was undertaken using an optogenetic approach in transgenic mice that express Channelrhodopsin-2 (ChR2) in PVINs. In addition, pairs of PVINs and other unidentified populations were recorded to directly assess individual connections. Adult mice (30 ± 3 wks, both sexes) were deeply anaesthetized with ketamine (100 mg/kg, i.p.) and decapitated before parasagittal spinal cord slices were prepared from the lumbar cord. Whole cell patch clamp recordings were made from PVINs (identified by ChR2-YFP) and slices photostimulated using brief whole-field illumination (488nm, 1ms). Synaptic responses were recorded using CsCl- (inhibitory responses), and K-gluc based internals (excitatory responses). During inhibitory recordings from PVINs, photostimulation caused an immediate photocurrent in the recorded cell but also caused optically-evoked postsynaptic currents oPSCs in many recordings occurring at short latency (4.9 ± 0.2 ms) and with limited jitter at onset (0.4 ± 0.1 ms). Allowing the time to recruit AP discharge following PVIN photostimulation (1.3 ms, cell attached), these latencies and limited jitter are consistent with direct monosynaptic connections (Lu and Perl 2003). Furthermore, oIPSCs were mixed GABA/Glycine inhibitory postsynaptic currents (ie, bicuculline and strychnine sensitive) and occurred in 61% (67/110) of PVINs. We were also able to demonstrate that photostimulation of PVINs mediated inhibition of primary afferents (ie presynaptic inhibition) and caused oEPSCs in other PVINs (81% - 89/110) that had longer latency (11.5 ± 1.5 ms), higher jitter (5.7 ± 4.1 ms), and were bicuculline sensitive. Together, these findings confirm PVINs are coupled through inhibitory synaptic connections (feed-forward inhibition) and also provide presynaptic inhibition to the same afferent fibers that excite them (feed-back inhibition). Together, these properties are consistent with the connectivity of PVINs in other CNS regions and suggest they are ideally configured to drive oscillations in the sensory circuits of the dorsal horn.

Disclosures: R.J. Callister: None. **M.A. Gradwell:** None. **D.I. Hughes:** None.

Poster

390. Pain: Descending Modulation

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Program #/Poster #: 390.01/X7

Topic: D.03. Somatosensation: Pain

Support: MEXT/JSPS KAKENHI 16H06817
MEXT/JSPS KAKENHI 16K11679

Title: Modulatory effect of psychophysical stress on orofacial nociception at the rostral ventromedial medulla in the rats

Authors: *M. KUROSE[1], M. HASEGAWA[2], Y. NAKATANI[1,3], S. SHIMIZU[1,3], N. FUJII[2], K. YAMAMURA[1], K. OKAMOTO[4]

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Abstract: *Introduction* Psychological stress is known to facilitate orofacial nociception. Preclinical studies revealed that psychophysical stress conditionings increased nociceptive neural activities in the medullary dorsal horn (Vc). The basis for increased Vc response could be due to functional changes of descending pain pathways from the rostral ventromedial medulla (RVM): however it remains unclear if psychophysical stress had modulatory effect on neural excitability at the RVM. *Material and Methods* Sprague-Dawley male rats were subjected to repeated forced swim stress (FST, 10 min/day) to induce psychophysical stress. After 3 day FST, we recorded neural activities from the RVM and suprahyoid muscle activity in the presence or absence of noxious heat stimulation to the facial skin. Although three types of neural activity termed as On-, Off- and Neutral-cells were identified, we focused on neural properties of On-cells because evidence revealed that On-cells have roles of pain facilitation. In separate study, we tested the effect of FST on Fos expression in the RVM. Results from FST rats were compared with those of sham rats. *Results* Noxious heat stimulation increased On-cell activities in FST and sham rats: however, quantitative analysis revealed that response magnitude of neural and muscle activities evoked by heat stimulation in FST rats appeared to be greater than that of sham rats. Further, FST rats displayed significant prolonged after-discharges compared with sham rats. The number of Fos positive cells in the RVM was significantly greater in FST rats than in sham rats. These findings indicated facilitatory roles of FST on neural activity in the RVM, although functional relationships between On-cell and Fos positive cells remain unclear. Repeated FST alone had no effects on On-cell activity and Fos expression in the absence of heat stimulation. Further, FST increased muscle activity evoked by heat stimulation compared to sham rats. *Discussion* The functional changes of descending pain controls indicated by increases in On-cell activities and Fos responses in the RVM can explain possible mechanisms for pain exacerbation at the orofacial region under psychophysical stress conditions.

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Poster

390. Pain: Descending Modulation

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Topic: D.03. Somatosensation: Pain

Support: NIH Grant DA1005562

NSF GRFP

Title: Diverse cell types within the vlPAG exhibit unique adaptations to membrane firing properties after inflammation

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Abstract: The periaqueductal grey (PAG) is an important integration site within the descending pain modulatory pathway that receives diverse inputs from throughout the brain. The ventrolateral region (vlPAG) is a key target for opioid induced analgesia mediated via its projection to the rostroventromedial medulla (RVM). The vlPAG is a highly heterogeneous region with diverse cell types that have yet to be characterized fully in terms of their response to noxious stimuli and opioids. Using whole-cell patch-clamp recordings of vlPAG neurons, we measured intrinsic membrane firing properties in naïve rats and defined 5 distinct cell-types: onset-spiking, fast-spiking, transiently fast-spiking, random-spiking, and dopamine neurons. Complete Freund's Adjuvant (CFA) injections into the hindpaw induced inflammation and hyperexcitability at 2 h post-CFA in a subpopulation of vlPAG neurons, including increased firing frequencies and altered firing patterns. The sensitized characteristics varied between individual cell types suggesting that subsets of neurons are specifically activated by the CFA. To address this variability we are evaluating the use of Fos, a protein marker for strong neuronal activity, as a tool to identify neurons within the vlPAG that are activated by inflammation. Fos expression is robustly increased 2 h after CFA, with a significant reduction 6 d after CFA that remains significantly greater than the naïve animals—suggesting dynamic changes in neuronal activity between early and persistent inflammation. In FosGFP transgenic rats activation of the cFos promoter drives expression of GFP, allowing us to identify and record membrane firing properties from Fos-positive and Fos-negative neurons *in vitro*. Studies will compare inflammation-induced changes at acute (2 and 24 h) and after persistent inflammation (6 d). Additionally, the studies use retrograde fluorescent CTb labeling from the RVM to examine intrinsic properties of vlPAG neurons that project to the RVM. The effects of opioids on the intrinsic membrane properties of these cell types will also be examined.

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Poster

390. Pain: Descending Modulation

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INPRFM grant NC12165994.0

Title: D2-like receptor agonist inhibits high-voltage activated calcium-current in small IB4 positive dorsal root ganglion neurons and produces specific analgesia in mechanonociceptive test

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Abstract: *Background:* The intrathecal (i.t.) administration of quinpirole, a dopamine D2-like receptor agonist, produces significant analgesia during mechanonociceptive stimulus but not with thermonociceptive stimulus in intact rats (Almanza et al., *Pharmacol Biochem Behav*, 2015: 137: 119-125). To find a cellular mechanism for the analgesic specific-effect through D2-like agonist on mechanonociception, we evaluated the effect of quinpirole on high voltage-activated (HVA) Ca^{2+} current (I_{Ca}) and the action potential (AP) discharge of small-diameter neurons of the dorsal root ganglia (DRG) from rats. *Methods:* Rat DRG neurons (P21-P25) were isolated and kept in culture (DMEM + N2 complement) less than 24 h. The I_{Ca} and the AP firing were recorded with the patch-clamp technique. Small-diameter DRG neurons (< 30 µm) were classified in two populations depending on its binding to isolectin B_4 (IB_4+/-), due to non-peptidergic IB_4+ and peptidergic IB_4- have been implicated in mechanonociceptive and thermonociceptive stimulus signaling, respectively. All the experiments in the present work were approved by our Institutional Ethics Committee. *Results:* Quinpirole inhibited significantly the HVA I_{Ca} in 11/21 small IB_4+ neurons, meanwhile, HVA I_{Ca} in peptidergic small IB_4- neurons were almost unaffected, only in 4/34 IB_4- neurons a modest but significant decrease of HVA I_{Ca} was measured. AP from small-IB_4+ and IB_4- neurons were recorded, quinpirole didn't affect DRG's AP firing produced by square or ramp current injection, in neither IB_4+ (n = 24) nor IB_4- (n = 34). *Conclusions:* Activation of D2-like receptors in non-peptidergic small-diameter DRG neurons (IB_4+) inhibits the HVA I_{Ca}, such inhibition could diminish neurotransmitter release at the spinal cord level leaving unaffected the DRG neurons AP-firing. These results could explain, at least in part, the specific analgesia of the D2-like agonist to mechanonociceptive stimuli. *Significance*: We present evidence about D2-like receptors activation modulates mechanonociception at primary afferent level through the inhibition of HVA I_{Ca} in IB_4+ neurons. This finding could convey to rational analgesic therapy for patients with mechanical hyperalgesia or allodynia.

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Poster

390. Pain: Descending Modulation

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Topic: D.03. Somatosensation: Pain

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Title: Disinhibition of rostral insular cortex produces pronociceptive activity in brainstem pain-modulating neurons and behavioral hyperalgesia

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Abstract: The insula processes information related to pain and bodily state, transmitting that information to higher centers, including amygdala and other cortical areas. However, it also has descending projections to brainstem regions. Activating the rostral agranular insular cortex (RAIC) in rats has shown to produce hyperalgesia, while inhibiting the RAIC has an antinociceptive effect. It has been suggested that the RAIC influences nociceptive processing via a descending pain modulation influence, but the underlying neural circuit remains largely unknown. The rostral ventromedial medulla (RVM) exerts a descending modulatory influence via pain-facilitating "ON-cells" and pain-inhibiting "OFF-cells". The aim of this study was to test the hypothesis that disinhibition of the RAIC produces hyperalgesia via activation of RVM ON-cells and/or suppression of the activity of RVM OFF-cells. We recorded heat-evoked paw withdrawal and activity of identified ON- and OFF-cells in lightly anesthetized Sprague-Dawley rats. Retrograde tracing demonstrated that the densest projection from the insula to the RVM arises in the RAIC. Following a microinjection of the $GABA_A$ receptor antagonist bicuculline (40 pmol/400 nL) in the RAIC, OFF-cell firing decreased and ON-cell firing increased for a prolonged period of time. NEUTRAL-cells, RVM neurons without a known role in pain modulation, were not affected by disinhibition of the RAIC. In addition, the threshold for paw withdrawal to heat decreased after bicuculline injection. These data suggest that the insular cortex exerts it effects on pain at least in part via the pain-modulating neurons in the RVM.

Disclosures: Y. Zhang: None. **M.M. Heinricher:** None.

Poster

390. Pain: Descending Modulation

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Topic: D.03. Somatosensation: Pain

Support: NIH Grant NS098660

Title: Parabrachial complex relays light-related information to brainstem pain-modulating circuitry

Authors: M. E. MARTENSON[1], E. E. HANSON[2], *M. M. HEINRICHER[3]
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Abstract: Individuals with chronic pain often experience increased sensitivity across multiple sensory modalities. This "multisensory hypersensitivity" is only now starting to be recognized as having an understandable neural basis. We recently showed that some pain-modulating neurons in the rostral ventromedial medulla (RVM) respond to visual light in lightly anesthetized rats, with activation of pain-facilitating ON-cells and suppression of pain-inhibiting OFF-cells. The pathways through which light information gains access to pain-modulating circuitry are thus an important step in understanding the interactions between pain and other sensory modalities. Initial studies showed that non-image forming pathways convey light-related information to the RVM. The goal of the present studies was to identify additional steps in the circuit linking light to pain. ON-cells (known to exert a net facilitating effect on spinal nociceptive processing) and OFF-cells (known to exert a net inhibiting effect on spinal nociceptive processing) were recorded in the rostral ventromedial medulla (RVM). These neurons have historically been defined by changes in activity during noxious somatic stimulation. Approximately half of the pain-facilitating ON-cells sampled, and a similar proportion of pain-inhibiting OFF-cells showed a change in firing with light. We investigated three possible relays through which information about ambient light could reach the RVM: the lateral parabrachial complex, central nucleus of the amygdala, and medial nucleus of the amygdala. Light-evoked changes in ON- and OFF-cell firing were attenuated by inactivation of the lateral parabrachial complex, but not the central or medial amgydala. The parabrachial complex receives interoceptive and exteroceptive sensory information, and is thought to convey threat signals to higher structures such as the amygdala. However, it also transmits information about noxious somatic stimuli to the RVM, where it regulates activity of pain-modulating systems. The present findings thus extend that idea, indicating that the parabrachial complex transmits a range of potentially threatening sensory information to descending control systems as well as to ascending systems

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Poster

390. Pain: Descending Modulation

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Title: Altered brainstem pain modulation circuit connectivity in chronic pain

Authors: *E. P. MILLS[1], Z. ALSHELH[1], F. DI PIETRO[1], R. AKHTER[2], G. M. MURRAY[2], C. C. PECK[1], L. A. HENDERSON[1]
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Abstract: Background and Aims: Evidence suggests that altered functioning within the brainstem pain modulation network contributes to the maintenance of some chronic pain conditions, for instance painful temporomandibular disorder (TMD), a chronic facial pain condition. TMD patients' abnormal responses to psychophysical paradigms like conditioned pain modulation[1], where "pain inhibits pain", indicate that patients with TMD may not effectively engage pain modulation systems. Despite these psychophysical findings, it is unknown whether the function of the brainstem pain modulation circuitry is altered in TMD subjects. The aim of this investigation is to use resting state functional magnetic resonance imaging (fMRI) to characterize brainstem modulation circuits in individuals with TMD and matched pain-free controls.
Methods: A resting state fMRI scan was performed on subjects with painful TMD (n=15, mean age: 35.3) and pain-free controls (n=45, mean age: 34.1). Using SPM12, fMRI images were realigned, movement and physiological noise removed and the brainstem isolated. The brainstem fMRI images were then spatially normalized to a brainstem-only template using the SUIT toolbox. Two key regions of the brainstem pain modulation network, namely the rostral ventromedial medulla (RVM) and subnucleus reticularis dorsalis (SRD), were selected as "seed" regions. The connectivity of these regions with each brainstem voxel was calculated and compared between TMDs and controls in a voxel-by-voxel analysis ($p<0.05$, small volume correction).
Results: Compared to controls, TMD subjects display enhanced functional connectivity between the RVM and the oralis division of the spinal trigeminal nucleus (SpV: mean ± SEM functional connectivity: *control*: 0.06±0.03, *TMD*: 0.30±0.07) and SRD (*control*: 0.10±0.03, *TMD*: 0.29±0.06). Using the SRD as a second connectivity seed, we found that TMD subjects show negative connectivity with another region important for pain modulation, the midbrain periaqueductal gray matter (*control*: 0.12±0.06, *TMD*: -0.22±0.08). Additionally, TMD subjects

show reduced SRD connectivity with the caudalis division of SpV (*control*: 0.29±0.05, *TMD*: 0.05±0.07).
Conclusions: These results reveal that TMD is associated with alterations in resting functional connectivity within the pain modulation network. The observed alterations primarily involve the pathways involving the SRD, a region critical for the well-described "pain inhibits pain" response. Dysfunction in this network may contribute to the abnormal pain processing reported in TMD subjects and the maintenance of pain in these individuals.
[1]*King et al., (2009) Pain, 143(3):172-178*

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Poster

390. Pain: Descending Modulation

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Topic: D.03. Somatosensation: Pain

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Title: Dopaminergic regulation of the circadian rhythm of pain perception

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Abstract: Pain is associated to desynchronization of circadian and biological rhythms and some diabetics, multiple sclerosis and arthritics patient refer different intensity of the pain along the day. Nowadays it is well-known that hypothalamic A11 area controls the pain through the dopaminergic receptors present in the dorsal horn of the spinal cord. We investigated: 1) the pain rhythmicity, 2) if the lesion dopaminergic A11 area can alter the diurnal pain behavior and 3) the dopaminergic receptors implicated in the pain rhythmicity regulation. Male Wistar rats were used to evaluate the threshold and rhythms of the pain in a model of mechanical allodynia using the next experimental groups: 1) Control L:D (Light:Dark 12h:12h); 2) D:D (Continuous darkness to 15 days); 3) Injured (Injection of 10 μg/μL of 6-OHDA in the A11 area) and 4) Antagonist (Intrathecal administration of SCH23390, L-741,626, GR-103691 and L-745,870). The results showed a rhythmic activity in the pain perception with 24 h period, 9:35 hours of maxim threshold and 2.67 of amplitude; the hypothalamic lesion A11 changed the maxim threshold to 13:00 h and decreased amplitude to 0.39 h. The administration of the SCH23390, L-

741,626, GR-103691 and L-745,870 dopaminergic antagonists decreased the threshold amplitude to 0.96, 1.84, 0.88 and 0.53, while the acrophase changed to 5:30, 13:83, 13:16 and 4.08 respectively. All together results demonstrate a direct regulation of circadian behaviour in the pain perception by the A11 area through differential activation of all kind of the dopaminergic receptors expressed in the lumbar spinal cord

Disclosures: C. Piña-Leyva: None. **L. Rodriguez-Sosa:** None. **B. Florán-Garduño:** None. **J.A. Gonzalez-Barrios:** None.

Poster

390. Pain: Descending Modulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 390.08/X14

Topic: D.03. Somatosensation: Pain

Title: The effect of transcranial direct current stimulation on conditioned pain modulation in healthy older adults

Authors: *B. WIND[1], A. MEEK[2], M. GODZA[3], Z. A. RILEY[1], K. M. NAUGLE[1]
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Abstract: Recent evidence suggests aging is associated with reduced endogenous pain inhibitory capacity, placing older adults at risk for developing persistent pain. Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique capable of modulating excitability of cortical and cerebellar neurons. Recent work shows tDCS of the motor cortex (M1) improves pain inhibitory capacity in healthy young adults. The purpose of this study is to examine the effects of anodal tDCS on descending pain inhibition in healthy older adults. This study has enrolled 11 older adults, with enrollment ongoing. Subjects completed three sessions on separate days that include one of the following 15-minute experimental conditions during each session: (1) anodal M1 tDCS, (2) anodal cerebellar tDCS, and (3) sham tDCS. Order of experimental conditions were randomly assigned per session. A dynamic quantitative sensory test called conditioned pain modulation (CPM) was used to assess pain inhibitory capacity pre and post tDCS. A 3 (condition: M1, Cerebellum, Sham) x 2 (time: pre-tdcs, post-tdcs) repeated measures ANOVA was conducted to compare CPM scores. Results showed a main effect of time, p=.016. Regardless of condition, pain inhibition on the CPM test decreased from pre-tdcs (15.3±3.0) to post-tdcs (3.7±2.4). These results indicate both tDCS of M1 and cerebellum are ineffective in enhancing endogenous pain inhibitory capacity on the CPM test in healthy older adults.

Disclosures: B. Wind: None. **A. Meek:** None. **M. Godza:** None. **Z.A. Riley:** None. **K.M. Naugle:** None.

Poster

390. Pain: Descending Modulation

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Program #/Poster #: 390.09/Y1

Topic: D.03. Somatosensation: Pain

Support: NIH Grant NS019296
NIH Grant DE025137

Title: Presynaptic inputs to serotonin-containing neurons of the mouse rostral ventromedial medulla

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Abstract: It has been recognized that 5-HT-containing neurons in the rostral ventromedial medulla (RVM) project to spinal dorsal horn and modulate spinal nociceptive transmission. Recent studies have demonstrated that active 5-HT-dependent descending facilitation after nerve and tissue injury contributes to central mechanisms underlying the development of secondary hyperalgesia and the maintenance of persistent pain and. Although a large number of early studies indicate comparative afferent projections to the RVM in the rat, cat and monkey brain by using conventional tracing approaches, the circuits controlling 5-HT neuronal activity in the RVM remains uncharacterized. To identify local and long-range inputs that strictly target 5-HT neurons in the RVM, we applied a genetically restricted two-viral tracing strategy to target 5-HT neurons of Sert-Cre mice. At 4 w after the first microinjection of helper virus (AAVDJ-EF1a-DIO-HTB) into the RVM, we sterotaxically delivered a genetically modified rabies virus pseudotyped with the avian virus envelope protein (EnvA-ΔG-RV-mCherry) in the same region. To compare afferent inputs to the RVM, we also microinjected the retrograde tracer fluoro-gold into the RVM of wildtype mice. We found that AAV-infected expression of TVA was restricted to the RVM and most of them were 5-HT-immunoreactive. Similar to the local distribution of FG-labeled neurons, we observed a substantial numbers of RV-infected presynaptic neurons within the RVM and medial parts of the pontine reticular nucleus, suggesting local control of 5-HT neurons from other non-5-HT neurons. We also confirmed that there were a large number of presynaptic neurons in the ventrolateral regions of the midbrain periaqueductal gray (PAG) and the dorsomedial nucleus (DMN) of hypothalamus. A small to moderate number of presynaptic neurons were observed in the locus coeruleus and the pontine reticular tegmentum. Different

from FG-labeled neuronal distribution, we found few presynaptic neurons in primary sensory cortex and dorsal raphe. We did not observe presynaptic neurons in the preoptic area, medial prefrontal cortex, anterior cingulate cortex, insular cortex, amygdala, paraventricular nucleus of hypothalamus and parabrachial nuclei. Our results dissect the whole-brain monosynaptic inputs specifically onto 5-HT RVM neurons. The descending 5-HT system mainly receives synaptic inputs and regulation from local neurons in the RVM and higher structures including the ventrolateral PAG and the hypothalamic dorsomedical nucleus linking descending pain modulation and neuroendocrine/autonomic response during stress, respectively.

Disclosures: C. Bian: None. **R. Hu:** None. **Y. Du:** None. **J. Yang:** None. **J. Asgar:** None. **W. Guo:** None. **R. Dubner:** None. **K. Ren:** None. **F. Wei:** None.

Poster

390. Pain: Descending Modulation

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 390.10/Y2

Topic: D.03. Somatosensation: Pain

Support: NIH Grant 12385358

Title: Analysis of the role of glutamatergic and GABAergic ventrolateral periaqueductal grant (vlPAG) neuronal subpopulations in a mouse model of persistent inflammatory pain

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Abstract: It has been estimated that 100 million adults suffer from chronic pain in the United States, with an annual societal cost of approximately 600 billion dollars. Endogenous analgesic pathways represent an alternative route for the development of new therapies for chronic pain. Pharmacological and electrical stimulation studies have demonstrated the role of the ventrolateral periaqueductal gray (vlPAG) in descending pain modulation. It has been proposed that tonic GABAergic neurotransmission at the level of the vlPAG serves to inhibit efferent excitatory projections that mediate descending analgesia. Disinhibition of vlPAG excitatory neurons is thought to allow subsequent activation of rostromedial ventral medulla (RVM) neurons that project to the spinal cord dorsal horn and inhibit nociception. Nevertheless, the experimental manipulations used in prior studies lack cell-type specificity, preventing unambiguous determination of the role of specific subsets of vlPAG neurons in analgesia. Techniques such as chemo- and opto-genetics now afford us the opportunity to selectively

manipulate identified subclasses of vlPAG neurons allowing for precise identification of circuit components critical for analgesia. With the GABA disinhibition hypothesis as our model, we hypothesized that stimulation of excitatory vlPAG neurons or a reduction of in vlPAG GABAergic tone would result in analgesia. We find chemogenetic stimulation of glutamatergic (Vglut2) or inhibition of GABAergic (Vgat) vlPAG neurons results in an elevation of withdrawal thresholds to noxious stimuli in naïve animals. In the context of persistent inflammatory pain, we find that optogenetic stimulation of Vglut2 or chemogenetic inhibition of Vgat vlPAG neurons results in attenuation of inflammation-induced hyperalgesia. To further characterize the circuitry, anatomical tracing experiments supports the notion that glutamatergic PAGàRVM projections, but not GABAergic projections, are responsible for engaging descending analgesic pathways that result in analgesia in both naïve and inflammatory states. We provide direct experimental evidence for the proposed analgesic role for glutamatergic projections from the PAG to the RVM. In brief, our findings support the GABA disinhibition hypothesis, highlighting the role of local tonic GABAergic neurotransmission at the level of thevlPAGas an analgesic gatekeeper.

Disclosures: J.G. Grajales-Reyes: None. **V.K. Samineni:** None. **B.A. Copits:** None. **M.R. Bruchas:** None. **R.W. Gereau:** None.

Poster

390. Pain: Descending Modulation

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Program #/Poster #: 390.11/Y3

Topic: D.03. Somatosensation: Pain

Support: Nukada Institute for Medical and Biological Research neuropathic pain research grant

Title: Endogenous oxytocin release in periaqueductal gray eased inflammatory pain through long term suppression of pain perception neurons in the spinal cord

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Abstract: When a pain stimulus is added to healthy animal body, oxytocinergic (OT) neurons in perventricular nucleus (PVN) are activated. OT neurons in PVN project to periaqueductal gray (PAG), and the concentration of OT increases in response to pain input. In view of those, we hypothesize that pain input induces firing of OT neurons in PVN, hence releasing OT locally in PAG. Additionally, previous study reported that the increase of OT concentration in PAG suppressed pain perception. Further, In PAG, administration of OT to PAG resulted in increase of firing of OT receptor (OTR) expressing neurons. Therefore, we can infer that OT achieve

analgesia through facilitation of firing of OTR-expressing neurons in PAG. Based on the background above, my project aims to characterize i) the firing dynamics of neurons in PAG in response to endogenous OT release in PAG, examine if ii) OT release in PAG induces the suppression of pain perception neurons in the spinal cord, and check iii) if inflammatory pain can be eased by it.

Disclosures: M. Iwasaki: None. **A. Charlet:** None.

Poster

390. Pain: Descending Modulation

Location: SDCC Halls B-H

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Topic: D.03. Somatosensation: Pain

Support: NIH Grant NS038261
NIH Grant NS081121
NIH Grant NS106902

Title: KOR activation in the central amygdala increases spinal dorsal horn neuronal activity through an action on amygdala CRF neurons

Authors: *G. JI[1,2], E. NAVRATILOVA[3], F. PORRECA[3], V. NEUGEBAUER[1,2]
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Abstract: Patients with functional pain syndromes (FPS) often experience intermittent episodic pain related to “triggers” including stress. Stress-induced activation of kappa opioid receptors (KORs) has been shown to promote negative affective states, including depression and anxiety. KORs are found in multiple brain regions relevant to pain modulation, including the central nucleus of the amygdala (CeA). The amygdala is a limbic brain area that plays a key role in emotional responses and averse affective states and disorders such as learned fear, anxiety, depression, and pain. We hypothesize that KOR signaling in the amygdala promotes functional pain responses through an action that involves corticotropin releasing factor (CRF) neurons. Our previous work identified the CRF system as a key player in pain-related amygdala plasticity and pain modulation. In this study, systems electrophysiology was used to determine the consequences of KOR activation in the amygdala on spinal dorsal horn neurons in transgenic CRF-Cre rats and the contribution of amygdala CRF neurons. Extracellular single-unit recordings of wide dynamic range (WDR) neurons were performed in the spinal dorsal horn of adult anesthetized (isoflurane, 2%) CRF-Cre rats. Background activity and evoked responses (spikes/s) to brief (15 s) innocuous and noxious mechanical test stimuli applied to the hindpaw were measured before and after stereotaxic administration of a KOR agonist (U-69,593) in the

right CeA (ipsilateral to spinal recording site) by microdialysis. U-69,593 increased the responses of WDR neurons to innocuous and noxious mechanical stimuli and this effect was inhibited by optogenetic silencing of CRF neurons in the CeA in CRF-Cre rats injected with AAV-EF1a-DIO-eNpHR3.0-EYFP into the CeA. Optical silencing was done with yellow light pulses (590 nm) delivered through an optical fiber inserted into the CeA. The data provide direct evidence for facilitatory effects of KOR activation in the amygdala on spinal nociceptive processing through a mechanism that engages amygdala CRF neurons.

Disclosures: G. Ji: None. **E. Navratilova:** None. **F. Porreca:** None. **V. Neugebauer:** None.

Poster

390. Pain: Descending Modulation

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Topic: D.03. Somatosensation: Pain

Support: NIH Grant NS038261
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NIH Grant NS106902

Title: Synaptic transmission in different amygdala neuronal cells types under normal condition and in a neuropathic pain model

Authors: *T. KIRITOSHI[1], V. A. YAKHNITSA[1], V. NEUGEBAUER[1,2]
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Abstract: The central nucleus of the amygdala (CeA) is comprised of distinct populations of GABAergic neurons identified by molecular markers such as corticotropin releasing factor (CRF) and somatostatin (SOM), and plays a key role in emotional aspects of pain. The CeA receives polymodal sensory information via the basolateral nucleus (BLA) and purely nociceptive information via parabrachial nucleus (PB). Pain-related changes at these synapses have been shown in different pain models by our group and others but cell-type specific differences in synaptic inputs remain to be determined. In this study, we analyzed synaptic input from PB and BLA to different CeA cell types under normal condition and in a neuropathic pain model. Whole-cell patch clamp recordings were obtained from lateral CeA neurons in brain slices from sham rats and neuropathic rats (4 weeks after L5 spinal nerve ligation, SNL model). Transgenic Crh-Cre rats and posthoc immunohistochemistry of recorded neurons were used to identify neuronal cell-types in the lateral CeA of the right hemisphere. In current clamp mode, action potential firing patterns were measured by injecting depolarizing currents. For selective activation of PB inputs to CeA neurons in amygdala brain slices, a viral vector (AAV) encoding

channelrhodopsin 2 (ChR2) under the control of the CaMKII promoter (AAV5-ChR2-CaMKII-eYFP) was injected stereotaxically into the right PB. BLA inputs were activated by electrical stimulation in the BLA. In voltage clamp mode, amplitudes of excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) and IPSC/EPSC ratio were measured. We found differences of EPSC and IPSC amplitudes and IPSC/EPSC ratio in different cell types. For example, regular spiking (RS) CRF-CeA neurons received stronger excitatory and weaker or no inhibitory input compared to SOM-CeA and non-RS cells. EPSCs of RS-CRF cells increased in slices from SNL whereas non-RS cells showed pronounced IPSCs. These results suggest that cell-type specific differences in synaptic transmission in CeA neurons under normal conditions and differential changes in neuropathic pain.

Disclosures: T. Kiritoshi: None. **V.A. Yakhnitsa:** None. **V. Neugebauer:** None.

Poster

390. Pain: Descending Modulation

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Topic: D.03. Somatosensation: Pain

Support: NIH Grant NS038261
NIH Grant NS081121
NIH Grant NS106902

Title: Group II metabotropic glutamate receptors, particularly mGluR2, in the amygdala regulate sensory and affective responses in a rodent model of arthritis pain

Authors: *M. MAZZITELLI[1], P. CONN[3], V. NEUGEBAUER[1,2]
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Abstract: Pain is a multidimensional experience with an important averse-affective dimension. The amygdala, a limbic brain area, plays a critical role in the emotional-affective aspects of behaviors and pain and in pain modulation. The central nucleus of amygdala (CeA) serves major output functions, and neuroplasticity in the CeA is mechanistically linked to pain-related behaviors in different pain conditions. The activation of Gi/o-coupled group II metabotropic glutamate receptors (mGluRs), which consist of mGluR2 and mGluR3, can decrease neurotransmitter release and regulate synaptic plasticity. Evidence from preclinical studies suggests that mGluR2/3 may be a target for neuropsychiatric disorders and can inhibit pain-related processing and behaviors, but the contribution of mGluR2 and 3 in the amygdala to pain-related behaviors remains to be determined. This knowledge gap was addressed here in a rodent

model of arthritis pain. Audible and ultrasonic vocalizations (averse affective response) and mechanical withdrawal thresholds were measured in adult rats before and 6 h after the induction of a kaolin/carrageenan-mono-arthritis in the left knee joint. Systemic application (intraperitoneally; 30 min before behavioral tests) of a group II mGluR agonist (LY379268 disodium salt) decreased the vocalizations and increased the spinal reflex thresholds of arthritic rats but had no effect under normal conditions. To determine the contribution of mGluRs in the amygdala, a group II mGluR antagonist (LY341495 disodium salt), a positive allosteric modulator for mGluR2 (PAM, LY487379 hydrochloride), or a negative allosteric modulator for mGluR2 (NAM, VU6001966) was applied stereotaxically into the right CeA (contralateral to the arthritic knee) by microdialysis. Stereotaxic administration of the group II antagonist and the mGluR2 NAM in the CeA reversed the effects of the systemically applied group II mGluR agonist. Stereotaxic administration of the mGluR2 PAM alone in CeA was able to mimic the effect of the systemically applied group II mGluR agonist in arthritic rats. These results suggest that group II mGluRs, and particularly mGluR2, in the amygdala can regulate pain-related behaviors and play a major role in the effects of systemic group II agonists.

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Poster

390. Pain: Descending Modulation

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Topic: D.03. Somatosensation: Pain

Support: NIH Grant NS038261
NIH Grant NS081121
NIH Grant NS106902

Title: Sex differences in fear extinction learning ability predicting pain behaviors

Authors: *P. D. PRESTO[1], G. JI[1,2], V. NEUGEBAUER[1,2]
[1]Pharmacol. and Neurosci., [2]Ctr. of Excellence for Translational Neurosci. and Therapeut., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: Sex differences in pain and disorders such as depression and anxiety are now being recognized. Pain and fear may share neurobiological mechanisms such as plasticity in emotional networks that include the amygdala. The amygdala plays a key role in fear conditioning and has emerged as an important node of emotional-affective aspects of pain modulation. Impaired fear extinction learning, which involves prefrontal cortical control of amygdala processing, has been linked to conditions such as posttraumatic stress disorder (PTSD). Here we tested the hypothesis that fear extinction learning ability can predict certain aspects of pain-related behaviors of rats

and that these may be different in female and male rats. We correlated fear extinction learning in adult male and female rats with behavioral outcome measures (sensory thresholds, vocalizations, and anxiety-like behaviors) before and >6h after induction of an arthritis pain model (kaolin/carrageenan-induced knee joint arthritis). Auditory fear conditioning, extinction, and extinction retention tests were conducted using two chambers. On Day 1 rats were habituated to context A followed by fear conditioning (2 US-CS pairs). On Day 2, rats were habituated to context B followed by extinction training (30 CSs). On Day 3, rats were habituated to context B followed by extinction retention measurement (5 CSs). There was no difference in fear learning between male and female rats. The majority of rats (78% male, 73% female) showed a quick decline of freezing level during extinction training and retention (FE+) whereas a smaller group of rats (22% male, 27% female) maintained a high freezing level (FE-). Male and female FE- rats had lower open-arm preferences in the elevated plus maze (EPM) or shorter center duration in the open field test (OFT) than FE+ rats, reflecting anxiety-like behavior, but there were no significant differences in sensory thresholds and vocalizations between FE+ and FE- types under normal conditions. In the arthritis pain model, male and female FE- rats developed higher levels of vocalizations and anxiety-like behavior than FE+ rats, but there were no differences in mechanical reflex thresholds. Female FE- rats had stronger vocalizations than FE- males. The data may suggest predictive value of fear extinction ability for emotional-affective pain aspects in male and female rats, and greater vulnerability of female than male rats with lower extinction ability.

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Poster

390. Pain: Descending Modulation

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Topic: D.03. Somatosensation: Pain

Support: NIH Grant NS038261
NIH Grant NS081121
NIH Grant NS106902

Title: KOR activation in the central amygdala enhances pain behaviors through an action on amygdala CRF neurons

Authors: *V. NEUGEBAUER[1,2], P. PRESTO[1], E. NAVRATILOVA[3], F. PORRECA[3], G. JI[1]
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Abstract: The amygdala plays an important role in emotional responses and affective states and disorders such as anxiety and depression. The amygdala also contributes to emotional-affective aspects of pain and pain modulation. Neuroplastic changes that lead to increased neuronal activity in the amygdala output region (central nucleus, CeA) have been found in different pain models. Corticotropin releasing factor and its CRF1 receptor have been linked to pain-related amygdala changes and behavioral consequences. Mechanisms of pain-related activation of amygdala CRF neurons are still not well understood. The dynorphin/kappa opioid receptor (KOR) system has been linked to stress related conditions such as anxiety and depression, and KOR activation produces dysphoria in humans and aversive behaviors in animals. Evidence points to the amygdala as a major site of action where KOR is expressed. Here we tested the hypothesis that KOR activation in the amygdala under normal conditions promotes pain-related emotional responses and anxiety-like behaviors through activation of amygdala CRF neurons. In adult transgenic CRF-Cre rats, emotional-affective responses were determined by measuring the duration of audible and ultrasonic vocalizations evoked by brief (15 s) innocuous and noxious mechanical test stimuli (compression of the paw). Anxiety-like behavior was assessed in the elevated plus maze (EPM) and open field test (OFT). Stereotaxic administration of a KOR agonist (U-69,593) in the right CeA by microdialysis increased audible and ultrasonic vocalizations and decreased the center duration of OFT. Systemic (intraperitoneal) application of U-69,593 also increased vocalizations and anxiety-like behavior. These effects were inhibited by optogenetic silencing of CRF-CeA neurons in CRF-Cre rats injected with AAV-EF1a-DIO-eNpHR3.0-EYFP. For optical silencing of CRF neurons yellow light pulses (590 nm) were delivered through an optical fiber implanted into CeA and attached to a head stage for wireless stimulation. The data provide direct evidence for facilitatory effects of KOR activation in the amygdala on pain-related emotional responses and anxiety-like behaviors through a mechanism that engages amygdala CRF neurons.

Disclosures: V. Neugebauer: None. **P. Presto:** None. **E. Navratilova:** None. **F. Porreca:** None. **G. Ji:** None.

Poster

390. Pain: Descending Modulation

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Topic: D.03. Somatosensation: Pain

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NIH Grant NS106902

Title: SK-channel function in different amygdala neuronal cell types under normal condition and in a neuropathic pain model

Authors: *V. A. YAKHNITSA[1], T. KIRITOSHI[1], V. NEUGEBAUER[1,2]
[1]Pharmacol. and Neurosci., [2]Ctr. of Excellence for Translational Neurosci. and Therapeut., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: The central nucleus of amygdala (CeA) plays a key role in the regulation of the emotional-affective component of pain. CeA neurons include neurochemically and functionally distinct cell types: GABAergic neurons express corticotropin releasing factor (CRF), somatostatin (SOM) and/or PKCdelta. Activation of CRF neurons and SOM neurons in the CeA contributes to anxiety-like behavior and emotional responses. Work from our group and others showed that increased activation of CeA neurons in acute and chronic pain models drives emotional-effective responses and anxiety-like behaviors. Here we tested the hypothesis that dysfunction of small-conductance calcium-activated potassium (SK) channels contributes to increased excitability of CRF-CeA and/or SOM-CeA neurons in a rat model of chronic neuropathic pain. Whole-cell voltage- and current-clamp recordings were made from latero-capsular CeA neurons in brain slices from behaviorally tested sham rats and neuropathic rats (spinal nerve ligation SNL model) 3-4 weeks after surgery. In transgenic Crh-Cre rats, rAAV5/Ef1a DIO-YFP or mCherry was injected stereotaxically into the CeA to label CRF somata. CRF-CeA neurons and non-CRF-CeA neurons were recorded, filled with biocytin through the patch pipette, and stained immunohistochemically for co-localization with SOM. In slices from sham rats, all neurons showed an apamin-sensitive medium afterhyperpolarization (mAHP). In brain slices from neuropathic rats that had developed mechanical hypersensitivity, increased vocalizations, and anxiety-like behavior, CRF-CeA neurons lacked an mAHP and SOM-CeA had a reduced mAHP, and both showed increased excitability measured as the number of spikes in response to depolarizing current injections (F-I function) compared to neurons from sham rats. The results suggest that SK-channel dysfunction may be more pronounced in CRF than SOM amygdala neurons in a neuropathic pain model.

Disclosures: V.A. Yakhnitsa: None. **T. Kiritoshi:** None. **V. Neugebauer:** None.

Poster

390. Pain: Descending Modulation

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Program #/Poster #: 390.18/Y10

Topic: D.03. Somatosensation: Pain

Support: NIH Grant NS038261
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NIH Grant NS106902

Title: Kappa opioid receptor mediated disinhibition of amygdala CRF neurons

Authors: *M. HEIN[1], V. YAKHNITSA[1], G. JI[1,2], E. NAVRATILOVA[3], F. PORRECA[3], V. NEUGEBAUER[1,2]
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Abstract: Neuroplastic changes in the central nervous system have been implicated not only in pain conditions associated with an identifiable injury, but also in functional pain syndrome (FPS), in which the pain cannot be attributed to tissue pathology. Mechanisms of FPS remain to be determined, but these conditions are typically triggered by stress, which can advance the pain condition from episodic to chronic. Corticotropin releasing factor and its CRF1 receptor in the amygdala have been linked to emotional-affective behaviors and pain modulation. The amygdala is also a major site of opioid receptors, including $G_{i/o}$-coupled kappa opioid receptors (KOR). KOR activation by its endogenous ligand dynorphin or agonists can have adverse effects. Here we tested the hypothesis that KOR activation disinhibits CRF neurons in the central nucleus (CeA) in uninjured rats. CeA serves major amygdala output functions. Brain slice electrophysiology was used to determine the effects of a KOR agonist (U-69,593) on CRF-CeA neurons. To visualize these neurons, AAV-EF1a-DIO-mCherry was injected into the right CeA of transgenic CRF-Cre rats (4 weeks old). To allow optical activation of glutamatergic afferent input from the lateral parabrachial area (LPB), AAV5-ChR2-CaMKII-eYFP was injected into the LPB. Animals were allowed to recover for four weeks for viral expression. Whole-cell patch-clamp recordings of CRF-CeA neurons were used to measure neuronal excitability (frequency-current F-I relationship), excitatory and inhibitory synaptic currents (EPSCs and IPSCs) evoked by optical activation of LPB terminals in the CeA or by electrical stimulation in the basolateral amygdala, and synaptically-evoked spiking (LPB evoked E-S coupling). U-69,593 decreased glutamate driven IPSCs (feedforward inhibition) and E-S coupling but had no effect on EPSCs and on F-I relationships. The data suggest that KOR activation under normal conditions leads to synaptic disinhibition of CRF-CeA neurons, which could result in increased pain responses and anxiogenic behavior.

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Poster

391. Pain Imaging and Perception

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Topic: D.03. Somatosensation: Pain

Support: KAKENHI Grant Number 18K07561

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Title: Changes in resting-state functional connectivity after cognitive behavioral therapy for chronic pain

Authors: *A. YOSHINO, Y. OKAMOTO, G. OKADA, S. YOKOYAMA, R. JINNIN, M. TAKAMURA, N. ICHIKAWA, S. YAMAWAKI
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Abstract: Introduction
The efficacy of cognitive behavioral therapy (CBT) for chronic pain has been demonstrated across clinical trials. However, there are few resting-state functional magnetic resonance imaging (rs-fMRI) studies. We used the intrinsic connectivity network (ICN) analysis to examine changes of neural activities after CBT and to assess whether brain regions predict clinical responses.
Methods
We assessed rs-fMRI data on a group of 29 chronic pain patients and 30 age-matched healthy controls (T1). Patients were enrolled in a weekly 12-session group CBT (T2). We used regions of interest such as the dorsal attention network (DAN) and sensorimotor network (SN) extracted by ICN analysis, exhibited differences in connectivity strength between the patients and controls at T1, and compared T1 and T2. We also examined the correlations between clinical effects and rs-fMRI data. All participants gave their written informed consent before participation, according to a protocol approved by the Hiroshima University ethics committee.
Results
Abnormal ICN connectivity of the orbitofrontal cortex (OFC) and inferior parietal lobule within the DAN and of the paracentral lobule within the SN in patients with chronic pain normalized after CBT. Increased ICN connectivity strength after CBT in the OFC indicated greater improvements in pain intensity. Furthermore, ICN connectivity strength in the dorsal posterior cingulate cortex (dPCC) within the DAN at T1 was negatively correlated with CBT-related clinical improvements.
Conclusions
We consider that the OFC is important for improvements of pain intensity by CBT, and that the dPCC activation at pretreatment also plays a key role in improvement of clinical symptoms via CBT.

Disclosures: **A. Yoshino:** 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.; Atsuo Yoshino has previously received support for a neuroimaging research from Eli Lily (2016-2017).. **Y. Okamoto:** None. **G. Okada:** None. **S. Yokoyama:** None. **R. Jinnin:** None. **M. Takamura:** None. **N. Ichikawa:** None. **S. Yamawaki:** None.

Poster

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Topic: D.03. Somatosensation: Pain

Support: R61AT009310

Title: Daily caffeine consumption does not modulate acupuncture analgesia and fMRI signal changes evoked by acupuncture stimulation

Authors: *J. CAO[1,3], Y. TU[1], C. LANG[1], J. PARK[1], M. VANGEL[2], J. LIU[1], R. GOLLUB[1], J. KONG[1]
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Abstract: Introduction: Caffeine is a common central nervous system stimulant and is found in coffee, tea, chocolate, energy drinks, and even some medications. Since caffeine is a potent adenosine receptor (A1) antagonist and acupuncture achieves its analgesic effects through the local release of adenosine and activation A1, caffeine consumption may interfere with the efficacy of acupuncture when used to treat pain. In this study, we examined whether individuals' daily intake of caffeine influenced acupuncture analgesia as measured by heat and pressure pain thresholds and fMRI brain responses during acupuncture stimulation. **Methods:** 24 subjects completed this study. We divided the subjects into two groups (high and low consumption groups, n =12 respectively) based on their daily caffeine consumption. Then we investigated baseline pain thresholds before each treatment and compared the difference in analgesia between the real and sham acupuncture in the two groups. fMRI data were collected using a 3.0 T MRI scanner while subjects completed two 9-minute scans during which intermittent intervention was performed. Data analysis was performed using SPM 12 with a threshold of $p < 0.001$ uncorrected and $p < 0.05$ FDR corrected. **Results:** We found that real acupuncture increased subject's pain thresholds compared to sham acupuncture, however, no significant differences between the two caffeine groups before vs. after real and sham acupuncture interventions in pain threshold changes was found.Imaging results indicated that real acupuncture activated the bilateral insula, secondary somatosensory cortex, and right caudate; and deactivated the bilateral anterior cingulate cortex, medial prefrontal cortex, left superior temporal gyrus, and right hippocampus. None of the three caffeine covariates (daily dose, duration of consumption, and their interaction) were statistically significant for any measurement. No significant differences between two caffeine consumption groups in pain thresholds at baseline, pre- to post-intervention threshold changes after real acupuncture treatment, and fMRI signal changes during acupuncture needle stimulation. **Conclusions:** In reality, the invasiveness evoked by acupuncture stimulation in

humans and rodents differ significantly since most acupuncture studies applied in animals have used regular human acupuncture needles. Therefore, the invasiveness and pain produced by acupuncture needles used in animal research are much greater than those experienced by humans. In summary, our results suggest that daily caffeine consumption does not interfere with the analgesic effects associated with acupuncture in healthy populations.

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Poster

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Topic: D.03. Somatosensation: Pain

Support: DP1 MH103908
Keck Foundation grant

Title: *In vivo* imaging and recording of general anesthesia activated central analgesic neurons

Authors: *B. CHEN, JR, T. HUA, S. ZHAO, B.-X. HAN, P. THOMPSON, L. JIANGXIE, J. LU, F. WANG
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Abstract: One of the key functions of general anesthesia (GA) is analgesia. We hypothesized that the GA-induced analgesic process involves an active process in which endogenous central analgesic neurons are activated by GA. Indeed, our lab has identified neurons in the central amygdala (CeA) that are activated by GA, and these neurons (CeA_{GA}) have profound analgesic functions (See Abstract by Hua et al, SfN 2018). Here, we combine *in vivo* one- and two-photon calcium imaging, photo-tag recording to characterize the in vivo activities of CeA_{GA} neurons. Using the CANE technology developed in our lab, we expressed either GCaMP6m (for in vivo imaging) or ChR2 (for in vivo photo-tagged recording using optotrode arrays) specifically in CeA_{GA} neurons. We found that these neurons are strongly activated by isoflurane GA, and are inhibited by acute noxious mechanical or heat stimuli. To begin to understand how activities of CeA_{GA} neurons are controlled in the neuronal circuit level, we used a CANE-based monosynaptic rabies virus to identify presynaptic inputs to CeA_{GA} neurons. Preliminary studies revealed brain-wide inputs from a diverse source of regions to CeA_{GA} neurons.

Disclosures: B. Chen: 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.; DP1 MH103908,

Keck Foundation grant. **T. Hua:** 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.; DP1 MH103908, Keck Foundation grant. **S. Zhao:** None. **B. Han:** None. **P. Thompson:** None. **L. Jiangxie:** None. **J. Lu:** None. **F. Wang:** None.

Poster

391. Pain Imaging and Perception

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Program #/Poster #: 391.04/Y14

Topic: D.03. Somatosensation: Pain

Support: Facial Pain Research Foundation

Title: Free water as a neural marker of trigeminal neuralgia

Authors: *Q. ZHAO[1], C. SPECTOR[2], I. B. H. SAMUEL[1], J. NEUBERT[3], M. DING[1]
[1]J. Crayton Pruitt Family Dept. of Biomed. Engin., [2]Dept. of Orthodontics, [3]Dept. of Neurol., Univ. of Florida, Gainesville, FL

Abstract: Neuroinflammation has been implicated in the pathology of trigeminal neuralgia (TN), a debilitating chronic facial pain disorder, but quantitative analysis of neuroinflammation in human TN patients is still lacking. Recent studies propose that free-water measurement derived from MRI diffusion data provides an index of neuroinflammation. In this study, twenty seven patients (17 females) clinically diagnosed with TN enrolled and underwent diffusion MRI scanning. Among the patients, seventeen had TN1 diagnosis, and twelve (eight TN1 patients) had prior microvascular decompression (MVD) surgery. Selecting the pontine crossing tract (PCT), which links brainstem with the thalamus, as the region of interest, we found that the average fractional volume of the free water in PCT was positively correlated with disease duration, controlling for age ($r=0.50$, $p=0.76\times10^{-2}$). For TN patients without the MVD surgery (n=15), the association between PCT free water and disease duration became stronger ($r=0.89$, $p=7.84\times10^{-6}$), whereas for patients with prior MVD surgery (n=12), the association disappeared ($r=0.20$, $p=0.54$). For patients with TN1 diagnosis and no prior surgery (n=9), PCT free-water and disease-duration association was extremely strong ($r=0.97$, $p=1.10\times10^{-5}$), but for patients with TN1 diagnosis and prior surgery (n=8), the association again disappeared ($r=0.08$, $p=0.84$). These findings suggest that (1) free water in the pontine crossing tract can serve as a neural marker of trigeminal neuralgia especially in patients with TN1 and (2) MVD surgery disrupts the relationship between neuroinflammation and disease duration.

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Poster

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Topic: D.03. Somatosensation: Pain

Support: Grant by by the Ministry of Internal Affairs and Communications in Japan

Title: Pain sensation induced by transcranial magnetic stimulation in human

Authors: K. TANI, *S. TANAKA
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Abstract: Transcranial magnetic stimulation (TMS) is a non-invasive procedure of cortical stimulation. TMS has been widely used to investigate human cognitive and motor functions. Pain sensation is the most common side-effect of TMS (Rossi et al., 2009). The pain sensation induced by TMS may vary among individuals, and depend on scalp location, intensity and frequency of stimulation. The cutaneous sensation affects behavioral and cognitive performance during experiments and could be a potential confounding factor in TMS studies. However, systematic investigations on the pain sensation induced by TMS have not been reported. In the present study, we examined the difference in the pain thresholds induced by TMS over different scalp locations. Six healthy right handed volunteers (3 females, 3 males) participated in the study after they provided the written informed consent. This study was approved by the ethical committee of Hamamatsu University School of Medicine and performed in accordance with the Helsinki Declaration. The experiment was pre-registered as a clinical trial (UMIN000029783). Single-pulse TMS was applied over the scalp locations just above the left primary motor cortex (M1) or the Broca's area (BA). A magstim 200 square machine and a figure-eight coil were used. The participants were asked to report verbally weather she/he felt the sensation of pain after each TMS. The primary outcome was the pain threshold. The pain threshold was defined as the stimulation intensity that induced the pain sensation five out of ten stimulations. As the result, the mean pain threshold in M1 stimulation was 53.8 % of the maximum machine output, whereas that in BA stimulation was 27.3 %. The difference was statistically significant (paired t-test, $t(5)=9.98$, $p<0.001$). The lower pain threshold in BA stimulation could be due to the trigeminal nerve stimulation. In conclusion, the present study shows that the TMS-induced pain sensation differs in the scalp locations. The present results contribute the further understanding on side-effect of TMS. This work was supported by the Ministry of Internal Affairs and Communications in Japan.

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Poster

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Topic: D.03. Somatosensation: Pain

Support: CIHR
Mayday fund

Title: Investigating brain network dynamics of pain perception using MEG measures of dynamic functional coupling

Authors: *J. A. KIM[1,2], R. L. BOSMA[1], K. S. HEMINGTON[1], N. R. OSBORNE[1], A. ROGACHOV[1], J. C. CHENG[1], B. DUNKLEY[3], K. D. DAVIS[1]
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Abstract: Introduction: Emerging evidence suggests that brain activity and regional communication is intrinsically dynamic. For example, variability in connectivity within and between nodes of the dynamic pain connectome (salience and default mode networks (SN, DMN) and ascending nociceptive pathway (ANP)), has been linked to variability in pain perception. Here, we leverage the temporal resolution of MEG and introduce a novel metric called dynamic functional coupling (dFCp) to measure brain dynamics. We used this new measure and conventional measures of static FCp within the SN, DMN, and ANP to determine 1) if pain sensitivity is related to dynamic or static FCp, and 2) if there are sex differences in these relationships.
Methods: Healthy participants underwent a 5 minute resting state MEG session and a psychophysiological battery that included measuring heat pain threshold (HPT). Resting state MEG data was processed using Fieldtrip. Atlas-guided beamforming was performed using linearly constrained minimum variance on the data to obtain time series data for regions of interest (ROIs). For FCp analysis, MEG data was divided into 10s epochs and a Hilbert transform was used to calculate instantaneous phase and amplitude in the canonical bands. Phase lag index (PLI) and amplitude coupling was calculated for pairs of beamformed node within and between the SN, DMN and ANP. Static FCp was calculated by averaging the FCp values across all the epochs and dynamic FCp was considered as the standard deviation of FCp values across all the epochs.
Results: In the whole group analysis, HPT was related to dynamic FCp within the SN, cross-network dynamic FCp between the SN and the DMN, as well as the cross-network dynamic FCp between the SN and the ANP. However, a relationship between HPT and cross-network static FCp between the SN and ANP was only present in men, and a relationship between HPT and

static FCp within the SN was only present in women.
Discussion: In this study we applied a new approach to understand the role of brain dynamics in pain sensitivity. We found a link between individual variability in pain sensitivity and dynamic FCp across all participants, whereas sex differences were apparent in measures of static FCp. We propose that dynamic measures of FCp (standard deviation of PLI and amplitude coupling over time) predominantly reflects "state" properties, whereas the static measures of FCp (average PLI and amplitude coupling over time) predominantly reflects "trait" properties. These MEG measures of brain connectivity can provide complementary insight into brain mechanisms of pain perception.

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Poster

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Program #/Poster #: 391.07/Y17

Topic: D.03. Somatosensation: Pain

Title: Evaluation of temporal dynamics of pain using electrocorticogram in rat formalin-induced pain model

Authors: Y. TAKAHARA, M. MATSUO, R. TAMONO, T. ASAKI, *K. OGAWA
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Abstract: Non-clinical pain evaluation method reflecting clinical assessments is of great importance for analgesic drug discovery. The various conventional methods are used for non-clinical pain evaluation, but these have some limitations. For example, the von Frey test assesses the pain thresholds on the basis of withdrawal response to filament stimulus, and the tail flick test does with the response latencies from heat stimulus. These methods can measure the pain thresholds quantitatively at the moment of the test, but it is difficult to assess pain intensity over time, while in human, patients with chronic pain suffer ever-changing ongoing pain without external stimulus. In this study, we aimed to establish the evaluation method for temporal dynamics of pain in rats using electrocorticography (ECoG) in a different way of stimulus-induced responses. We hypothesized that pain sensation affect the cortical neuronal activity and ECoG recording can detect the temporal dynamics of neuronal activity associated with pain. We recorded ECoG from right primary somatosensory cortex (S1) of Wistar rats before and after formalin injection into the plantar of the left hindpaw. The injection of formalin evoked a biphasic pain-like behavior as previously reported. And interestingly, significant increase in gamma band (30-90 Hz) ECoG activity in S1 area was also observed along with the biphasic pain-like behavior. Moreover, the enhancement of gamma band activity was highly correlated

with the amount of pain-like behavior, and lidocaine (local anesthetics) could attenuate the enhancement of formalin-induced gamma band activity. These results indicate that the gamma band activity in S1 area surrogates temporal pain intensity in rats and ECoG recording of gamma power in S1 area is a useful method for evaluating the temporal dynamics of pain in non-clinical study.

Disclosures: Y. Takahara: A. Employment/Salary (full or part-time):; SHIONOGI & Co., Ltd. **M. Matsuo:** A. Employment/Salary (full or part-time):; SHIONOGI & Co., Ltd. **R. Tamono:** A. Employment/Salary (full or part-time):; SHIONOGI & Co., Ltd. **T. Asaki:** A. Employment/Salary (full or part-time):; SHIONOGI & Co., Ltd. **K. Ogawa:** A. Employment/Salary (full or part-time):; SHIONOGI & Co., Ltd..

Poster

391. Pain Imaging and Perception

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Topic: D.03. Somatosensation: Pain

Support: NIH Grant DK82370
NIH Grant DK110669

Title: Predicting chronic pelvic pain symptom progression based on resting state functional connectivity

Authors: *S. FENSKE[1], J. J. KUTCH[2]
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Abstract: The pathophysiology of Urologic Chronic Pelvic Pain Syndrome (UCPPS) is poorly understood and no generally effective treatment targets have been identified. Studies have demonstrated that brain structure and function are important factors distinguishing UCPPS patients from healthy individuals, and we have recently shown that resting-state functional magnetic resonance imaging (rs-fMRI) at baseline may predict longitudinal progression of UCPPS symptoms. The goal of our current work is to optimize this predictive model of symptom change in UCPPS. In order to predict UCPPS symptom change, we developed a model associating baseline rs-fMRI data and reported symptom progression derived from a pain scale assessed every two weeks up to 1 year collected as part of the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network study. The training dataset included 52 UCPPS patients (34 women and 18 men). Each step of our pipeline: brain parcellation, feature reduction, classification, and cross validation, was improved by parameter optimization and investigation into alternative analysis techniques. The dimensionality of the connectivity feature

set was reduced in combination with a machine learning regression and goodness of fit were estimated by leave one out cross validation. Our preliminary results present an optimal model (r^2=0.32 , p=0.031) for 3-month longitudinal symptom change. This model follows a combination of the Power et al. anatomical atlas, univariate feature selection of 100 features, support vector regression, and leave one out cross validation. Top features are associated with connections to regions in the frontal lobe. These results highlight the ability of our model to predict changes in UCPPS symptom behavior as well as identify potential treatment targets. By optimizing and validating our predictive model, we have made a contribution to our understanding of the neural correlates related to UCPPS with potential clinical applications of a wider UCPPS population.

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Poster

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Topic: D.03. Somatosensation: Pain

Support: DFG PL 321/10-2, PL 321/11-2

Title: A machine learning approach to establish an EEG-based marker of chronic pain

Authors: S. TA DINH[1], M. M. NICKEL[1], L. TIEMANN[1], E. S. MAY[1], H. HEITMANN[1], V. D. HOHN[1], G. EDENHARTER[2], D. UTPADEL-FISCHLER[1], T. R. TOELLE[1], J. GROSS[3], *M. PLONER[1]
[1]Neurol., [2]Anesthesiol., TU Muenchen, Munich, Germany; [3]Univ. of Münster, Münster, Germany

Abstract: Chronic pain is a pathological condition associated with significant sensory, cognitive and affective abnormalities, has detrimental effects on quality of life and is a leading cause of disability worldwide. Converging lines of evidence from animals and humans indicate that the brain plays an important role in chronic pain. However, the brain mechanisms of chronic pain are not fully clear yet. Further insights into these mechanisms promise to advance the understanding of the neural basis of chronic pain. Moreover, a brain-based marker of chronic pain would be immensely helpful for the diagnosis, classification and treatment of chronic pain. Using electroencephalography (EEG) to establish such a brain-based marker of chronic pain is particularly appealing as it is safe, cheap, broadly available and potentially mobile. Moreover, an EEG-based marker of chronic pain might itself represent a target for novel therapeutic strategies such as neurofeedback or non-invasive brain stimulation techniques.We have recorded EEG during restful wakefulness in 101 chronic pain patients (age 58.2 ± 13.5 years (mean ± standard

deviation), 69 female) and 71 matched healthy controls (age 56.6 ± 14.2 years, 49 female). A previous analysis using well-established measures such as spectral power and graph theoretical network measures did not detect significant differences between patients and healthy controls. However, this lack of differences does not preclude that more complex patterns of EEG data contain predictive information about the chronic pain state. In the present study, we therefore applied a multivariate machine learning approach to differentiate chronic pain patients and healthy controls based on resting state EEG data. In particular, we have tried to classify the two cohorts using a support vector machine (SVM) with a range of features characterizing brain activity and brain connectivity. This includes dominant peak frequency, spectral power, connectivity and connectivity-based graph measures. A systematic and comprehensive exploration of the feature space with a simple linear SVM shows that we can distinguish between pain patients and healthy controls with an accuracy of about 66 percent. The features which are most consistently included by a sequential forward feature selection are the power in the theta and alpha frequency and the total connectivity in the beta frequency band. In a next step, deep learning will be used to autonomously generate meaningful features from our data. We aim to accomplish a better differentiation of the two groups as well as a characterization of the most meaningful features.

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Poster

391. Pain Imaging and Perception

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Topic: D.03. Somatosensation: Pain

Support: NIH-NIDCR grant DE019448

Title: Diffuse noxious inhibitory controls and brain networks are modulated in a testosterone-dependent manner in Sprague Dawley rats

Authors: *J. SILVA, Y. ZHANG, J. ASGAR, J. Y. RO, D. SEMINOWICZ
Dept. of Neural and Pain Sci., Univ. of Maryland Baltimore, Baltimore, MD

Abstract: Diffuse noxious inhibitory control (DNIC), which involves endogenous pain modulation, has been investigated as a potential mechanism for the differences in pain observed between men and women. We used a capsaicin-induced DNIC behavioral assay and resting state functional magnetic resonance imaging (rsfMRI) to assess the effect of testosterone on pain modulation and related brain circuitry in rats. Male, female, and orchidectomized (GDX) male

rats had a capsaicin injection into the forepaw to induce DNIC and mechanical thresholds were observed on the hindpaw. Rats were scanned using a Bruker 7T MRI and under isoflurane anesthesia ≤ 1.5%. Functional scans (TR = 1500 ms, in plane resolution = 450 μm, slice thickness 1 mm) were acquired during 15.5 minutes. rsfMRI scans were done before and after capsaicin injection to analyze the effects of DNIC on periaqueductal gray (PAG), anterior cingulate cortex (ACC) and nucleus accumbens (NAc) connectivity to the whole brain. The strength of DNIC was higher in males compared to females and GDX males. PAG connectivity with prelimbic cortex (PrL), ACC and insula was stronger in males compared to females and GDX males, whereas females and GDX males had increased connectivity between the right ACC, hippocampus and thalamus. GDX males also showed a stronger connectivity between right ACC and NAc, and right NAc with PrL, ACC, insula and thalamus. Our findings suggest that testosterone plays a key role in reinforcing the endogenous pain inhibitory system, while circuitries related to reward and emotion are more strongly recruited in the absence of testosterone.

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Poster

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Topic: D.03. Somatosensation: Pain

Support: ERC Fusimage
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Title: Functional ultrasound neuroimaging of the trigeminal ganglion in the context of ocular pain

Authors: *S. PEZET[1], M. THIBAUT[1], L. RAHAL[2], F. JOUBERT[3], A. RÉAUX-LE-GOAZIGO, 75012[3], M. TANTER[4]
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Abstract: Introduction
Ocular pain, in particular corneal pain, is a core symptom of inflammatory or traumatic disorders affecting the anterior segment of the eye. Its increasing prevalence, morbidity, and the resulting

social burden has caused chronic ocular pain to be recognized as a serious public health issue. To date, the management of chronic ocular pain remains a therapeutic challenge in ophthalmology, The absence of therapy imposes a deepening of our fundamental knowledge on the anatomy of the corneal nociceptive system. The cornea is the most densely innervated tissue of the body, as it is innervated by a large number of C and Aδ fibers, the terminal endings of primary sensory neurons (about 150-200) located in the trigeminal ganglion (TG). Preclinical models of corneal injury are characterized by ocular pain and neuronal and microglial activation, both in the TG and the trigeminal sensory complex (Launay et al., 2016). While several preclinical models of corneal injury or dry eye disease have recently been developed, neuroimaging of the TG has never been performed in animal models of ocular pain due to its deep seated location.

The aim of this study was to determine if fUS imaging a novel and highly sensitive neuroimaging modality relying on unequaled spatiotemporal resolution (1 ms, 100µm) could image the functional activations of the TG induced by mechanical or chemical stimulations of healthy cornea in anaesthetized rat.

Results

Our results show that, despite the deep seated location of the TG, fUS is able to image and detect the haemodynamic fluctuations of TG in the rat model. Surprisingly, the TG receives a significant blood supply, which was confirmed using *in toto* vascular staining (using DiI staining). Application of a dynamic mechanical stimulations (5x10 sec) on a healthy cornea induced a rapid and specific activation in the ophthalmic branch of the ipsilateral TG. Such a vascular response was completely prevented by a topical instillation of an anesthetic, oxybuprocain, which blocks the nociceptive corneal nerve fiber activity. In addition, one drop of the potent TRPV1 receptor agonist, Capsaicin (20µM) applied on the cornea induced a rapid and a large phasic haemodynamic response in the ipsilateral TG.

Discussion

This study shows that fUS imaging is feasible and accurate to evaluate neurovascular coupling in the rat TG. This method represents a unique tool for 1) evaluating trigeminal and central neurovascular response in various preclinical model of ocular pain and 2) screening and developing effective therapeutic molecules to alleviate ocular pain.

Launay PS et al., Neurobiol Dis. 2016 Apr;88:16-28.

Disclosures: S. Pezet: None. **M. Thibaut:** None. **L. Rahal:** None. **F. Joubert:** None. **A. Réaux-Le-Goazigo:** None. **M. Tanter:** None.

Poster

391. Pain Imaging and Perception

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 391.12/Z4

Topic: D.03. Somatosensation: Pain

Support: Fusimage
ESPCI
CNRS
INSERM

Title: Imaging of spinal cord functional activation and somatotopy in rats using functional ultrasound (fUS) imaging

Authors: *J. CLARON[1], L. RAHAL[1,2], V. HINGOT[1], M. THIBAUT[2], O. COUTURE[1], M. TANTER[1], S. PEZET[2]
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Abstract: Acute pain is a physiological phenomenon characterized by activation of nociceptors in the peripheral nervous system (PNS). Spinal cord plays a major role in nociception as the first synapse of the nociceptive system. This is the site of activation of second order neurons, through glutamatergic neurotransmission (mediated through AMPA and NMDA receptors (NMDAR)), but also of local and descending inhibitory tonus. In the spinal dorsal horn, entry of primary sensory fibers terminals follow particular somatotopy, thereby allowing to separate them by choosing different stimulation areas[1]. Functional Ultrasound (fUS) imaging is a highly resolved technique that can image functional connectivity (FC) in the adult rat brain[2]. Also, using contrast agents, we performed super-resolution in the living rat brain.
This project aimed at studying the spinal functional activation and organization using fUS imaging. In the neuroimaging field, studies using functional magnetic resonance imaging (fMRI) measure blood-oxygen level dependent (BOLD) signal[2]. fUS imaging measures hemodynamic fluctuation corresponding to spinal blood volume (SBV). Therefore, while fUS imaging gives us an *in vivo* spinal vascular organization, due to the neurovascular coupling, it also allows the indirect measurement of neuronal activity.
Our results, obtained or not in combination with micro-bubbles for Ultrasound Localization Microscopy describe in details the vasculature of the rat spinal cord. fUS experiments without contrast agents imaged functional activations induced by either Aβ, Aδ or C fibers and showed that peripheral stimulation of Aδ or C fibers induce a strong increased SBV in the ipsilateral dorsal horn. Pharmacological blockade of NMDAR showed that this is an NMDA-dependent mechanism. Those results are in agreement with other techniques like optical imaging[3], electrophysiology[4] or fMRI but with a much larger field of view and spatiotemporal resolution. Finally, repetitive activation of C-fibers showed spinal hyperexcitability and increased hemodynamic response.
In conclusion, we can image specific activations in ipsilateral dorsal horn. Because fUS is allowing a large field of view, it is possible to follow up high resolution somatotopy. This indicates fUS imaging as a new tool in pain and spinal cord imaging. FC alterations under conditions such as chronic pain will lead to a better fundamental understanding of the spinal cord role.
1. Nash, 2013 doi : 10.1016/j.pain.2012.11.008
2. Osmanski, 2014 doi : 10.1038/ncomms6023

3. Zhao, 2008 doi : 10.1016/j.neuroimage.2007.11.010
4. He, 2015 doi : 10.3390/brainsci5040400
5. LeBars, 1979 doi : 10.1016/0304-3959(79)90049-6

Disclosures: J. Claron: None. **L. Rahal:** None. **V. Hingot:** None. **M. Thibaut:** None. **O. Couture:** None. **M. Tanter:** None. **S. Pezet:** None.

Poster

391. Pain Imaging and Perception

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 391.13/Z5

Topic: D.03. Somatosensation: Pain

Support: National Natural Science Foundation of China (31600890)

Title: physical pain modulates reward-related brain activities in medial prefrontal cortex

Authors: *C. WANG, J. GAO, X.-W. DONG
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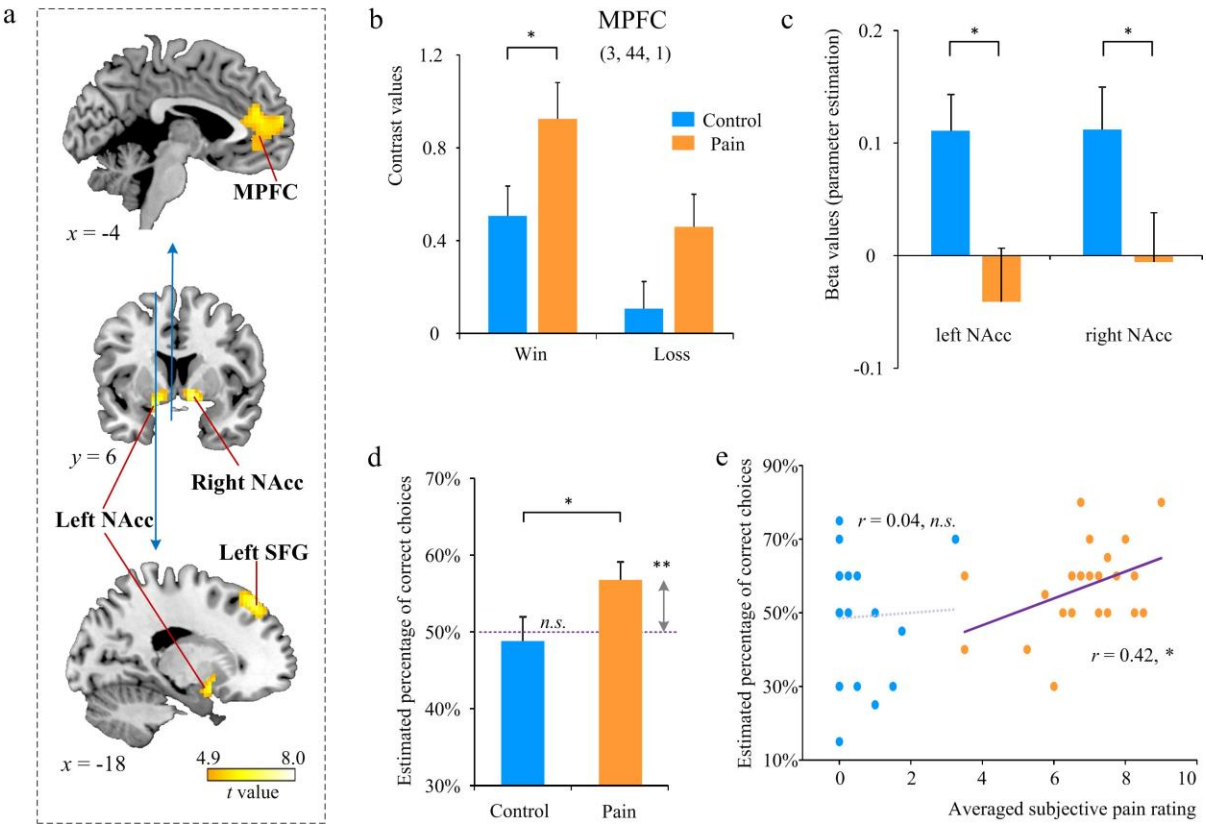
Abstract: Pain modulates motivation and reward-related brain activities. For example, individuals in physical pain are motivated to perform reward-seeking behaviors. However, it is unclear about the mechanism of how pain modulates reward-seeking behavior in humans. By using fMRI technology, the present study investigated the influence of physical pain on reward-related activities of the brain regions in reward/motivation neural circuitry. A total of 50 healthy university students (age = 22.4 ± 2.5) were recruited and were then applied with either Capzasin (pain group) or hand cream (control group). During brain imaging, they were asked to play a card guess game in which they would receive monetary gain by a correct guess or monetary loss by an incorrect guess. Results showed that, the medial prefrontal cortex (MPFC) and bilateral nucleus accumbens (NAcc) were activated when individuals received win feedback in both groups (Fig. a). Interestingly, Results revealed that physical pain increased the reward-related neural activity in the mPFC (Fig. b), but not in the bilateral NAcc. Moreover, the functional connectivity between mPFC and NAcc was decreased in pain condition (Fig. c). Accompanied with the enhanced activation of mPFC responding to monetary reward, participants in pain overestimated their correct choices in the card-guess game (Fig. d) and such positive bias was correlated with subjective rating of pain intensity (Fig. e). These findings demonstrate that the reward-related activity of the mPFC is subject to the modulation of physical pain. We postulate that the modulation of the mPFC activity by pain may result in alterations of both emotional response to and evaluation of reward. The study extends the understanding of the consequences of pain on human cognition and behavior.



Disclosures: C. Wang: None. **J. Gao:** None. **X. Dong:** None.

Poster

391. Pain Imaging and Perception

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 391.14/Z6

Topic: D.03. Somatosensation: Pain

Support: Medical Research Council grant (MR/M013901/1) to PH

Title: Thermal grill model of human pain perception facilitates late but not early somatosensory evoked potentials

Authors: *F. SACADURA[1], T. BROOKES[2], B. BECK[3], F. FARDO[3], P. HAGGARD[3]
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Abstract: Aims: The thermal grill illusion (TGI) involves a paradoxical burning heat sensation evoked by alternating non-noxious warm and cold temperatures. The TGI has been proposed as

an experimental model of pain in humans. Reduced intracortical inhibition has been reported in studies of chronic pain patients. We therefore combined TGI conditioning with electrical stimulation of digital nerves of index and middle fingers to investigate whether TGI stimulation affects somatosensory-evoked potentials (SEPs), and measures of intracortical inhibition based on under-additivity of responses to double-digit stimulation. **Methods**: 32 participants received electrical stimulation to the right index, right middle or both fingers simultaneously, during four fingertip thermal stimulation conditions: warm index/cold middle (TGI), warm/neutral, neutral/cold or neutral/neutral. Importantly, thermal and electrical stimuli were adjusted according to individual pain and detection thresholds. To measure the TGI, participants adjusted the temperature of a further stimulus to the left hand to match perceived temperature of the target right middle finger. **Results**: We found a significant temperature overestimation of the cold stimulus when paired with a warm stimulus, confirming TGI. We found no thermal effects on intracortical inhibition in early sensory SEP components (N20, P27, N33, P45, N80). However, thermal stimulation modulated later cognitive SEP components (P100, N140). Specifically, TGI conditioning increased the N140 amplitude to individual finger stimulation, but not double-digit stimulation. **Conclusions**: Our results suggest TGI conditioning increases the gain of later, "attentional" somatosensory processing, and also increases intracortical inhibition.

Disclosures: F. Sacadura: None. **T. Brookes:** None. **B. Beck:** None. **F. Fardo:** None. **P. Haggard:** None.

Poster

391. Pain Imaging and Perception

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Program #/Poster #: 391.15/Z7

Topic: D.03. Somatosensation: Pain

Support: NIH Grant R01 NR015314-01A1

Title: Thalamus in chronic low back pain: Insights from vertex-based morphometry and connectivity-based diffusion tensor tractography

Authors: *H. PENG[1], J. G. CRAGGS[2], A. SMITH[2], K. BOLAND[3], D. VILCEANU[4], C. M. CIRSTEA[5]
[1]Psychological Sci., Univ. of Missouri Columbia, Columbia, MO; [2]Physical Therapy, [3]Psychological Sci., [4]Anesthesiol. and Perioperative Med., [5]Physical Med. & Rehabil., Univ. of Missouri, Columbia, MO

Abstract: Background: Chronic low back pain (CLBP) is the leading cause of activity limitation and work absence in the U.S. No therapies are cited in CLBP as having persuasive evidence of improvement free of undesirable side-effects. This is a considerable issue for the

U.S. healthcare system: total cost related to CLBP exceeds $100 billion per year. A better understanding of the neural bases of CLBP is mandatory. Structural brain changes in CLBP are a relatively new concept. Decreased gray matter (GM) volume of certain brain regions, including thalamus -a key brain region in pain matrix- has been reported, yet not present in all studies. Likewise, there is limited evidence of reduced fractional anisotropy (FA) of the thalamic projections, which is generally attributed to altered microstructural architecture. We used vertex-based morphometry and connectivity-based diffusion tensor (DTI) tractography to test the hypothesis that the CLBP is associated with decreased thalamic GM and altered integrity of the thalamic projections to the frontal and parietal lobes. **Methods:** High-resolution T1-weighted MRI and DTI images were obtained in 45 CLBP and 57 age/sex-matched healthy controls. Vertex-based analysis (FIRST, FSL, Oxford, UK) was used to quantify the thalamic surface. PROBTRACKX (5000 samples, 0.2 threshold) was applied to determine the probability of connectivity of each thalamic voxel with the mask of each cerebral lobe (connectivity probability>50%). Thalamic surface and FA values of thalamo-frontal and thalamo-paritetal projections were compared between groups. Pain measures (McGill pain questionnaire) were correlated to DTI measures. **Results:** As predicted, in CLBP compared to controls, the left thalamus exhibited significant regional (anterior superior, posterolateral) changes in shape, suggestive of GM reduction in these thalamic parts. Likewise, the left thalamo-frontal projections showed lower FA (0.34±0.02 in CLBP vs. 0.37±0.02 in controls, $p<0.05$), reflective of reduced axonal density, thickness, or demyelination. Contrary to our prediction, there were no significant changes in the right thalamus shape or bilateral thalamo-parietal projections. No correlations with clinical scores were found. **Discussion:** Individuals with CLBP had distinct abnormalities in thalamic morphometry and thalamo-frontal projections compared with healthy controls. Although the precise mechanisms underlying such changes remain unclear, a reduction of GM volume and an altered white matter integrity may represent a degenerative pain-related process. Additional work is underway to decipher the functional implications of such changes.

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Poster

391. Pain Imaging and Perception

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Program #/Poster #: 391.16/Z8

Topic: D.03. Somatosensation: Pain

Support: Intramural Research Program, NCCIH, NIH

Title: Neural and behavioral correlates of noxious stimuli in a pain-insensitive patient: A case study

Authors: E. FRANGOS[1], J. LILJENCRANTZ[2], J. TUBBS[1], C. L. DABLE[1], B. WANG[1], D. SAADE[3], D. BHARUCHA-GOEBEL[3,4], C. BONNEMANN, MD[3], A. T. CHESLER[5], *M. C. BUSHNELL[6]
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Abstract: Objective: A patient with congenital insensitivity to pain presents with no known genetic mutations or gross brain abnormalities. Anecdotal evidence suggests that the patient's autonomic system is reactive to noxious stimuli (increased heart rate, sweating, nausea, skin pallor) despite the absence of reported pain. Therefore, we compared the neural, behavioral, and autonomic correlates of noxious heat stimuli in the patient to healthy controls (HCs).
Methods: The 17-year-old patient and 4 sex- and age-matched healthy controls had 2 sessions of noxious heat stimuli (high=49°C, low=46°C): a behavioral session with heat stimuli (10 trials each) and concurrent autonomic measures (electrocardiogram, skin conductance, respiration) and a functional MRI session with heat stimuli (30 trials each). Pain intensity (0=no sensation; 100=pain threshold; 200=intolerable) and unpleasantness (-100=extremely unpleasant; +100=extremely pleasant) were collected after each trial during the behavioral session. Behavioral and autonomic data were processed and statistically analyzed using, SPSS, AcqKnowledge, and Matlab. Functional MRI data where preprocessed and statistically analyzed using FSL with a cluster correction of $z>2.3$, $p<0.05$, fixed effects.
Results: The patient reported no sensations for all trials in both sessions. The average pain intensity and unpleasantness ratings for HCs were 145.4±10.5 and -43.5±14.6. The patient's pattern of autonomic activity (skin conductance response, heart rate, and heart rate variability) was comparable to that of HCs in response to noxious heat. Healthy controls had significantly greater activations within the insula, anterior cingulate, and primary and secondary somatosensory cortices in response to noxious heat compared to the patient ($z>2.3$, $p<0.05$). No region was significantly more active in the patient than controls. Compared to baseline, the patient had significant activation within the mid-anterior-ventral insula ($z>2.3$, $p<0.05$) in response to noxious heat.
Conclusions: The dearth of activation to noxious heat is consistent with the patient's reported insensitivity to pain. The significant activation within the insula in the patient corresponds to the conserved autonomic responses to noxious input, as this region is a site of visceral afferent input. Furthermore, the insular activation suggests that the central nervous system is reactive to noxious input potentially ruling out a peripheral etiology.

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Poster

391. Pain Imaging and Perception

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 391.17/Z9

Topic: D.03. Somatosensation: Pain

Support: Grants-in-Aid for Scientific Research No. JP 26460695

Title: Comparison of surfase-based cortical thickness and voxel-based morphometry in chronic pain patients

Authors: *T. LI, T. KAMMA, S. YANG, T. OTA, J. KURATA
Anesthesiol., Tokyo Med. and Dent. Univ., Tokyo, Japan

Abstract: [Purpose] Surface-based cortical thickness (CT) and voxel-based morphometry (VBM) are the most commonly investigated in gray matter tissues, which could potentially provide valuable information in the neuroplasticity of pain chronification. However, it remains unclear which of those modalities might be more sensitive in the diagnosis of chronic pain. We compared CT and gray matter volume (GMV) between chronic pain patients (CP) from various etiologies and healthy controls (HC), and sought for brain regions showing anatomical correlation with behavioral characteristics. [Methods] We recruited 62 CP (age, 42.3±13.9 yr; 26 males) and 39 HC (age, 36.4±13.1 yr; 29 males), who underwent high-resolution T1-weighted 3T MRI scans at two hospitals in Japan. Each subject completed short-form McGill Pain Questionnaire (MPQ), painDETECT questionnaire, Beck Depression Inventory (BDI), Brief Scale for Psychiatric Problems in Orthopaedic Patients (BS-POP), and Roland Morris Disability Questionnaire (RDQ). The structural data were processed via CAT12 Toolbox (in SPM) to analyze CT and GMV. Those parameters, at a group level, were compared with two-sample t-test with age and gender as covariates. Behavioral correlations were sought with Pearson Correlation Test. ROC curve analysis was performed to evaluate sensitivity and specificity of each parameter in distinguishing CP from HC. [Results] Compared with HC, CP showed decreased total gray matter volume ($P=0.002$), total intracranial volume ($P=0.014$), and mean cortical thickness ($P=0.007$). After a whole-brain comparison, CP showed decreased GMV in the right superior parietal lobule (SPL) ($P_{FWE}=0.018$), right posterior cingulate cortex (PCC), right lingual gyrus, and left superior frontal gyrus ($P<0.001$). Moreover, CP showed decreased CT in the right precuneus ($P_{FWE}=0.005$), bilateral PCC, and right interior temporal gyrus ($P<0.001$). Furthermore, CT in the right precuneus (AUC=0.717) showed slightly higher sensitivity than GMV in the right SPL (AUC=0.712) in distinguishing CP from HC. Age and pain duration was correlated with GMV, whereas painDETECT, MPQ, BDI, BS-POP, RDQ were correlated with CT in CP patients ($P<0.05$). [Conclusion] CT of the right precuneus showed a high potency in diagnosing chronic pain comparable to that of GMV in the same region. Moreover, CT showed

correlations with more behavioral parameters associated with chronic pain than GMV. CT might be a more suitable alternative to GMV in characterizing specific anatomical changes in pain chronification.

Disclosures: T. Li: None. **T. Kamma:** None. **S. Yang:** None. **T. Ota:** None. **J. Kurata:** None.

Poster

391. Pain Imaging and Perception

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 391.18/Z10

Topic: D.03. Somatosensation: Pain

Support: CIHR
Mayday Fund

Title: Sex-specific abnormalities in functional connectivity in the descending pain modulation system contribute to chronic pain

Authors: *N. R. OSBORNE, J. C. CHENG, R. L. BOSMA, K. S. HEMINGTON, A. ROGACHOV, J. A. KIM, R. D. INMAN, K. D. DAVIS
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Abstract: Introduction: Pain is a major public health issue that is difficult to treat when chronic. This is likely due to individual differences which are not addressed by current treatments. Individual variability in pain is thought to involve the brain's descending pain modulation system (DPM). In healthy people, brain activity in a key node of the DPM system, the subgenual anterior cingulate cortex (sgACC), is associated with pain habituation (Bingel et al., 2007). Furthermore, functional connectivity (FC) between the sgACC and the DPM system is stronger in women than in men, while sgACC FC with the salience network (SN) is stronger in men than women (Wang et al., 2014). This sgACC FC may explain why healthy women, but not men, show strong adaption and habituation to sustained, repeated pain stimuli (Hashmi and Davis, 2009). However, sex-specific FC of the sgACC with the DPM and SN in patients with chronic pain is not known. Here, we assessed sgACC FC in patients with a chronic pain condition that is more prevalent in men (ankylosing spondylitis (AS)). The study aims were to determine whether sgACC FC with the DPM and SN in AS 1) is abnormal compared to healthy controls and 2) show the sex-differences we previously identified in healthy individuals.
Methods: Patients with AS and age/sex-matched healthy controls provided informed written consent to the study. All participants underwent a 3T MRI session to acquire resting state fMRI data. FC was determined from the sgACC to nodes of the SN and DPM.
Results: We found that FC between sgACC and a region of the SN (anterior MCC) was greater in women with AS compared to healthy women. However, there were no significant differences

in sgACC FC 1) in men with AS compared to healthy men, and 2) between men and women with AS.
Discussion: Our findings reveal that patients with chronic pain have abnormal functional communication between regions of their DPM and SN, and that these abnormalities are sex specific. The increased connectivity of the sgACC with the SN in women with AS is a pattern more typically seen in healthy men than in healthy women, and thus may reflect increased attention to pain in women with AS. Future studies will explore whether FC of the sgACC with the SN represents a vulnerability or propensity to develop chronic pain in AS. This study also provides a foundation to further examine whether chronic pain treatment outcomes are related to individual differences in DPM or SN dysfunction.

Disclosures: N.R. Osborne: None. **J.C. Cheng:** None. **R.L. Bosma:** None. **K.S. Hemington:** None. **A. Rogachov:** None. **J.A. Kim:** None. **R.D. Inman:** None. **K.D. Davis:** None.

Poster

391. Pain Imaging and Perception

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 391.19/Z11

Topic: D.03. Somatosensation: Pain

Support: SFB936/A06

Title: Generalization in placebo analgesia

Authors: *L. KAMPERMANN, C. BUECHEL
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Abstract: Placebo analgesia refers to a perceived pain relief due to cognitive modulation induced by expectation and experience. The phenomenon is frequently augmented using a conditioning step adding a positive treatment experience aspect. However, there is only little research on how experiences of pain relief are adaptively transferred to similar but novel situations in the future. The principle of generalization, well-studied in the fear domain, has so far only scarcely been linked to placebo analgesia as one form of appetitive learning. However, it is intuitive to assume generalization in this domain as well: having experienced a substantial pain relief from one treatment in the past, one might expect similar outcomes if the features of a novel treatment resemble the ones experienced before. Using a placebo paradigm including conditioning in healthy humans during functional MRI, we treated heat induced tonic pain on capsaicin pretreated skin. We conditioned participants to expect better treatment from one human (depicted by a face cue; CS+) by pairing it with a stronger temperature decrease, i.e. pain relief, than another human face cue (CS-), which was followed by a more subtle decrease. Following conditioning, participants were tested on a circular continuum of eight face cues ranging from

CS+ to CS-, controlled for their perceived similarity using a simple model of the human visual cortex. In this phase, all faces were paired with the more subtle treatment. Pain relief ratings in the generalization phase showed a significant placebo effect, given by stronger relief ratings for the CS+ vs. CS-, accompanied by decaying (placebo) relief with increasing dissimilarity to the CS+. This gradient in the placebo effect was better explained by a Gaussian fit than a uniform null model ($p < .001$). On the neuronal level, general pain relief was associated with changes of neuronal activity in regions commonly associated with pain and placebo analgesia. Modelling neuronal representations as Gaussian tunings of activity ranging from CS+ to CS- as a Gaussian shaped curve, we identified regions representing generalization at the neuronal level. We conclude that learned analgesic associations are transferred to novel situations to the degree they resemble previous experiences, thus following the principle of generalization. This finding yields important clinical implications, as controlling similarity of treatment features could be used to foster placebo effects wherever beneficial treatment experiences might be transferred to novel situations.

Disclosures: C. Buechel: None.

Poster

391. Pain Imaging and Perception

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 391.20/Z12

Topic: D.03. Somatosensation: Pain

Support: Department of Veteran Affairs Merit award

Title: Topological changes in brain connectivity following visceral inflammation

Authors: *L. M. COLON-PEREZ[1], M. FEBO[2], R. M. CAUDLE[3], J. ZUO[4], Q. ZHOU[4]
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Abstract: Recently, the relationship between brain and external immune signaling has elicited interest from researchers. The brain-immune system relationship is complex one with evidence that alterations in one elicits changes in the other and vice versa. In particular, visceral inflammation may provide measurable and significant changes in brain connectivity and organization. To this end Sprague Dawley rats underwent colonic infusion of 20mg trinitrobenzene sulfonic acid and were scanned following visceral inflammation. On the scanning day, rats were induced using 3-4% isoflurane after induction, the anesthesia was lowered to 1.5% isoflurane during scanning. MR images were acquired in a 4.7T/33cm horizontal bore magnet. The MR imaging consisted on resting state scan using a 2-shot spin echo EPI sequence and an anatomical image for image overlay and reference-to-atlas registration. The images were aligned

with a rat brain template, corrected slice timing delays and slight displacements, and time series spikes were removed. Linear and quadratic detrending, spatial blurring, intensity normalization was also performed, and voxelwise temporal band-pass filter (between 0.01 Hz and 0.1 Hz) was applied to the fMRI data. Time series fMRI signals were extracted from each region of interest (ROI) based on the atlas-guided seed location (150 total areas, equally divided in left and right representations of each region) to create symmetrical connectivity graphs. The graphs were thresholded for each subject to create matrices with equal densities (e.g., z values in the top 15% of all possible correlation coefficients). Our results show differences in several brain regions such as: striatum, thalamus, amygdala, hypothalamus and several cortical areas in network features such as node degree, clustering coefficient, and modularity. A t-test of functional connectivity between control and inflamed rats employing the infralimbic cortex displayed positive correlation with 1283 voxels (41.87 mm^3), while 1488 voxels (48.56 mm^3) displayed negative correlation out a total of 68526 voxels (2236.17 mm^3) in the entire brain (correlations $Z > 2.38$, $p < 0.05$). Meanwhile a similar analysis using the thalamus displayed positive correlation with 1804 voxels (58.87 mm^3), while 1269 voxels (41.41 mm^3) displayed negative correlation out a total of 68526 voxels (2236.17 mm^3) in the entire brain (correlations $Z > 2.38$, $p < 0.05$). Our results imply that visceral inflammation elicits several changes in brain connectivity in several autonomic relevant brain regions in turn inducing large scale alterations of topological indices of network connectivity.

Disclosures: L.M. Colon-Perez: None. **M. Febo:** None. **R.M. Caudle:** None. **J. Zuo:** None. **Q. Zhou:** None.

Poster

391. Pain Imaging and Perception

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Program #/Poster #: 391.21/Z13

Topic: D.03. Somatosensation: Pain

Title: The relationship of Sensorimotor Peak Alpha Frequency to regions across the brain is modulated by pain

Authors: *A. FURMAN, S. KRIMMEL, J. ZHANG, M. KEASER, R. GULLAPALLI, D. SEMINOWICZ
Univ. of Maryland Baltimore, Baltimore, MD

Abstract: Objective markers of pain sensitivity represent a key tool for identifying individuals who may be at high risk for developing chronic pain. Sensorimotor peak alpha frequency (PAF), the frequency band within the 8-12 Hz alpha range that displays maximal power, may represent one such marker; previous work from our lab has shown that EEG-recorded sensorimotor PAF could predict reported pain intensities to a painful event occurring either 45 minutes or 4 days

later. Understanding how sensorimotor PAF modulates brain activity represents an important avenue for increased understanding of the mechanisms that generate individual differences in pain sensitivity. To address this question we use combined EEG-fMRI (n=10) to probe the relationship between sensorimotor PAF and brain activity during a baseline, eyes-closed resting state scan and a capsaicin heat-pain, eyes-closed resting state scan. Following EEG artifact correction, we extracted PAF from electrodes over sensorimotor cortex and created a PAF timeseries by calculating PAF within 2 second epochs that matched the MRI TR (237 time steps for each scan). We convolved this PAF timeseries with a canonical HRF and treated it as a regressor, predicting each voxel's timeseries. At baseline, PAF positively predicted fMRI in supramarginal gyrus, sensorimotor cortex, cuneus, thalamus, dorsal anterior cingulate cortex (ACC) and negatively in subgenual ACC, precuneus, and lateral prefrontal cortices. These effects tended to be small to moderate. After inducing capsaicin heat-pain, PAF positively predicted a similar set of regions as without capsaicin, but the effects were much larger. After capsaicin, PAF negatively predicted fMRI in rostral ACC, angular gyrus, precuneus, and lateral prefrontal cortex with effect sizes largely equivalent to baseline. In conclusion, we provide analysis from a pilot study relating PAF to spontaneous fMRI signals across the brain and demonstrate the feasibility of this approach. Our results suggest that sensorimotor PAF coupling across the brain is altered by pain, giving a possible mechanism through which PAF affects pain sensitivity.

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Poster

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Location: SDCC Halls B-H

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Program #/Poster #: 391.22/Z14

Topic: D.03. Somatosensation: Pain

Support: NSERC
CIHR
Canadian Institutes of Health Research Strategy for Patient Oriented Research 'Chronic Pain Network'
Alberta Children's Hospital Foundation
Vi Riddell Pediatric Pain Initiative

Title: Abnormal hippocampal and amygdalar volume in children with chronic pain

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Abstract: Pediatric chronic pain affects one in five children. Despite its prevalence, there are few studies investigating brain abnormalities in adolescents with chronic pain. More specifically, there have been many studies investigating brain structure and function in adults with chronic pain. However, these data cannot be extrapolated to pediatric populations. Therefore, there is a need to investigate whether the brain abnormalities present in adults with chronic pain are also present in pediatric pain populations. Recent pain studies in rodents and adult populations have identified that the hippocampus (HC) and amygdala (Amyg) are abnormal in chronic pain, and that abnormalities in these regions predict the transition to chronic pain, and even treatment responsiveness. Specifically, smaller HC/Amyg predict whether an adult with subacute back pain will transition to chronic pain. Here, we explicitly test whether adolescents with chronic pain have smaller HC/Amyg volumes. All procedures have been approved by local ethics committees. Nine adolescents with chronic headache, including migraine (3 girls, 6 boys; 14.77+/- 2.9 (mean+/-SD) years old) and 14 healthy controls (9 girls, 3 boys; 12.80 +/- 2.0 (mean+/-SD) years old). Participants underwent brain scanning at the Alberta Children's Hospital on a General Electric 3 T MR750w system using a 32-channel head coil. A high-resolution 3-dimensional T1-weighted structural MRI scan was acquired for every participant (TR= 8.2 ms, TE= 3.2 ms, 0.8mm isotropic). The structural brain images were analyzed using Freesurfer v6.0. Images underwent standard preprocessing, and bilateral HC and Amyg volumes were extracted. An unpaired two-tailed t-test with Welch's correction for uneven variances was used to determine whether the HC and Amyg volumes were different between healthy adolescents and those with chronic pain. Statistical threshold was set at $p<0.05$. We found that the left Amyg and bilateral HC were significantly smaller in those with chronic pain, compared to healthy adolescents. These data indicate that pediatric chronic pain is associated with abnormal limbic brain regions. Future studies should investigate the functional and behavioural significance of these structural abnormalities.

Disclosures: M. Moayedi: None. **S. Lee:** None. **L.J. Ayoub:** None. **J. Vinall:** None. **C. Lebel:** None. **M. Noel:** None.

Poster

391. Pain Imaging and Perception

Location: SDCC Halls B-H

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Program #/Poster #: 391.23/Z15

Topic: D.03. Somatosensation: Pain

Support: Japan Society for the Promotion of Science, Grants-in-Aid for Scientific Research No. JP26460695

Title: A pivotal role for the cerebellum in connectomic plasticity of chronic pain patients

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Abstract: [Introduction] Although some plastic changes in brain network function are involved in chronification of pain, evidence from comprehensive analysis of brain connectomics has been scarce to date. Here we examined functional connectivity across all the brain networks in chronic pain patients to be compared with that in healthy controls, and further sought for its correlations with behavioral parameters. [Methods] We recruited 22 chronic pain patients and 17 healthy volunteers. Each subject underwent several psychophysical tests and multimodal magnetic resonance imaging (MRI) on a 3T scanner, including resting-state functional and 3-dimensional high-resolution MRI. Functional connectivity was comprehensively examined across all the pairs of predefined 116 regions of interest from the Automated Anatomical Labeling atlas and compared between the groups (FDR $p<0.05$) using SPM12 and CONN software. Correlations were sought between functional connectivity and various psychophysical parameters while age and sex were set as covariates (FDR $p<0.05$). [Results] We found six networks with significantly increased and one with decreased functional connectivity, all of which involving part of the cerebellum, in chronic pain patients vs. controls. The cerebellum also showed changes in functional connectivity depending on psychophysical parameters: with the right temporal pole depending on pain intensity ($r=0.703$); right orbitofrontal cortex on pain duration ($r=0.723$); right caudate nucleus on Pain Catastrophizing Scale scores ($r=-0.693$); left amygdala on painDETECT scores ($r=-0.712$); and right parietal lobule on McGill Pain Questionnaire scores ($r=-0.708$). [Discussion] Although roles for the cerebellum in pain processing remain to be determined, many earlier neuroimaging studies have shown cerebellar responses to noxious stimulation. The current study implied that chronification of pain involved specific changes in network plasticity between the cerebellum and emotion- or sensory-related regions in association with various behavioral profiles. The cerebellum might possibly play a pivotal role in the supraspinal mechanisms of pain chronification.

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Poster

391. Pain Imaging and Perception

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Center for Cognitive Aging and Memory
Cognitive Aging and Clinical Translational Research Program
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Title: Pain modulation is associated with cingulate morphology in older adults with musculoskeletal pain

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Abstract: Introduction: Musculoskeletal pain negatively impacts older individuals and is often associated with deficient pain modulation. Multiple brain regions are responsible for pain modulation, but the cingulate cortices (CC) play a significant role with abnormal morphology reported across various pain conditions. While age-related changes in CC structure and function have also been reported, the relationship between CC morphology and pain modulation in older individuals is not currently understood. The present study aims to determine CC volumetric correlates of pain modulation in older adults using self-reported and experimental pain measures. **Methods:** Volunteers (n=40, mean age=71.4 years) within the Neuromodulatory Examination of Pain and Mobility Across the Lifespan (NEPAL) study completed the Graded Chronic Pain Scale (GCPS), a pain modulation procedure (punctate Temporal Summation (TS)), and a structural T1-weighted MRI. We used Freesurfer's *recon-all* to perform whole-brain structural segmentation and obtain volumetric statistics. The residual method was applied to control for intracranial volume with adjusted volumes of interest (VOIs) created for the anterior (A)CC gray matter (GM), mid-ACC GM, mid (M)CC GM, mid-posterior (P)CC GM, PCC GM, caudal ACC WM, rostral ACC WM, and PCC WM. We conducted bivariate and partial correlations of adjusted VOIs with GCPS intensity and disability subscales and TS scores. **Results:** MCC GM volumes were positively correlated with GCPS pain disability ($r = 0.341$, $p = 0.036$), but no correlations were found for any VOI with GCPS pain intensity ($ps > 0.05$). Volumes of mid-PCC GM ($r = -0.38$, $p = 0.018$), mid-ACC GM ($r = -0.390$, $p = 0.021$), left caudal ACC WM ($r = -0.356$, $p = 0.036$), and right rostral ACC WM ($r = -0.355$, $p = 0.036$) were negatively correlated with TS scores. Controlling for sex, age, and education resulted in comparable findings. **Conclusion:** The CC's key role in cognition, affect, and emotion likely accounts for its associations with pain disability, a construct encompassing pain's overall impact on everyday life. The findings that lower GM and WM CC volumes were associated with higher TS scores suggests that brain plasticity may be negatively impacted by pain, above and beyond the impact of aging. In younger samples, pain modulation using TS has been implicated as a key mechanism underlying chronification of pain; and this process may also occur in older individuals. Future research is needed to directly link the role of brain morphology to chronic pain in older adults. Understanding the neurocorrelates of pain in aging has the potential to inform treatment and improve quality of life in later years.

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Poster

391. Pain Imaging and Perception

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Topic: D.03. Somatosensation: Pain

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UAB CTSA Grant UL1TR001417

Title: Associations of pain catastrophizing with pain-related brain structure in individuals from different race groups with or at risk for knee osteoarthritis

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Abstract: Research provides compelling evidence that non-Hispanic Blacks (NHB) engage in pain catastrophizing (a maladaptive tendency to negatively evaluate one's ability to cope with pain) more often than non-Hispanic Whites (NHW), while NHWs more often use other strategies (e.g., ignoring sensations). These differences may represent an important contributor to poorer pain-related treatment outcomes and greater functional disability in NHBs. Functional neuroimaging studies have revealed that individuals with high levels of trait pain catastrophizing show increased cerebral responses to pain in several pain-related brain regions (e.g., insula, primary somatosensory cortex [S1]), but associations between brain structure and pain catastrophizing remain largely unexplored. Moreover, to date, no neuroimaging studies have investigated the extent to which the influence of pain catastrophizing on pain-related brain regions contribute to ethnic group differences in pain responses. The current project is a sub-study of an ongoing observational cohort investigation conducted by the University of Florida (UF) and the University of Alabama at Birmingham (UAB). Participants were 176 community-dwelling adults between 45 and 85 years old with and without knee pain, including 76 NHB (15

without knee pain) and 100 NHW (33 without knee pain). All participants completed the pain catastrophizing subscale of the Coping Strategies Questionnaire-Revised and Magnetic Resonance Imaging (MRI) data were obtained. High-resolution anatomical MRI (T1-weighted MP-RAGE, 1mm3 resolution) data were acquired at both sites on a 3 Tesla Philips Achieva. Images were processed using FreeSurfer 6.0 (Fischl, 2012). Mean thickness values for each cortical region (i.e., insula, S1; DKT parcellation) were exported to SPSS software, version 24 (IBM, Chicago, IL) for analyses. Partial correlation analyses within race groups were conducted and adjusted for the following covariates: age, education, body mass index, and study site. Results revealed higher pain catastrophizing was associated with thinner left insula ($p < .05$) in NHB participants with or at risk of knee osteoarthritis (OA), while higher pain catastrophizing was associated with thinner S1 bilaterally (ps $< .05$) in NHW participants with or at risk for knee OA. However, there were no significant associations in brain regions (i.e., insula, S1) for either race in the control groups (participants without knee pain) (ps $> .05$). These results suggest that pain catastrophizing might have differing effects on pain-related central pathways and may contribute to ethnic group differences in individuals with or at risk for knee OA.

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Poster

391. Pain Imaging and Perception

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 391.26/Z18

Topic: D.03. Somatosensation: Pain

Support: Nelson Foundation

Title: Specific neural correlates underlie improved clinical outcomes in chronic pain patients following interdisciplinary pain management

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Abstract: Chronic pain is associated with maladaptive brain functional and anatomical changes. However, brain mechanisms underlying successful pain rehabilitation remain unknown. Here we use anatomical and resting-state functional MRI (rs-fMRI) to examine brain changes associated with improved pain, emotional and ability outcomes in chronic pain patients following a 4 week interdisciplinary pain management program.

25 patients with chronic pain underwent anatomical and rs-fMRI scans at baseline and at the conclusion of the treatment which included pain education, biofeedback, occupational and physical therapy. Behavioral and functional properties were measured using self-reported questionnaires and included pain intensity, central sensitization, pain related anxiety, depression, and disability. We performed a Principle Component Analysis to identify similarities in clinical outcomes. Changes in functional connectivity were assessed using standard graph-theoretical measures. Differences in global and local anatomical properties were computed using standard FSL tools.

Patients showed significant improvement of pain, emotional and functional parameters. PCA identified two factors accounting for 69% of the variance. Factor 1 correlated with pain and emotional improvements including pain intensity, central sensitization, depression, pain related anxiety and catastrophizing. Factor 2 correlated with increased pain coping and daily function. Brain networks of patients exhibited small world properties with high efficiency and clustering not changing following treatment. Their functional connectivity showed increased similarity to healthy subjects following treatment. Factor 1 correlated with decreased Default-Mode Network connectivity with the Saliency Network, Fronto-Parietal network and subcortical regions. No significant connectivity changes were observed for Factor 2.

Patients showed significant increase in Gray Matter Volume following treatment which correlates with Factor 1, but not Factor 2. Factor 1 significantly correlates with increased Grey Matter Density in the right DLPFC, part of FPN, and rostral ACC. Factor 2 was associated with increased GMD in the Thalamus and the Ventral Striatum, centered in the NAC and extending into the Caudate and Putamen.

These results suggest that pain rehabilitation is associated with changes in two behavioral clusters with unique central representations, and provide insight into key neural mechanisms underlying successful pain rehabilitation, which may facilitate the development of behavioral-specific and personalized treatments.

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Poster

391. Pain Imaging and Perception

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Topic: D.03. Somatosensation: Pain

Support: Keio Gijuku Academic Development Funds
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Title: Altered functional connectivity underlying time discounting in chronic pain

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Abstract: Time discounting (TD), a topic of behavioral economics, is a tendency to assign less value to future gains than to present ones. High TD rate is represented in an impulsive personality, which is a risk of substance abuse and addiction. Impulsiveness is also a characteristic aspect of chronic pain (CP) patients. However, little is known about involvement of TD in CP. Furthermore, neuro-mechanism underlying TD is unclear at all. Hereby we investigated the resting-state brain function associated with TD both in CP patients and healthy subjects. Nineteen chronic neck pain patients (pain intensity > 4/10, pain duration > 3 months) and 19 healthy subjects matched for age and gender were recruited. We identified similarity and differences in the brain functional connections underlying TD in healthy and CP with FDR-adjusted p value (< 0.05) and cluster threshold (< 2 links). Finally, we demonstrated mediation analyses to investigate relationship among behavior, brain function, and TD. Behaviorally, TD showed significant correlation with meaningfulness in healthy ($R = 0.41$, $p < 0.01$), but not in CP patients ($R = 0.35$, $p = 0.49$). TD in patients only correlated with pain intensity ($R = 0.56$, $p < 0.01$). Connectivity analysis based on 333 regions of interest (ROIs) in healthy identified 45 links and 51 ROIs significantly relating to TD. When predetermined 13 communities were assigned to each ROIs, the default mode network (DMN) was the community with the most connections (6 links between 8 ROIs) and every links were included in the prefrontal cortex (PFC). The specific network within DMN significantly correlated to TD ($R = 0.89$, $p < 0.01$) and mediated the effect of meaningfulness on TD significantly (indirect effect (IE) = -0.62, 95% confidential interval (CI) = [-1.24, -0.21]). On the other hand, 10 links and 12 ROIs were identified in CP patients as the network significantly related to TD, and they had no overlap with the links in healthy subjects. The right dorsolateral prefrontal cortex (DLPFC) in the cinguro-opacular network was a notable ROI gathering the most connections (7 links) of them. The network of the DLPFC was significantly correlated to TD ($R = 0.83$, $p < 0.01$) and mediated the effect of pain on TD (IE = 0.49, 95% CI = [0.16, 1.12]). CP patients have different behavioral associations and resting-state brain networks underlying TD compared to healthy subjects. While the DMN in PFC is a key network associated with TD in healthy, the DLPFC plays a significant role in TD in CP patients.

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Poster

391. Pain Imaging and Perception

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Program #/Poster #: 391.28/AA2

Topic: D.03. Somatosensation: Pain

Support: National Center for Complementary and Integrative Health AT007987

Title: Predicting placebo pills response in randomized controlled trials

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Abstract: Objective
Placebo response is universally observed in randomized controlled trials (RCTs), yet the individual characteristics rendering a chronic pain patient 'placebo responder' remains minimally understood. We used a prospective neuroimaging-based RCT performed in chronic back pain patients to build a model predictive of placebo response. In a second neuroimaging-based RCT, we evaluate the interaction between placebo response and medication treatment in individuals stratified as placebo responders vs non-responders based on our predictive model. Here, we present the model and its accuracy in a second cohorts of patients.
Methods
A first prospective cohort study (N = 63) included a neuroimaging session and a large battery of questionnaires assessing personality traits prior to repeated 2-weeks placebo treatment periods. A predictive model of placebo response was built from a logistic regression weighting the parameters dissociating placebo responders and non-responders prior to treatment. We applied the model in a second cohort study, were chronic back pain patients (N = 90) were stratified into placebo responders and non-responders based on brain scan and questionnaire results at entry. These participants were randomized so that 40% received placebo treatment, 40% active medication (naproxen 500mg bid), and 20% no treatment. The treatment duration of this second study was fixed to 6 weeks.
Results
In a first prospective neuroimaging-based RCT, we identified brain parameters and personality traits that predict the propensity for placebo response. Placebo response depended both on functional coupling of the ventrolateral prefrontal cortex (VLPFC) and the dorsolateral prefrontal cortex (DLPFC) with the periaqueductal grey (PAG) and the precentral gyrus (PreCG); and a set of psychological factors including interoceptive awareness and emotion regulation. We used these parameters to stratified patients from the second prospective neuroimaging-based RCT at

entry. This second study is now closed to enrolment and the model accuracy will be presented at the conference.

Conclusions

We showed that functional connectivity between the VLPFC and DLPFC with the PAG and the PreCG as well as interoceptive awareness and emotion regulation predicted placebo pills response. The generalizability of the model is currently tested in a second group of chronic back pain patients.

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Poster

392. Somatosensation: Thalamic and Cortical Processing

Location: SDCC Halls B-H

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Program #/Poster #: 392.01/AA3

Topic: D.04. Somatosensation: Touch

Support: BMBF/FKZ 01GQ1002
ERC 633428
DFG SFB 1089

Title: Cortical output is driven by transcolumnar pathways in deep layers

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Abstract: Pyramidal tract neurons (PTs) broadcast the results of cortical processing to a variety of downstream targets throughout the brain. Unraveling mechanisms that drive activity in this major output cell type of the neocortex will thus be essential for understanding how cortical computations orchestrate sensory-guided behaviors. The origin of the PTs' sensory-evoked responses - which are the most broadly-tuned within a cortical column, and which can be as fast as those in the major input layer (L4) of the cortex - remains, however, enigmatic within the present concepts of cortical circuit organization. We find that thalamocortical input drives two orthogonally organized cortical circuits in parallel: the columnar pathway in L4, and a transcolumnar pathway in the deep layers (L5/6). We show that the transcolumnar pathway drives PTs during sensory stimulation, potentially to switch these neurons into an excited state that is required for transforming inputs from recurrent and top-down circuits into behavioral responses.

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Poster

392. Somatosensation: Thalamic and Cortical Processing

Location: SDCC Halls B-H

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Program #/Poster #: 392.02/AA4

Topic: D.04. Somatosensation: Touch

Support: NSERC
HFSP

Title: Towards a microcircuit-based understanding of multiplexed spike codes in the neocortex

Authors: *M. TRAN, L. PRINCE, D. GREY, H. CHASIOTIS, B. RICHARDS
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Abstract: Information about the world is communicated by populations of neurons through their action potentials, or spikes. Deciphering the 'neural code', i.e. the patterns of spikes used to carry information, is a major goal in neuroscience. Currently there exists two hypotheses about how spikes convey information: 1) through the rate of spikes (i.e. a rate code) or, 2) through the precise timing of spikes (i.e. a temporal code). Evidence suggests that neocortical circuits can use both codes, specifically that both the rate and temporal synchrony of spikes are important for information transmission in sensory circuits. An unresolved question is whether there is any microcircuit-based multiplexing, i.e. a separation between the information transmitted by rate and synchrony codes at the microcircuit level. Here, we test the hypothesis that two broad classes of inhibitory interneurons in the neocortex, somatostatin positive (SST+) and parvalbumin positive (PV+) interneurons, are specialized for integrating rate and synchrony codes respectively. To test this, we combined optogenetics with patterned illumination to experimentally control spike rate and synchrony of channelrhodopsin-2 positive (ChR2+) pyramidal neurons in L2/3 of the barrel cortex ex vivo. Cellular responses were then analyzed using machine learning and information theoretic analyses to determine how much information each class of cell carries when receiving information encoded using rate or synchrony codes. We find that there are differences in the amount of information carried by different neuron subtypes, supporting the idea of microcircuit-based multiplexing in the neocortex.

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Poster

392. Somatosensation: Thalamic and Cortical Processing

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Program #/Poster #: 392.03/AA5

Topic: D.04. Somatosensation: Touch

Support: Boğazici University BAP Project: 17XP2

Title: Basal forebrain stimulation modulates vibrotactile responses of rat SI neurons based on cell type, layer, and in a time-dependent manner

Authors: *B. VARDAR, B. GÜÇLÜ
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Abstract: Cholinergic system is thought to increase the effectiveness of sensory inputs in cortex by promoting attention and arousal. Basal forebrain (BF) is the main source of cholinergic projections to the cortex. We electrically stimulated BF of ketamine-anesthetized rats while recording single-unit (n=87) spike activity in the hindpaw representation of SI cortex. The vibrotactile responses were measured with (ON) and without (OFF) BF stimulation (0.5-ms bipolar pulses at 100 Hz; duration: 0.5 s; amplitude: 50 µA). BF stimulation just preceded the vibrotactile stimuli applied on the glabrous skin of the hindpaw (bursts of 5-, 40-, and 250-Hz sinusoidal displacements; duration: 0.5 s; amplitude: 50 µm) in each trial. Each condition was repeated for 10 trials and long-term changes were assessed by performing the entire procedure once again after a 30-minute break. Average firing rates were calculated for different time periods (R_b: background; R_o: during the initial 100-ms of vibrotactile stimulus duration, R_d*: during the last 400-ms of vibrotactile stimulus duration). To quantify entrainment, vector strengths (VS) were calculated for the entire vibrotactile stimulus duration. The short and long-term (after 30 min) effects of BF activation were analyzed for different cells (regular-spiking (RS) and fast-spiking (FS)), vibrotactile frequencies and cortical layers (III, IV, V, VI) by using repeated measures ANOVA. BF activation had both short- and long-term significant effects on entrainment. When first and second runs were compared in terms of VS change in ON vs. OFF, a significant interaction was found between time block, layer and cell type ($p=0.009$). For example, VS of both RS and FS neurons in layer III increased, albeit in different amounts, due to BF stimulation in the second run. However, BF stimulation decreased VS of both neurons in the first run. BF activation did not cause significant main effects (regardless of cell type and layer) on the firing rate measures (including R_b). On the other hand, significant interaction effects between time block (first run ON vs. second run ON), layer and neuron were found on R_d*-R_b and R_o-R_b ($p=0.027$ and $p=0.04$, respectively). These results indicate that BF activation enhances VS in the second run, thus vibrotactile periodicity becomes more prominent. Consistent with previous studies, the effects of BF activation on firing rate developed over time. The main

novelty of current study is that the long-term effects of cholinergic activation are shown to be also dependent on cell type and layer, probably due to the projection pattern from BF.

Disclosures: B. Vardar: None. **B. Güçlü:** None.

Poster

392. Somatosensation: Thalamic and Cortical Processing

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Topic: D.04. Somatosensation: Touch

Support: NIH Grant P01NS074972
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Title: The cellular composition and connectivity of neocortical layer 1

Authors: *B. SCHUMAN[1], R. MACHOLD[1], Y. HASHIKAWA[1], J. FUZIK[1], G. J. FISHELL[2], B. RUDY[1]
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Abstract: Animals must sense and internally represent their surroundings to guide and adjust the behaviors necessary for survival. This process requires the means to interpret sensory information in different internal and environmental contexts. In the mammalian brain, the integration of sensory and contextual signals depends upon computations performed in the neocortex. The outermost neocortical layer (layer 1; L1), contains the distal tuft dendrites of pyramidal cells and receives contextual input from a diversity of regions, including higher-order thalamic nuclei, higher-order cortical areas, neuromodulatory sources, contralateral cortex, and other sensory cortical areas carrying cross-modal input. In addition, L1 contains the dendrites of L2 VIP and chandelier interneurons, and the axons of somatostatin (SST) interneurons in L2-6. Importantly, L1 contains cells that are exclusively inhibitory interneurons and are known to be functionally important for the processing and gating of contextual information. However, the precise role that L1 interneurons play in integrating contextual and sensory information is a mystery because their identities are poorly understood. Starting with utilizing a combination of transgenic mouse lines and genetic tools to investigate the identity of these cells, we found that L1 contains four distinct populations of interneurons, including one - canopy cells - first described here. These interneuron populations can be separated and genetically targeted by their expression of neuropeptide Y (NPY), neuron-derived neurotrophic factor (NDNF), vasoactive intestinal peptide (VIP), and strong expression of the α7 nicotinic receptor (α7). Whole cell recordings in brain slices revealed that each cell population has a distinct intrinsic

electrophysiology, morphology, and connectivity, strongly suggesting that a division of labor exists for L1 interneurons in processing contextual information.

Disclosures: B. Schuman: None. **R. Machold:** None. **Y. Hashikawa:** None. **J. Fuzik:** None. **G.J. Fishell:** None. **B. Rudy:** None.

Poster

392. Somatosensation: Thalamic and Cortical Processing

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 392.05/AA7

Topic: D.04. Somatosensation: Touch

Title: The missing piece of somatosensory evoked potentials (SSEPs): Difference between activation and phase resetting according to a stereo-EEG perspective

Authors: *M. DEL VECCHIO[1], F. CARUANA[2], I. SARTORI[3], V. PELLICCIA[3], G. LO RUSSO[3], G. RIZZOLATTI[2], P. AVANZINI[2]
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Abstract: In the present study, we mapped the spatio-temporal dynamics of cortical responses to ipsilateral median nerve stimulation using intracerebral recordings (stereo-EEG) in 38 drug-resistant epileptic patients by examining the increase of power in gamma band. Overall, 50 hemispheres have been explored (28 right, 22 left) including 5872 cortical sites of which 4466 were localized in the grey matter according to the anatomical reconstruction (2783 in the right hemisphere, 1683 in the left hemisphere). The 37 responsive leads were almost exclusively located in the parietal operculum and in particular in its dorso-caudal part corresponding to area OP1. Active leads were found bilaterally in the frontal operculum and in the long gyri of the right insular cortex while only residual activity was found in right inferior parietal cortex (2 leads), left short gyri of insular cortex (2), right premotor dorsal cortex (1) and in primary somatosensory cortex (2). Since previous findings are mostly based on SSEPs recording and identified active clusters in primary somatosensory cortex but inconsistently across subjects and topography, inter-trial coherence (5-145 Hz) was computed for all leads to bridge the gap with existing EEG/MEG literature about somatosensory processing. Results indicated a weak broadband gamma phase-resetting in SI and PMd while opercular and insular regions showed a diffuse response in sub-gamma frequencies. The phase synchronization in gamma band can not be considered as a measure of neuronal recruitment in absence of a significant gamma power increase, thus suggesting that it reflects a transcallosal echo originating from the activation of the contralateral homologue cortical area. This point was hindered so far by methodological constraints. The use of stereo-EEG, instead, allows one to distinguish power increase from phase

synchronization phenomena, offering a valuable insight for interpreting non invasively recorded findings and thus complementing the classical view about the somatosensory system organisation.

Disclosures: F. Caruana: None. **I. Sartori:** None. **V. Pelliccia:** None. **G. Lo Russo:** None. **G. Rizzolatti:** None. **P. Avanzini:** None.

Poster

392. Somatosensation: Thalamic and Cortical Processing

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 392.06/AA8

Topic: D.04. Somatosensation: Touch

Title: The building blocks of first order thalamic nuclei

Authors: *J. YI[1], R. PERIN[1], Y. SHI[2], H. MARKRAM[2]
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Abstract: The lateral geniculate nucleus (LGN) and the ventrobasal (VB) thalamic nuclei in rodents, receiving visual and somatosensory modalities respectively, share similarities in relay morphology and electrical properties. Further comparisons have not been thoroughly investigated. The three distinct morphological types of relay neurons of the LGN are organized within the nucleus based on regional preferences (Krahe et al., 2011). However, the microscopic organization of the VB is currently unknown. In addition, interneurons of the LGN represent 15-20% of the neuronal population (Arcelli et al., 1997). The VB has been reported to contain a local interneuron population percentage ranging from less than 1% (Arcelli et al.,1997) to (3.7%; Cavdar et al., 2014). Given the sparse distribution of interneurons in the VB, their properties and contributions to thalamic functions have been overlooked. However, the morphologies of their LGN counterparts suggest a profound influence on thalamocortical relay excitatory tone throughout the nucleus (Hamos et al., 1985). Thus, a comparison of relay organization and interneuron functionality remains to be completed. Here, we obtained acute slice whole cell recordings and morphologies of VB relay cells and local interneurons of juvenile Wistar han rats and GAD67-eGFP mice. We confirm that relay and interneuron cell types across the LGN and VB are identical. Furthermore, we demonstrate the presence of feedforward inhibition from local interneurons in the VB in response to medial lemniscus electrical stimulation. By isolating excitatory and inhibitory postsynaptic currents, we elucidate two temporal arrangements of feedforward inhibition, paralleling the spatial and temporal arrangements of relay cells and interneurons of the LGN (Blitz and Regehr, 2005). We also investigated the microscopic structure of the VB in juvenile rats using the golgi-cox staining method in serial slices traversing the nucleus and charted the regional preferences of each morphological type. Our results suggests that there is a universality of the building blocks of first order thalamic nuclei, despite

their different densities of cell types. The distinctions between the LGN and VB lie in the proportion of relay cells to interneurons and functional topography. These differences in circuit complexity may be the determinants of how different sensory modalities are perceived. The data gathered from the single cell characteristics and organization of VB cell types are being integrated into an *in silico* model of the somatosensory cortex (Markram et al. 2015) to reconstruct and simulate thalamic nuclei and thalamocortical interactions.

Disclosures: J. Yi: None. **R. Perin:** None. **Y. Shi:** None. **H. Markram:** None.

Poster

392. Somatosensation: Thalamic and Cortical Processing

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 392.07/AA9

Topic: D.04. Somatosensation: Touch

Title: Steady-state responses in the somatosensory system interact with intrinsic oscillatory activity

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Abstract: Brain oscillations have been related to many aspects of human behavior. To understand whether there is a causal relationship, it is of great importance to develop methods for modulating ongoing neural activity. It has been shown that external rhythmic stimulation leads to an oscillatory brain response that follows the temporal structure of the presented stimulus and is assumed to reflect the synchronization of ongoing neural oscillations with the stimulation rhythm. This interaction between individual brain activity and the so called steady-state response (SSR) to rhythmic stimulation is the fundamental implication of neural entrainment.Here we investigate whether neural responses to rhythmic vibrotactile stimulation, measured with EEG, are dependent on ongoing individual brain oscillations, and therefore reflect entrained oscillatory activity. We measured aspects of the SSR such as amplitude magnitude and phase synchronization in response to rhythmic stimulation across various frequencies in the alpha (6 Hz - 14 Hz) and beta (16 Hz - 24 Hz) band. To inspect dose-response relationships, three different stimulation intensities were applied for each frequency relative to the individual sensory threshold. Individual alpha and beta frequencies over motor areas were determined for 28 healthy participants before receiving vibrotactile stimulation to the index finger of the dominant hand. We assessed for each participant the EEG electrode over the somatosensory area that would reveal the strongest frequency-dependent response across all stimulation conditions.We found that a higher stimulation intensity, compared to lower intensities, resulted in greater amplitudes of the SSR's, and a more pronounced phase synchronization with the stimulation signal.

Moreover, EEG responses to stimulation frequencies closer to individual peak frequencies within the alpha and beta band showed bigger amplitudes and revealed a higher degree of phase synchronization, compared to stimulation conditions with frequencies that were more distant to intrinsic oscillations. Our findings provide evidence that the efficacy of rhythmic vibrotactile stimulation to evoke a SSR is dependent on ongoing brain oscillations.

Disclosures: M.J. Wälti: None. **M.T. Bächinger:** None. **N. Wenderoth:** None.

Poster

392. Somatosensation: Thalamic and Cortical Processing

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 392.08/AA10

Topic: D.04. Somatosensation: Touch

Support: European Union Seventh Framework Programme - Human Brain Project
MINECO

Title: Quantitative 3D analysis of ventral posteromedial and posterior nuclei thalamocortical synapses in the mouse primary somatosensory cortex

Authors: *J. RODRIGUEZ MORENO[1], A. ROLLENHAGEN[2], A. SANTUY[3], A. MARCHAN-PEREZ[3], J. DEFELIPE[4], J. LÜBKE[2], F. CASCA[1]
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Abstract: First-order thalamocortical (TC) pathways provide the main access route for subcortical inputs to the neocortex, while higher-order TC pathways are believed to mainly regulate functional connectivity between cortical areas. These two main types of TC pathways often converge the same cortical columns in complementary laminar patterns. For example, in the vibrissal domain of the rodent primary somatosensory cortex (S1BF), first-order axons from the dorsomedial portion of the ventral posteromedial nucleus (VPMdm) place their synaptic terminals within the barrel domains of layers 4-3, while axons from the higher-order posterior nucleus (Po) target mainly layer 5a and 1. Here, we 3D-reconstructed, measured and compared the main structural parameters of synaptic terminals of axons selectively labeled in S1BF from VPMdm or Po, and their postsynaptic target structures.Virtually all VPMdm and Po synapses are located in boutons. However, VPMdm boutons show significantly different size, mitochondrial content, and number of synaptic vesicles than Po boutons. A much higher percentage of VPMdm axonal boutons are multi-synaptic. The main (80%) postsynaptic target of boutons from both nuclei are dendritic spines of different morphologies, some of which (5-10%) form large dendritic protrusions inserted into the TC bouton. Both populations have postsynaptic densities of similar area. Along with specific receptor distributions, the above structural features may

significantly contribute to differences in efficacy and/or dynamic properties between the VPMdm and Po synapses in S1BF.

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Poster

392. Somatosensation: Thalamic and Cortical Processing

Location: SDCC Halls B-H

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Program #/Poster #: 392.09/AA11

Topic: D.04. Somatosensation: Touch

Support: U01NS090576
ERC NEUROPATTERNS
Flag-Era JTC SLOW-DYN

Title: Population activity patterns in the ventral posteromedial nucleus of the thalamus across behavioral states

Authors: A. SATTIN[1,2], M. MORONI[2,3], A. ANTONINI[1], S. BOVETTI[1], A. FORLI[1,2], F. NESPOLI[1,2], A. BERTONCINI[5], C. LIBERALE[5], S. PANZERI[2,3], *T. FELLIN[4,1,2]
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Abstract: Neuronal activity in primary sensory areas of the thalamus varies as a function of the brain or behavioral state. However, whether these changes are associated with state-dependent reorganization of spatial activity patterns in thalamic networks is unclear. To address this issue, we performed functional imaging in the ventral posteromedial (VPM) nucleus of awake, head-restrained mice while monitoring whisking, locomotion, and pupil diameter. We expressed the genetically encoded calcium indicator GCaMP6s in transgenic mice expressing the enzyme Cre recombinase in the majority of VPM neurons and we imaged GCaMP6s signals using two-photon microscopy through a GRIN microendoscope. We found that periods with no locomotor activity and no whisker movement (resting periods) were characterized by calcium activities that were sparsely distributed both across neurons and time. In contrast, during periods of locomotion and whisking in air (active periods), which were frequently preceded by the dilation of the eye pupil, we observed an increase in the frequency and amplitude of calcium events across VPM neurons compared to resting periods. During both resting and active periods, neural ensembles displaying significantly correlated activity over hundreds of microns were observed. On average,

each functional ensemble involved a small fraction of the total number of neurons in the field of view. Moreover, for some of the recorded ensembles their appearance correlated with the behavioral state of the animal, i.e specific ensembles were selectively observed during resting periods while other distinct ensembles were selectively engaged during active periods. Altogether, these results show that in primary somatosensory thalamic nuclei the animal's behavioral state is dynamically and specifically encoded in the spatiotemporal structure of spontaneous neural activity patterns.

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Poster

392. Somatosensation: Thalamic and Cortical Processing

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 392.10/BB1

Topic: D.04. Somatosensation: Touch

Support: VR Grant 2017-01717

Title: Intracranial stereo-EEG reveals gamma-band modulation in the human somatosensory cortex during naturalistic gentle touch to the hairy skin

Authors: *E. ERIKSSON HAGBERG[1], D. KRÝSL[2], J. NILSSON[2], K. MALMGREN[3], B. RYDENHAG[3], J. WESSBERG[1]
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Abstract: The perception of touch to hairy skin is mediated by slow conducting C-tactile (CT) afferents and fast conducting Aβ afferents. Aβ signals are conveyed to primary (S1) and secondary (S2) somatosensory cortices, whereas previous studies using functional magnetic resonance imaging indicate that CT afferents project to the posterior insula but not to S1 or S2. CTs respond strongly to caress-like stimuli, and are believed to play a key role in signaling pleasantness of touch.
This ongoing study, aims to define the changes in brain oscillations following naturalistic gentle touch to the hairy skin in humans. Furthermore we want to investigate if brain responses due to Aβ and CT afference can be distinguished from one another. We hypothesize that oscillatory changes due to Aβ input should occur at a short latency post stimulation onset, whereas any brain responses, e.g., in the posterior insula, following CT activation should have a late onset.

Data was obtained from 5 adult patients (2 female) with focal refractory epilepsy undergoing intracranial stereo-electroencephalography (SEEG) as part of their pre-surgical evaluation. All patients had electrode implantation in the left hemisphere. The number of electrodes and placement was strictly individualized based on the clinical work up of the patients.
SEEG was recorded during soft brush stroking on the forearm. A trained experimenter manually delivered the stimuli with a hand held soft brush. A minimum of 100 repetitions on both contra- and ipsilateral forearm was carried out. The brushing velocity was CT-optimal, i.e. around 3 cm/s. The timing of brush contact with the skin was recorded using a fiber-optic sensor attached to the brush.
The data was analyzed using a bipolar montage between neighboring electrodes. Electrodes showing epileptiform activity was discarded from the analysis. Preliminary results confirmed that gentle touch to the hairy skin induces alfa and beta event-related desynchronization (ERD) and event-related synchronization (ERS) in primary sensorimotor regions (S1 and primary motor cortex), which we have previously seen using magnetoencephalography (MEG). However, SEEG also revealed gamma band ERS in S1 following gentle touch, which we were unable to detect with MEG in a previous study. One patient in the current sample, had an electrode located in the posterior insula, which showed early onset alfa and beta ERD, and gamma ERS to both contra- and ipsilateral stimulation. Thus far, we have failed to identify any brain activity that appears to be exclusively driven by CTs.

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Poster

392. Somatosensation: Thalamic and Cortical Processing

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 392.11/BB2

Topic: D.04. Somatosensation: Touch

Title: Ultrasonic vocalization and c-Fos expression in rats stimulated by 'affective' touch

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Abstract: The sense of touch consists of two general functional qualities: discriminative and affective, delivered by myelinated fast-conducting Aβ fibers, and unmyelinated slow-conducting C- tactile afferents, respectively. Gentle stimulation of C- tactile afferents is shown to induce a pleasant feeling and activates emotion-related brain regions such as the insular cortex. In the present study, tactile stimulation was delivered manually with an artist’s brush to the right dorsal trunk of male Wistar rats in slow, moderate, and fast speed (3, 9, and 18 cm/s, respectively).

Each rat received stimulation with the three speeds in different experimental sessions separated by one week with the order of the speeds counterbalanced. Ultrasonic vocalizations emitted throughout the experimental sessions, consisting of 10 minutes of habituation, 5 minutes of stimulation and 5 minutes of post-stimulation periods, were recorded as indices of the affective state. Ninety minutes after the last stimulation, rats were anesthetized and perfused for c-Fos staining. The number of c-Fos expressing cells in rostral periaqueductal gray (PAG), trunk region of primary somatosensory cortex (S1) and posterior insular cortices (PIC) of both hemispheres were counted. The number and duration of ultrasonic vocalizations were analyzed with repeated measures ANOVA to test whether different stimulation speeds altered the affective state of the rats. Rats did not emit calls with positive affect (50 kHz) during the experimental sessions. Results showed that fast stimulation of hairy skin increased the number of negative (22 kHz) vocalizations compared to moderate and slow speeds ($p= 0.011$ and $p= 0.001$, respectively). Similarly, rats produced longer 22-kHz calls when exposed to fast stroking than slow stroking ($p= 0.004$). The unilateral tactile stimulation was found to elevate c-Fos expression in contralateral S1 significantly ($p= 0.002$), and in contralateral PIC marginally ($p=0.051$). Finally, rats receiving stimulation with different speed in their last experimental session, did not significantly differ in terms of c-Fos expression in PAG, S1 and PIC of both hemispheres. These results suggest that the stimulation of hairy skin with gentle strokes at different speeds alters the emotional state of rats as vocalization behavior, albeit in a negative affective manner. On the other hand, static marker of neural activity tested here seems to be not much sensitive to such changes. Since affective touch is mostly relevant during social interaction with the conspecifics, information encoded by C-tactile afferents was probably processed differently in this study compared to previous work on humans.

Disclosures: E. Tunckol: None. **R.S. Canbeyli:** None. **B. Guclu:** None.

Poster

392. Somatosensation: Thalamic and Cortical Processing

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 392.12/BB3

Topic: D.04. Somatosensation: Touch

Support: R01HD084362-01A2

Title: Architectonic characteristics of the grizzly bear thalamus and superior colliculus

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Abstract: The current work is part of a broader comparative effort to determine how the thalamus has evolved in different lineages with alterations in brain size, sensory specialization, and behavior. In this study, we analyzed the architectonic characteristics of the grizzly bear (*Ursus arctos*) thalamus and superior colliculus using various histological stains. The thalamus was cut coronally at a thickness of 60 microns and saved in series, which were processed for cytochrome oxidase (CO), acetylcholinesterase (AChE), vesicular glutamate transporter 2 (VGLUT2), and Nissl substance. The borders of thalamic nuclei were readily visible in Nissl stained sections while subdivisions within nuclei were revealed in tissue sections processed for AChE, CO, and VGLUT2. Despite being much larger than the thalamus of other well studied carnivores such as ferrets and cats, many features of the grizzly bear thalamus were similar to those of other carnivores. These features include a well laminated lateral geniculate nucleus (LGN), architectonically distinct divisions within the lateral posterior/pulvinar complex, and a modular ventral posterior nucleus (VP). However, the modularity of VP was more complex than that observed in other carnivores, and even more complex than in non-human primates and humans. The superior colliculus shares a similar laminar pattern to that observed in most studied mammals. Thus, despite its size, the grizzly bear has retained many features of a general scheme of organization for the carnivore thalamus and superior colliculus, with some specializations of the ventral posterior nucleus.

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Poster

392. Somatosensation: Thalamic and Cortical Processing

Location: SDCC Halls B-H

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Program #/Poster #: 392.13/BB4

Topic: D.04. Somatosensation: Touch

Support: Barkley Trust (Barlow)

Title: Cortical fNIRS hemodynamics during saltatory pneumotactile glabrous hand stimulation in neurotypical adults

Authors: *J. GREENWOOD[1,2,3], M. HOZAN[1,2,3], M. K. SULLIVAN[1,3], S. M. BARLOW[1,2,3]
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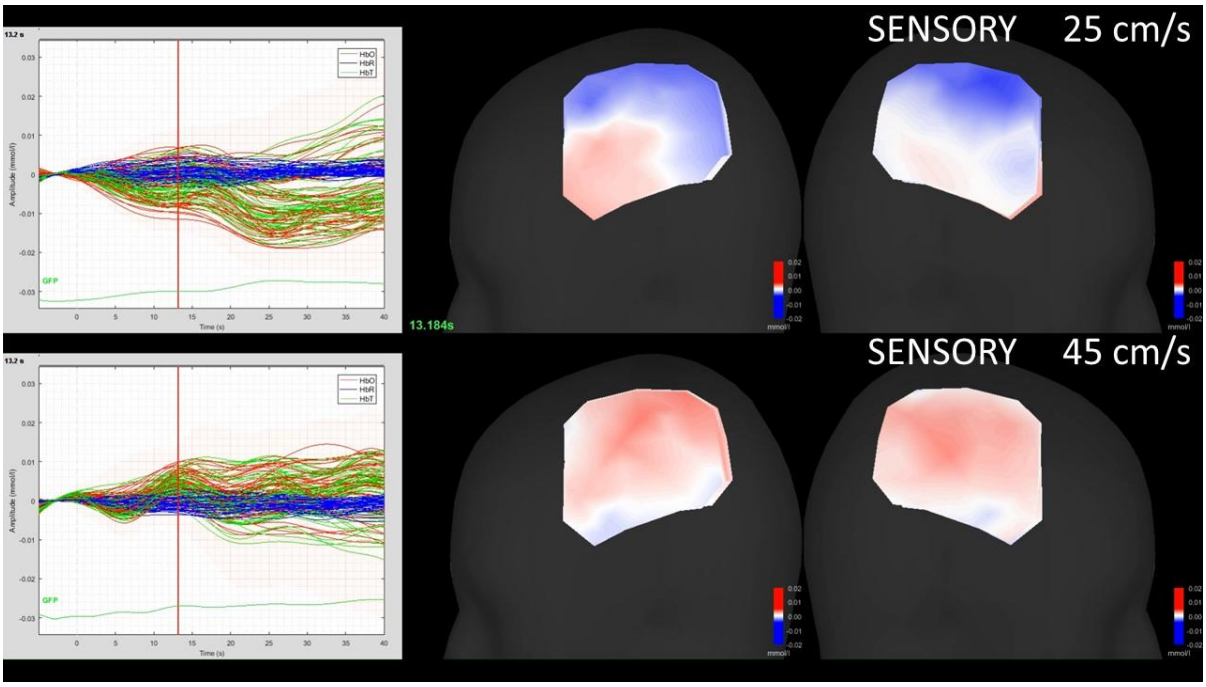
Abstract: BACKGROUND: The hemodynamic integrity and responsiveness of the human cerebral cortex can be assessed noninvasively using fNIRS to map the dynamic changes in the concentration of HbO and HbR during somatosensory stimulation (Rosner & Barlow, 2016, Somatosensory & Motor Research).

OBJECTIVE: Assess the effects of controlled saltatory pneumotactile velocity arrays applied to the glabrous hand on evoked cerebral hemodynamic signals using fNIRS. Extending previous fMRI BOLD studies (Oh, 2016, PLOSone), hypotheses include: 1)Primary activation of the hand region of S1/S2/M1 of contralateral hemisphere, 2)Reduced and delayed activation of ipsilateral hemisphere, and 3)Significant HRF scaling dependent on saltatory velocity
METHODS: Prospective study involving 20 adults (19-35 yrs), repetitive saltatory stimulation (60 ms pulses, 8-channels TAC-Cell array) presented in randomized blocks (20s ON, 20s OFF; 10 reps/velocity) at 25 and 45 cm/s on the right glabrous hand initiated at P1 of D2-D5 and traversing the palmar surface of the hand to P1 of D1. The fNIRS signals were acquired (NIRx) at 7.8125 Hz using 16 dual-tipped conical LED optodes (760 & 850 nm) and 20 fiber-optic detectors (52 chan pairs) placed bilaterally over sensorimotor cortex. Anatomic MRI (3T; MPRAGE; 0.5x0.5x1.0 mm) was acquired for fNIRS co-registration using a Polhemus head digitizer. Individual/group comparisons processed using NIRS_SPM.
RESULTS: Based on 5 right-handed subjects (2M/3F, mean age=26.8 yrs), a primary evoked HbO/HbR response peak occurs at 13 seconds on the contralateral hand representation in pre-central (M1) and post-central (S1, S2) cortex, with a significantly larger evoked HbO/HbR response during the higher velocity (45 cm/s) saltatory stimulation condition. The ipsilateral hand sensorimotor cortex manifest an attenuated HRF.
CONCLUSIONS: Non-invasive fNIRS methodology reveals cortical somatosensory topography in response to pulsed saltatory pneumotactile dependent on traverse velocities applied to the glabrous hand.



Disclosures: J. Greenwood: None. **M. Hozan:** None. **M.K. Sullivan:** None. **S.M. Barlow:** None.

Poster

392. Somatosensation: Thalamic and Cortical Processing

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 392.14/BB5

Topic: D.04. Somatosensation: Touch

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NSF-GRFP 1058262
NSF EPSCOR Award #1632738

Title: Primary and higher order inputs are differentially integrated by distinct classes of thalamic reticular neurons

Authors: *R. MARTINEZ-GARCIA, B. VOELCKER, S. L. PATRICK, T. STEVENS, B. W. CONNORS, S. J. CRUIKSHANK
Brown Univ., Providence, RI

Abstract: Primary and higher-order thalamic nuclei are dynamically regulated by inhibitory neurons of the thalamic reticular nucleus (TRN). Knowledge of the cellular composition of TRN, as well as the anatomical and functional organization of its afferent inputs, are fundamental to understanding thalamocortical processing. We previously showed that the somatosensory sector of TRN is composed of neurochemically distinct neuron types that are located in topographically separate zones. Most cells along the medial and lateral edges of TRN expressed somatostatin (SOM) whereas cells in the central zone generally expressed calbindin (Calb). We also found that thalamic inputs to TRN segregated topographically in fairly close alignment with the observed neurochemical pattern. The ventral posterior medial nucleus (VPM), a primary somatosensory region, synapsed on cells in the central Calb-rich zone of TRN. In contrast, higher-order posterior medial (POM) axons targeted the SOM-dense medial and lateral edges of TRN.
We have begun to address how these neurochemically and topographically distinct TRN cell types integrate their respective synaptic inputs. Thus we tested the dynamic features of their glutamatergic synaptic currents and the intrinsic membrane properties and firing characteristics that ultimately shape their postsynaptic outputs. We observed that evoked synaptic currents in central TRN cells (triggered by VPM input, above) were strong but had fast decay kinetics, and these currents depressed deeply with repetitive activation. Conversely, synaptic currents in TRN edge cells (originating from POM) were weaker but had slow decay kinetics, and were more stable in magnitude during repetition. Intrinsically, central TRN cells had leaky membrane

conductances, while edge cells were more electrotonically compact. Finally, central cells were highly bursty, whereas edge cells exhibited weak or no bursting.
We are now testing how these zone-specific synaptic and intrinsic properties interact, potentially leading to distinct integrated responses to primary and higher order thalamic input. We predict that Calb-expressing central TRN cells would be ideal for responding phasically to sensory transients. In contrast, SOM-expressing edge cells should be more capable of integrating temporally distributed higher order signals.

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Poster

392. Somatosensation: Thalamic and Cortical Processing

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Title: L4 SST function during near-threshold sensory detection

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Abstract: In sensory cortex, the earliest stages of stimulus-evoked activity are shaped by rapid and powerful inhibition by parvalbumin-expressing interneurons (PV INs) situated in layer 4. Thalamocortical feedforward inhibition serves important computational roles (Gabernet et al., 2005; Yu et al., 2016), but this comes at the cost of completely suppressing principal neuron responses to weaker, less coincident stimuli (Ollerenshaw et al., 2014). Somatostatin-expressing interneurons (SST INs) in L4 are known to show a unique axon targeting profile, with restricted arbors that preferentially innervate L4 PV INs rather than spiny stellate cells or excitatory apical dendrites (Xu et al., 2013; Munoz et al., 2016). Despite these connectivity findings, the possible functional role of L4 SST INs in reducing PV-mediated feedforward inhibition during relevant behaviors remains untested. We use Channelrhodopsin-assisted patching (Munoz & Rudy, 2014) to target L4 SST INs in the barrel cortex of awake mice performing a near-threshold detection task, and recorded phasic stimulus-evoked activity. Using viral expression of halorhodopsin in SST INs and targeted light delivery, we photoinhibit L4 SST signals during cue presentation and relate this manipulation to changes in behavioral performance. We test the hypothesis that SST INs in L4 may act to sensitize L4 principal neurons to weak stimuli by suppressing

thalamocortical feedforward inhibition, and that this function distinguishes SST INs in this layer from the apical dendrite-targeting SST INs of other layers.

Disclosures: N.D. Johnson: None. **K. Manson:** None. **I. Kruglikov:** None. **B. Rudy:** None.

Poster

392. Somatosensation: Thalamic and Cortical Processing

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 392.16/BB7

Topic: D.04. Somatosensation: Touch

Support: NIH Grant NS077989

Title: Distinct firing properties of adult thalamic reticular neurons

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Abstract: GABAergic neurons of the thalamic reticular nucleus (TRN) form powerful projections to distinct thalamic nuclei, thereby regulating thalamocortical and corticothalamic processing. Rather than forming a uniform structure, TRN neurons are organized into functionally-specific sub-networks. Furthermore, TRN neurons can be classified based on molecular, anatomical, and physiological properties. While previous work has revealed distinct firing patterns in juvenile animals, it remains unclear if these properties are maintained in the adult. Moreover, the involvement of distinct TRN neuronal subtypes in generating rhythmic thalamic activity is not well understood. To address these questions we performed cell-attached and whole-cell recordings from neurons in the TRN and the adjacent ventrobasal nucleus of the thalamus (VB), in slices derived from 3-4 months old mice. Based on their action potential characteristics, TRN neurons could be classified into two main groups. The majority (73 %) of neurons generated T-type channel-dependent bursting and generated multiple bouts of rebound spiking, while the remaining neurons cells lacked bursting and generated only single bouts of rebound spiking with fewer spikes. Interestingly, a sub-group of bursting neurons generated long-lasting plateau potentials (615.5±115.3 ms duration) that mediated persistent action potential activity (99.1±12.7 Hz). In slice preparations that preserved intrathalamic connectivity, bursting cells generating plateau potentials were prominently recruited by thalamoreticular inputs and, in turn, resulted in rhythmic and persistent inhibition in VB, which was highly synchronous for neighboring neurons. Persistent inhibition in VB was fully blocked by AMPAR antagonists but was insensitive to antagonists of group I mGluRs and TRPC4 channels, suggesting plateau potentials in different classes of GABAergic thalamic neurons are mediated by distinct

mechanisms. Together our data identify specific cell classes in the adult TRN and show that bursting cells generating plateau potentials are prominently involved in mediating rhythmic thalamic activity.

Disclosures: J. O'Malley: None. **F. Seibt:** None. **M. Beierlein:** None.

Poster

392. Somatosensation: Thalamic and Cortical Processing

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 392.17/BB8

Topic: D.04. Somatosensation: Touch

Support: Amgen Scholars Program, Amgen Foundation
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Title: A comparison of topography in projections from somatosensory thalamus to cortex

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Abstract: Sensory systems often make use of topography to organize the exterior world into an internal representation. The clear organization of the whisker representation in the primary somatosensory cortex (S1) facilitates investigation of cortical processing. In the mouse whisker-barrel system, touch information passes to S1 through two parallel pathways: one through a primary thalamic nucleus (the ventral posterior medial nucleus, VPM) and another through a secondary thalamic nucleus (the posterior medial nucleus, POm). While VPM clearly encodes whisker information to the cortex in a spatiotemporally precise manner, POm response patterns remain less clear. Furthermore, VPM displays a distinct one-to-one thalamic barreloid to cortical barrel somatotopy in its projections to S1, yet single axon tracing studies have shown that individual POm neurons innervate multiple barrel columns in S1. Because POm projects with less spatial precision than VPM, POm might not be simply relaying touch information, but rather may be involved in more complex information processing. We aimed to quantify the topography in VPM-S1 and POm-S1 projections to better understand how these nuclei might differentially impact S1 processing. We injected two fluorescent tracers into neighboring whisker regions of S1 to retrogradely label both VPM and POm cell bodies. Next, we reconstructed the injection sites and the barrel field in three dimensions to determine injection locations and sizes. In both VPM and POm, the number of cells projecting to each cortical location and the degree to which cell populations overlapped was quantified using confocal and epifluorescence microscopy.

Preliminary results confirm the strict somatotopy of the VPM-S1 projection and suggest that the POm-S1 projection is more widespread. Determining the degree of somatotopy preserved in the thalamocortical projections from VPM and POm to S1 will provide a clearer basis for understanding their functional differences and how these differences can affect cortical processing.

Disclosures: H.C. Macomber: None. **G.M. Pierce:** None. **R.M. Bruno:** None.

Poster

392. Somatosensation: Thalamic and Cortical Processing

Location: SDCC Halls B-H

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Program #/Poster #: 392.18/BB9

Topic: D.02. Somatosensation

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FONCYT, BID 1728 OC.AR. PICT-2016-1799 (PPP)
FONCYT, BID 1728 OC.AR. PICT 2015-2594 (VB) and NIGMS P30 GM110702 (EGR)

Title: Systemic administration of cocaine, caffeine or their combination alters the pacemaker activity of somatosensory thalamic neurons in mice

Authors: *M. C. RIVERO-ECHETO[1,3], P. P. PERISSINOTTI[2], C. GÓNZALEZ-INCHAUSPE[2], J. L. CADET[4], E. E. GARCIA-RILL[5], V. BISAGNO[6], F. J. URBANO[7]
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Abstract: Caffeine (Caf) is a worldwide consumed stimulant that is widely used in a range of commercially available products. Cocaine (Coc) is an addictive stimulant drug that can cause dependence and several long-term consequences when abused. In South America there is an endemic use of a drug known as “PACO” in which it was found to be chemically composed by a Caf:Coc ratio of 1:4. We tested the hypothesis that Caf:Coc combination alters intrinsic and synaptic properties of thalamocortical neurons. We treated male C57/BL6 mice with binge administrations (3 injections 1 hr apart, i.p.) of Coc (10 mg/kg), Caf (5mg/kg) and their combination (Coc+Caf). Patch-clamp records from ventrobasal (VB) neurons from thalamocortical slices in vitro were obtained 24-hour after either one day (i.e., Acute Binge, AB) or 13-day (i.e., Chronic Binge, CB) binge administrations. An AB of Caf decreased the number

of action potentials (APs) after a post-inhibitory rebound (PIR), whereas a CB of Coc + Caf increased APs frequency compared to other treatments (Ctrl n=6, Caf n=3, Coc n=5, Coc+Caf n=5; ANOVA p<0,05). Since IH (HCN-mediated) and IT (T-type calcium currents) act synergistically to underline VB pacemaker activity, we studied these currents after AB or CB treatments. Coc or Coc+ Caf increased IH current density 24 hour after an AB, but not in a CB treatment. Interestingly, CB Coc+ Caf administration decreased IH current density and shifted steady-state voltage-dependence of HCN channels activation towards hyperpolarizing holding potentials. Coc+ Caf decreased IT current density after an AB treatment (Ctrl n=32, Caff n=10, Coc n=5, Coc+Caff n=7; KW analysis H:13, p<0.001, Dunn´s Post-Hoc test p<0.05), while a CB treatment resulted in greater in IT current density (Control n=21, Caff n=12, Coc n=10, Coc+Caff n=16; KW analysis H:13, p<0.001, Dunn´s Post-Hoc test p<0.05) . We then studied intracellular calcium concentration $[Ca^{2+}]_{int}$ dynamics elicited by a rebound, low threshold spike depolarization using fura-2 ratiometric calcium imaging. Our results showed that $[Ca^{2+}]_{int}$ increased after an AB of Coc+Caf (Ctrl n=6, caff n=6, Coc n=6, Coc+Caf n=6; ANOVA p<0.05). We also found that both Glutamatergic and GABAergic synaptic transmission onto VB neurons was affected by either AB or CB treatments. In conclusion our results showed a greater impact of the Coc+ Caf combination on intrinsic and synaptic properties of thalamocortical neurons, compared to the individual effect of each psychostimulant.

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Poster

392. Somatosensation: Thalamic and Cortical Processing

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 392.19/BB10

Topic: D.04. Somatosensation: Touch

Support: 1R21NS096461-01A1

Title: Focused ultrasound neuromodulation: Refining optimal parameters in a swine model

Authors: *W. LEGON[1], T. WANG[1], J. W. L. ELIAS[2]
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Abstract: There is a significant interest to use ultrasound for neuromodulation because of its high spatial resolution and transcranial application. Acoustic energy has long been known to influence the activity of electrically-excitable tissues including muscle, peripheral nerves, and the central nervous system. Perhaps the most exciting, unharnessed potential for transcranial ultrasound is for noninvasive neuromodulation and brain mapping. Ultrasound has been used safely and effectively for cortical neuromodulation in mouse, rat, and rabbit and only a few

studies have been performed in large brain animal models including sheep, pig and monkey. There is a need for a consistent, large-brain animal model to comprehensively study and refine low-intensity neuromodulation of the brain. Here, we conduct a systematic analysis of ultrasound parameters to identify the optimal low-intensity sonications that inhibit as well as excite brain function in large brain. In addition, we test the ability of low-intensity focused ultrasound to modulate axonal tracts and test for the safety of these parameters. Preliminary evidence suggests that prolonged duration (> 5 min), low duty cycle (< 20%) ultrasound is effective at suppressing cortical activity whereas short duration (< 2 min) high duty cycle (> 70%) ultrasound is effective for inducing cortical excitation. There is as yet, no definitive data suggesting that low-intensity ultrasound can modulate white matter tracts. Histology demonstrates that these sonication parameters do not result in any damage.

Disclosures: T. Wang: None. **J.W.L. Elias:** None.

Poster

392. Somatosensation: Thalamic and Cortical Processing

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 392.20/BB11

Topic: D.04. Somatosensation: Touch

Support: R01 NS098781A1

Title: Multimodal validation of a thermal mechanism of transcranial focused ultrasound neuroinhibition

Authors: *B. ELAHIAN[1], D. DARROW[2], P. O'BRIEN[3], T. NETOFF[1], E. EBBINI[3]
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Abstract: INTRODUCTION: Focused ultrasound delivered at low intensities (LIFU) has been reported to evoke responses in the motor, sensory, and visual systems through both inhibition and excitation without inducing histologic changes. The mechanism of activation and reversible lesioning in the central and peripheral nervous system is not well understood. A recent report has shown that ultrasound can induce a startle response that may be a confound in measuring ultrasound effects in the central nervous system. Several mechanisms of ultrasound neuromodulation have been proposed; most generally discount the role of thermal energy. Here we investigate the mechanism and characterize the effects of LIFU neuromodulation of the somatosensory evoked response using a high-precision dual-mode ultrasound mediated through a phased-array that can generate arbitrary waveforms. **METHODS**: Somatosensory-evoked potentials (SSEPs) were elicited in Sprague-Dawley rats by electrical stimulation of the leg. The SSEPs provides a clear neural response and pathway in which to study the effects of LIFU.

Concurrent ultrasound imaging and therapy was applied through a dual-mode 32-channel phased array with a 3.2 MHz carrier frequency. The focused ultrasound was used to targeting the ventral posterolateral nucleus (VPL), through which SSEPs pass on their way from the spinal cord to the cortex. To test the thermal effects on VPL, a 50 mW laser was applied with stereotactic placement of a fiberoptic catheter. **RESULTS**: Ultrasound applied to the VPL contralateral to electrical stimulation reversibly, and with graded amplitude, suppressed the SSEP. Ipsilateral application had no effect. The amplitude of tFUS was found to significantly impact the SSEP amplitude and shape, but LIFU delivered with a constant intensity (spatial-peak temporal average) reproduced a constant effect. Laser-mediated, reversible thermal lesions reproduced suppression of the first two peaks of the SSEP waveform. **CONCLUSION**: Neuroinhibition of a somatosensory circuit by LIFU at the thalamic relay was demonstrated in a fully anesthetized rat with lateral specificity. Spatial specificity indicated that LIFU with a phased array did not act primarily through a startle response. Only suppression of the SSEP was observed, no excitation caused by ultrasound was observed. The creation of a Thermal lesion using light corroborated our hypothesis that thermal effects of ultrasound is the dominant mechanism of LIFU neural suppression.

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Poster

392. Somatosensation: Thalamic and Cortical Processing

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 392.21/BB12

Topic: D.04. Somatosensation: Touch

Title: Somatosensory cortical responses to Aristotle tactile illusions

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Abstract: The purpose of this study was to investigate neural activity related to tactile illusions in the human brain, which can help us understand better the neurological mechanisms of tactile sensation. The study employed two well-known tactile illusion conditions (Aristotle and reverse illusions) in which subjects' twisted fingers were lightly touched with different numbers of mechanical stimuli at different locations. In the Aristotle condition, a single stimulus was given to the middle of two twisted fingers (index and middle), possibly leading to the illusory perception of two stimuli. In the reverse condition, two stimuli were synchronously given to the both ends of twisted fingers (index and middle), possibly leading to the illusory perception of a single stimulus. In addition to these conditions of generating a mismatch between the actual and

perceived number of stimuli, the resting state and a control condition (asynchronous stimulations on both fingers) were included in the experiment. Ten subjects participated in the experiment and their fMRI images were obtained using a 3T scanner (3T Magentom Prisma, Siemens, Germany). SPM12 was used to analyze BOLD signal patterns for each condition and to contrast brain responses to each type of stimuli against those in the resting state. This contrast analysis revealed significant neural activations in the bilateral postcentral gyrus (S1) and bilateral inferior parietal lobe ($p<0.001$). Furthermore, a contrast analysis between the Aristotle and asynchronous conditions, with the same number of perceived but different number of physical stimuli, found a significant difference in the activation level of contralateral S1 ($p<0.001$). This may indicate that S1 might not drive tactile illusion alone. Another contrast analysis between the reverse and asynchronous conditions, with the same number of physical but different number of perceived stimuli, found significant differences in bilateral thalamus and contralateral middle frontal gyrus (MFG) as well as contralateral S1 ($p<0.001$). It suggests that tactile illusion may involve interactions among many brain regions along with somatosensory cortical areas. The follow-up studies on functional connectivity between these regions would further verify this conjecture about the neural underpinnings of tactile illusions.

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Poster

392. Somatosensation: Thalamic and Cortical Processing

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 392.22/BB13

Topic: D.04. Somatosensation: Touch

Support: Leibniz-ScienceCampus Primate Cognition Seed Fund

Title: Central sulcus depth profiles of a large human cohort: Incidence of a divided central sulcus and description of the pli de passage fronto-parietal moyen

Authors: *R. SCHWEIZER[1,2], H. GRAETZ[1], J. STROPEL[1], J. FRAHM[1]
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Abstract: Introduction
The structural MRI data of 1200 subjects of the Human Connectome Project Young Adults were utilized to obtain the depth profiles of the central sulcus (CS). These profiles were examined for a comprehensive description of the general location and extent of the pli de passage fronto-parietal moyen (PPfpm), a deep gyrus in the fundus of the central sulcus at the somato-motor hand area. The data additionally allows us to obtain the incidence rate of the rare cases of the PPfpm complete, in which the deep gyrus rises up to the brain surface, thereby forming a

connection between the pre- and postcentral gyrus and causing a divided CS. The only large scale exploration of its incidence rate, performed in by Heschl in 1876/77, included 1087 brains and resulted in 6 cases of a hemisphere with a completely divided CS.

Methods

Unprocessed T1-weighted images were downloaded from the 1200 subject release of the Human Connectome Project. Applying the Morphologist 2015 pipeline within BrainVISA 4.5.0., each hemisphere's central sulcus was detected, manually confirmed and screened for a divided CS as identified by a brain surface pre- to postcentral gyrus connection in the hand knob area and verified on T1-weighted image. The CS depth profile (in mm) for each analyzed hemisphere was automatically determined by the morphologist pipeline (100 values: 0 = medial start, vicinity of the mid-hemispherical cleft, 100 = lateral end, vicinity of the sylvian fissure) and screened with peakdetect.py for the number of local maxima.

Results

Among the 1110 brains (2220 hemispheres) surveyed, 7 hemispheres showed a divided CS with the pli de passage fronto-parietal moyen reaching from the fundus to the brain surface. This observation corresponds to an incidence rate of 0.6 %. The averaged extracted CS depth profile of 1088 hemispheres shows an almost U-like shape with a small incision in the midway position. The average central sulcus depth was 17.11 mm ± 1.05 mm (mean ± SD). The screening for local maxima resulted in 65% of the hemisphere showing one, 8% two and 27% no local maxima.

Conclusion

The incidence rate of 0.6 % corresponds to the rate found by Heschl in 1877, showing a comparable occurrence of the divided CS over the last 140 years and between the different cohorts. This finding suites the hypothesis that this anatomical deviation, having no described clinical or behavioral implications, probably does not underlie a strong evolutionary pressure. The current algorithm detects a local maximum, i.e. a pli de passage fronto-parietal moyen, in approximately two thirds of the CS sulcus depth profiles.

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Poster

393. Auditory Processing: Sound Localization and Binaural Interactions

Location: SDCC Halls B-H

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Program #/Poster #: 393.01/BB14

Topic: D.06. Auditory & Vestibular Systems

Support: Science Foundation of Beijing Language and Cultural University (supported by "the Fundamental Research Funds for the Central Universities")

Title: Selective attention enhances cortical processing of binaural gap

Authors: *L. KONG[1], M. WANG[2], Z. XIE[3], L. LU[3], T. QU[4], L. LI[3]
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Abstract: Human listeners are extremely sensitive to changes in the similarity of sounds at the two ears (interaural correlation), which is critical to both localizing sound sources and detecting target auditory object against the noisy background. When an interaural time difference (ITD) in milliseconds is introduced, listeners' ability of processing the interaural correlation is associated with spatial unmasking of speech recognition in reverberant environments. Although selective attention is known to be involved in speech recognition under cocktail-party conditions, it is not clear whether the interaural correlation processing can also be modulated by attention. In this study, a binaurally uncorrelated fragment (i.e., binaural gap, interaural correlation = 0) is embedded in the binaurally identical noises and scalp event-related potentials (ERPs) evoked by the binaural gap was recorded when the participants were instructed to detect a binaural silent gap (active listening condition) or count visually presented target letters (passive listening condition). We found that the binaural gap remarkably evoked ERPs even when a 2-ms ITD was introduced. Under either the active or passive listening condition, the ERPs to the binaural gap with a 2-ms ITD were significantly weaker than those with a 0-ms ITD. More importantly, the ERPs to the binaural gap under the active listening condition were significantly larger than those under the passive listening condition. In contrast, the ERPs to the sound onset were not significantly affected by either ITD or attention. Thus, the interaural correlation processing is modulated by selective attention. The paradigm developed by this study is useful for investigating how the primitive auditory memory of fine-structure signals (Li et al., *PLoS ONE*, 8 (4) e63106, 2013) is top-down affected by higher-order processes.

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Poster

393. Auditory Processing: Sound Localization and Binaural Interactions

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 393.02/BB15

Topic: D.06. Auditory & Vestibular Systems

Support: R03DC013388

Title: Loss of interaural time difference sensitivity in rabbit inferior colliculus neurons following noise-induced hearing loss

Authors: *H. HARAGOPAL, R. DORKOSKI, G. WHALEY, L. PALMER, M. DAY
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Abstract: Sensorineural hearing loss (SNHL) is the most common form of permanent hearing loss, usually caused by cumulative overexposure to loud sounds. Individuals with SNHL show impairment in the abilities to both localize sound sources and use spatial cues to aid speech comprehension in noisy environments. However, the effects of SNHL on the circuits underlying sound localization are not known. Humans, and rabbits, localize sound in the horizontal plane (azimuth) via interaural time and level differences (ITDs and ILDs) of the sound waveform. These binaural cues are first encoded in the auditory brainstem, which projects to the inferior colliculus (IC). We measured responses of single-units in the IC of awake rabbits either with or without noise-induced hearing loss in response to auditory stimuli that varied in binaural cues in order to determine the effect of SNHL on sound localization coding. Rabbits were exposed to octave-band noise centered at 750 Hz at 133 dB SPL for 90 min under isoflurane anesthesia. Auditory brainstem response thresholds increased by approximately 30-45 dB between 0.5 and 16 kHz as measured two weeks after exposure, indicating extensive cochlear outer hair cell loss. Neurons from SNHL rabbits had greater sound-level thresholds to pure tones and noise stimuli than those from normal-hearing rabbits, as expected. Azimuth tuning curves were measured in response to noise stimuli with either natural ITD and ILD (ITD+ILD), or with one binaural cue fixed at zero (ITD-only and ILD-only). We quantified neural sensitivity to sound source azimuth (ITD+ILD stimulus) as the mutual information (MI) between firing rate and azimuth. There was a trend towards reduction in median MI for neurons from SNHL rabbits as compared to those from normal-hearing rabbits. We also computed MI between firing rate and ITD for noise stimuli that only varied in ITD (ILD fixed to zero). In this case, median MI was dramatically decreased for neurons from SNHL rabbits, with all MI values near zero, even though stimuli were above sound-level threshold. For neurons from SNHL rabbits, ITD+ILD azimuth tuning curves were very similar to ILD-only tuning curves, indicating neural sensitivity to azimuth was dominated by sensitivity to ILD. Our results suggest that 1) noise overexposure causes a loss of ITD sensitivity in the IC, and 2) any remaining information about sound source azimuth is due to sensitivity to ILD.

Disclosures: H. Haragopal: None. **R. Dorkoski:** None. **G. Whaley:** None. **L. Palmer:** None. **M. Day:** None.

Poster

393. Auditory Processing: Sound Localization and Binaural Interactions

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 393.03/BB16

Topic: D.06. Auditory & Vestibular Systems

Support: DGAPA project IN224414-2.

Title: EEG activity between right and left handed on a sound localization task: Pilot sex differences study

Authors: *M. CASTRO GONZÁLEZ[1], Y. DEL RÍO-PORTILLA[2]
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Abstract: Differences in brain organization and brain processing between right and left handed have been described, nevertheless is continue to be an issue of fact. There is also known that sound processing is different for both hemispheres in relation to sound localization. Sex differences have also be found especially on asymmetry especially on visuospatial attention. The aim of this study was to analyze brain response and behavioral response during task localization task before and after eye movements. We used twenty stimuli (musical note A, 2s each). Stimuli were presented in a classical random block design, for each group (left handed and right handed). After each run, subjects (n=30) respond on a keyboard according to where they heard the stimuli (right or left side) and at the same time to gaze on the direction they heard the stimuli (right or left side). For eye movements recording, we placed electrodes according EOG. Results showed significant differences on brain activity between left and right handed according to sound localization.
According on what we have been studding, on other experiments at the Laboratory, what we expected is an increase on absolute power over widespread brain regions for both hemispheres especially on high frequencies, alpha2, beta1, beta2 and gamma higher for right handed. For behavioral data analysis by comparing PC keyboard answers, we expect right responses for right handed in comparison with left handed, and left answers for left handed in comparison with right handed. Also for EOG analysis.
By the time we might say that brain processing during a sound localization task, is similar for right and left handed according to EEG results in other Laboratory experiments, so we could assume brain widespread response especially in high frequencies. Moreover on behavioral analysis for those studies, it seems that on keyboard answers and eye movements response, there is a difference denoting that for eye movements, laterality plays an important role that might be related to orientation reflex, so eye movement response is faster and spontaneous in relation to keyboard answers where subjects need to be more attend to the task to give an answer.
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María del Carmen Castro-González is granted by DGAPA project IN224414-2.

Disclosures: Y. del Río-Portilla: None.

Poster

393. Auditory Processing: Sound Localization and Binaural Interactions

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 393.04/BB17

Topic: D.06. Auditory & Vestibular Systems

Support: Irish Research Council GOIPG/2015/1656

Title: Decoding trajectories of multiple moving sound sources from EEG in cocktail party environment

Authors: *A. BEDNAR[1], E. C. LALOR[2]
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Abstract: We can clearly perceive the location of moving auditory objects in space. However, the cortical representation of auditory space, and auditory motion in particular, is not well characterized. Recently, we showed that in a simple acoustic scene with one sound source, auditory cortex tracks the time-varying location of a continuously moving sound. Specifically, we found that both the delta phase and alpha power of electroencephalographic (EEG) data can predict the azimuth of a moving sound source (Bednar 2017).
However, in natural settings, we are almost always presented with a mixture of multiple competing sounds and so we must focus our attention on the relevant source in order to segregate it from the background noise e.g. the 'cocktail party effect'. While many studies have examined the neural underpinnings of attentional selection in a cocktail party problem - especially in the context of sound envelope tracking by the cortex, it is unclear how we process and utilize spatial information in complex acoustic scenes with multiple sound sources. In this study we aimed to answer two questions: (1) Can we decode time-varying locations of multiple concurrently presented moving sound sources from EEG? (2) How does selective attention influence the sound trajectory tracking in cortex?
Subjects listened to two simultaneously presented noise stimuli having different spectral content over headphones. The stimuli were acoustically modified to be perceived as randomly moving on a semi-circular trajectory in the horizontal plane. Participants were asked to pay attention to one of the two presented stimuli and detect embedded targets. While subjects listened to the stimuli, we recorded their EEG using a 128-channel acquisition system. The data were analyzed by 1) deriving a linear mapping, known as a temporal response function (TRF), between the stimulus and a training set of EEG data, and 2) using the TRF to reconstruct an estimate of the time-varying sound source azimuth from a test set of EEG data.
Preliminary results show that locations of both attended and unattended sound sources are cortically represented and we can decode the trajectories of both sound stimuli from EEG with a

reconstruction accuracy significantly above chance level. We also found that selective attention significantly enhances the cortical representation of the sound trajectory of the attended stimulus. In addition, we found a correlation between our EEG-based trajectory reconstruction accuracy and behavioral performance in the target detection task. We believe this method has the potential to complement established sound envelope tracking-based attentional decoders in cocktail party environments.

Disclosures: A. Bednar: None. **E.C. Lalor:** None.

Poster

393. Auditory Processing: Sound Localization and Binaural Interactions

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 393.05/CC1

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant NS104911

Title: Population responses underlying statistical inference in a map of auditory space in the barn owl's midbrain

Authors: *R. FERGER[1], M. V. BECKERT[1], K. SHADRON[1], D. SANCULI[2], W. M. DEBELLO[2], B. J. FISCHER[3], J. L. PENA[1]
[1]Neurosci., Albert Einstein Col. of Med., Bronx, NY; [2]Univ. of California Davis, Davis, CA; [3]Seattle Univ., Seattle, WA

Abstract: The barn owl is a nocturnal hunter with outstanding sound localization abilities. It has become a model organism to study neural circuits of extracting binaural cues for sound localization that are relevant for behavior. Neurons in the owl's midbrain are known to respond maximally to acoustic stimuli with a distinct combination of interaural time difference (ITD) and interaural level difference (ILD). Together, these neurons form a neural map of auditory space which supports sound-orienting behavior. However, open questions regarding how the neural population in the map is read out on a trial-by-trial basis remain unanswered. This work reaches beyond responses of single neurons to understand the relationship between the activity pattern across the neural population and behavior.
Recent work has shown that the read out of population activity by a population vector (PV) predicts orienting head saccades, and approximates Bayesian statistical inference by integrating the overrepresentation of frontal directions and the differential shape of spatial tuning curves across the map. We investigated whether the trial-by-trial variability of neural activity matched predictions made under a Bayesian model, which matches a PV to the behavioral output. When a stimulus becomes less reliable, for instance if ITD detection is disrupted by adding independent noise at the two ears, an animal’s performance becomes less accurate. The Bayesian model

predicts that this should manifest in a broadening of the population response in the map. An alternative hypothesis that could explain this behavioral effect is that the reduced reliability induces shifts in the population response, which are indistinguishable from a response to a different ITD, termed differential correlations. Using a microelectrode array to record multiple units across the map, we tested whether the trial-by-trial population activity better matched the Bayesian model or the presence of differential correlations. On single trials the activity matched the pattern of activity predicted by the Bayesian model implemented by a PV. This was consistent across a number of stimulus conditions. Furthermore, the correlation structure of neurons in the map did not support a trial-by-trial shift in the center-of-mass as predicted for differential correlations. This provides additional support for the PV model.

Disclosures: R. Ferger: None. **M.V. Beckert:** None. **K. Shadron:** None. **D. Sanculi:** None. **W.M. DeBello:** None. **B.J. Fischer:** None. **J.L. Pena:** None.

Poster

393. Auditory Processing: Sound Localization and Binaural Interactions

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Program #/Poster #: 393.06/CC2

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC007690
BRAIN Initiative Grant NS104911

Title: Co-variability across the neural population in the map of auditory space of the barn owl

Authors: *M. V. BECKERT[1], R. FERGER[1], K. SHADRON[1], B. J. FISCHER[2], J. L. PENA[1]
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Abstract: The midbrain of the barn owl contains a map of auditory space. However, the characterization of the map has been based mainly on single-cell or local multiunit recordings. This approach is blind to co-variabilities in response strength and spike timing that occur on a trial-by-trial bases. However, these correlations have profound effects on information encoded by neural populations, depending on their structure as well as the system they are embedded within. We therefore sought to expand upon previous work by measuring population activity in the map-of-space.
To this end we have developed multi-electrode array recordings, utilizing a microdrive capable of positioning electrodes deep within the brain, allowing access to the midbrain map. Using this approach, data were collected to quantify the similarity of tuning (signal correlation), trial-by-trial co-variability (noise correlation), as well as precision of spike timing (spike-time synchrony).
As anticipated, more distant neurons displayed weaker signal correlations. Additionally, noise

correlations were directly related to the signal correlation between pairs of neurons, similar to observations in the visual system. On the other hand there was no relationship between the level of spike-time synchrony and signal correlation. We assessed the impact of these correlations on information by training a decoder with neural data, which preserved the natural correlations. We compared this to a decoder that removes correlations by shuffling the trials. This work expands upon previous work that investigated the sound localization system of the barn owl using either recordings from single neurons or multiple nearby neurons.

Disclosures: M.V. Beckert: None. **R. Ferger:** None. **K. Shadron:** None. **B.J. Fischer:** None. **J.L. Pena:** None.

Poster

393. Auditory Processing: Sound Localization and Binaural Interactions

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 393.07/CC3

Topic: D.06. Auditory & Vestibular Systems

Support: DC007690

Title: The effect of anticipated cue reliability on behavioral and neural adaptation in barn owls

Authors: *K. SHADRON, M. V. BECKERT, R. FERGER, J. L. PENA
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Abstract: The brain actively updates the representation of the environment. An open question about this function is whether adaptation is weighted by the predicted statistics of sensory information. Here we asked whether anticipated cue reliability affects the rate of adaptation in the auditory system of the barn owl.
The midbrain of the barn owl contains a map of auditory space, which uses the interaural phase difference to compute sound location in azimuth. Previous work showed that space-specific neurons in this map are tuned to the frequency range that is most reliable for its preferred location. This effect is due to the acoustical properties of the head, causing higher frequencies to convey interaural phase difference more reliably in frontal space and lower frequencies in the periphery. We hypothesized that adaptation to sound location would differ between stimuli expected to be reliable or unreliable. We tested this hypothesis at the behavioral and neural-population levels. We measured the pupillary dilation response, an orienting response that readily adapts upon repetition of a stimulus. Tones were repeatedly presented through earphones to an awake barn owl, either from the front or periphery of the head to measure the habituation rate. To assess the strength of the novelty detection, a deviant in location was then presented to elicit a recovery of the PDR. Trials with higher cue reliability were compared to trials with low reliability to find trends in habituation. To assess this question at the neural-population level, we

conducted recordings of multiple neurons in the space map using a microelectrode array. Adapter and test stimuli were used to assess population and activity and tuning of individual cells before and after adaptation. Frontal and peripheral neurons were compared to test the hypothesis that anticipated reliable and unreliable stimuli lead to different adaptation rates.

Disclosures: K. Shadron: None. **M.V. Beckert:** None. **R. Ferger:** None. **J.L. Pena:** None.

Poster

393. Auditory Processing: Sound Localization and Binaural Interactions

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Program #/Poster #: 393.08/CC4

Topic: D.06. Auditory & Vestibular Systems

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Title: Subthreshold oscillations underlie diverse firing phenotypes and impart graded sensitivity to envelopes in neurons of the medial superior olive

Authors: *B. BONDY[1], N. GOLDING[2]
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Abstract: Neurons of the medial superior olive (MSO) compare the arrival time of low frequency sounds to the two ears (interaural time difference, ITD), cues used for sound localization. It has long been assumed that the MSO is composed of identical neurons, regardless of location along the tonotopic axis of the nucleus, but the data testing this assumption is sparse and conflicting. To understand the relationship between physiological properties and tonotopic location, we made >500 whole cell patch clamp recordings from MSO neurons in brainstem slices from Mongolian gerbils (P18-28), subsequently analyzing cell morphology and location within the nucleus. Unexpectedly, we found that MSO neurons could be separated into three groups based on firing pattern: phasic neurons, which fire a single, small-amplitude spike at stimulus onset, oscillators, which fire trains of overshooting spikes in a pause pattern, and tonic neurons, which fire large spikes regularly. All firing types could be found in both high and low frequency regions of the MSO. The membrane properties of MSO cells formed a continuum, with oscillators and tonic neurons exhibiting membrane time constants 3x and 16x those of phasic neurons. All MSO neurons received bilateral excitation and inhibition and sent axons outside the MSO. However, as with their slower membrane properties, oscillator/tonic neurons exhibited poorer temporal resolution in *in vitro* ITD experiments (halfwidths: phasic, 0.51±0.0 ms; oscillator, 0.92±0.2 ms; tonic, 2.1±0.4 ms). The differences in firing patterns were driven in

part by subthreshold oscillations. Large, slow (~25-130 Hz) oscillations in tonic neurons drove low-threshold spiking. Faster, smaller oscillations (~100-425 Hz) promoted spiking in oscillator neurons, while even faster oscillations (~400-750 Hz) were too small to evoke spikes in phasic neurons.To determine how different functional properties of MSO neurons might affect their ability to encoding auditory stimuli with different temporal features, we injected rectified sinusoidal shaped currents into cells over a range of amplitudes and frequencies, to mimic amplitude modulated envelopes. Phasic neurons were unable to respond to frequencies below ~200Hz, whereas most oscillators and all tonic neurons responded robustly across all frequencies. Oscillations in both oscillator and tonic neurons appeared to play a particularly important role in facilitating spiking during low frequency envelopes. In summary, our results show that MSO neurons are far more diverse than previously reported. We postulate that such diversity allows encoding ITDs of both envelopes and fine temporal structure of sounds.

Disclosures: B. Bondy: None. **N. Golding:** None.

Poster

393. Auditory Processing: Sound Localization and Binaural Interactions

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant T32DC012280
FRAXA Research Fellowship
Hearing Health Foundation, Emerging Research Grant

Title: Alterations to the sound localization pathway in fragile X syndrome

Authors: *E. MCCULLAGH, M. M. HUNTSMAN[1], A. KLUG[2]
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Abstract: Hypersensitivity to sound and impaired sound localization are some of the most common sensory symptoms described by people with autism. The sound localization pathway, a neural network within the auditory brainstem, enables us to not only localize the location of a sound source per se, but also to separate between multiple simultaneous auditory streams that enter our ears. Whenever we have a conversation in situations such as a crowded restaurant, a busy public place, or a room where background noises are present, our sound localization circuit helps us to parse this complex situation into multiple narrow spatial channels based on their location. Being unable to localize the source of a sound, and to focus on a conversation when distracting noises are present significantly impairs social interactions in autistic patients. Despite

its importance, our understanding of how the sound localization circuit is impaired in autism is largely unknown. To explore alterations in the sound localization pathway in autism, we can use a mouse model (*Fmr1* KO mice) for the most common genetic form of autism, Fragile X syndrome (FXS). We have shown that there are frequency-specific alterations to the auditory brainstem in FXS mice, we have continued this study by examining alterations to myelin fibers that innervate this area and contribute to the speed of sound processing. We have seen alterations in myelin in the brainstem of FXS consistent with possible alterations to sound localization ability. We have measured myelination using both Transmission Electron Microscopy (TEM) and Coherent Anti-Stokes Raman Scattering (CARS). Using these two techniques we can measure the diameter of myelinated axons as well as the thickness of the myelin in those areas. We have seen changes to the myelination in FXS in the fibers that innervate the medial nucleus of the trapezoid body (MNTB). Determining the cause of sound localization impairments in FXS will help determine future strategies for treatment of these impairments, and perhaps FXS in general.

Disclosures: M.M. Huntsman: None. **A. Klug:** None.

Poster

393. Auditory Processing: Sound Localization and Binaural Interactions

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Program #/Poster #: 393.10/CC6

Topic: D.06. Auditory & Vestibular Systems

Support: NSF BCS-1539376
NSF BCS-1539276

Title: Investigate what makes it “new” in the old-plus-new strategy of auditory scene analysis in the auditory cortex of marmoset monkeys

Authors: *Y. ZHOU[1], J. BRAASCH[2]
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Abstract: Auditory Scene Analysis (ASA) is an umbrella term that describes the auditory system’s remarkable ability to decompose signal-based acoustic features from a complex sound mixture and regroup them into perceptually relevant auditory objects and streams. A fundamental concept of ASA is the *old-plus-new* strategy, which states that the auditory system correctly interprets the sudden development of new elements in an acoustic mixture as additional, distinct elements added to an existing continuing sound source. Braasch and Hartung (2002) showed that human localization performance of a target sound (200-ms broadband noise) was worsened by a masker sound (different noise waveform) presented simultaneously from a

fixed frontal location. However, the listeners had no difficulties localizing the target if the masker preceded the target by 200 milliseconds. Their model proposed that the correct target localization was possible because listeners could use information from the preceding part of the masker to perceptually separate target and masker using the old-plus-new strategy of ASA. This study investigated the neural mechanisms of perceptual spatial release from masking and the way in which neuronal activities manifest the target spatial information in the presence of a competing masker sound. We used a similar spatial setup to the one used in the human study mentioned above. We collected single-unit activity from the auditory cortex of awake marmoset monkeys. We first measured the rate spatial tuning function of a neuron to a target sound (broadband noise or best-frequency tone) presented alone. We then compared how simultaneous or preceding noise or tonal masker altered the target-alone spatial tuning function. We observed that while the spiking activities of many cortical neurons show hemifield-field spatial tunings (front, back, left and right), the non-responsive regions are actively inhibited, suggesting cortical modulations of auditory spatial selectivity. We found that spatial tuning remains less affected with the preceding than simultaneous maskers, consistent with human studies. However, the extent of spatial release from masking is affected by the onset/sustained patterns of the masker responses and the extent of cortical inhibition/suppression in both target and masker responses. We propose a new model for the old-plus-new strategy in the spatial domain, which integrates two neural processes - adaption and suppression - to reveal the central mechanisms that make the target spatial information become “new” in a multi-source acoustic environment.

Disclosures: Y. Zhou: None. **J. Braasch:** None.

Poster

393. Auditory Processing: Sound Localization and Binaural Interactions

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Program #/Poster #: 393.11/CC7

Topic: D.06. Auditory & Vestibular Systems

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Brain Research Seed Funding by UCSC

Title: Characterization of auditory neurons in the mouse superior colliculus detected using virtual auditory space stimulation

Authors: *S. ITO[1], D. A. FELDHEIM[2], A. M. LITKE[1]
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Abstract: We characterized the properties of sound localization of the neurons in the mouse superior colliculus (SC) using large-scale silicon probe recording and virtual auditory space stimulation. We found that the SC contains neurons with responses tuned to localized areas in the virtual auditory space.
The brain computes the sound source location based on auditory signals that arrive at the two ears. The cues for the sound localization are binaural differences of the timing and the intensity, as well as a modulation of frequency spectra. These cues are induced by physical structures of the head and ears that can be modeled by a measured head-related transfer function (HRTF). The computed sound elevation and azimuth are mapped onto the SC. The SC also contains a visual map of space that is aligned with the auditory map. The alignment of the visual and auditory maps has been demonstrated in species such as the cat, the ferret and the barn owl (optic tectum), but in mice the properties of the SC auditory spatial map has not been well established. Characterization of the mouse SC auditory map will open the door for further investigations of the sound localization circuitry using genetic and neural recording tools available for use in mice.
In order to measure the mouse SC auditory map, we developed a system that presents auditory stimulation in a virtual space. First, we measured the HRTFs using a high-frequency speaker located 25 cm from a mouse head with a calibrated microphone coupled with the back of the ear canal. The measured HRTFs are then used to calculate the auditory stimulus with properties consistent with a specific source direction. This stimulus is presented to a mouse via earphones. This system is compatible with large-scale recording methods that require overhead acoustic obstacles and allows automated control of the sound directions in two dimensions.
This virtual auditory stimulation system revealed that there are neurons in the deep SC that are tuned to a specific (virtual) sound source direction. The properties of the auditory receptive fields and the topographic map will be presented. These results demonstrate that the mouse is a model to study mechanisms of auditory circuit formation and function.

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Poster

393. Auditory Processing: Sound Localization and Binaural Interactions

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Program #/Poster #: 393.12/CC8

Topic: D.06. Auditory & Vestibular Systems

Support: AIIMS, New Delhi, Intramural Grant No. - A-499

Title: Age-related changes in the total number of neurons and the GAD-immunoreactive neurons in human inferior colliculus: Stereological study

Authors: *I. PAL[1], P. KUMAR[1], T. G. JACOB[1], D. BHARDWAJ[2], T. S. ROY[1]
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Abstract: The inferior colliculus (IC) in an integration centre in the auditory pathway plays a critical role in the binaural processing of auditory information. Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter (produced by glutamic acid decarboxylase-GAD) in IC that is critically involved in the temporal processing of auditory information. During aging, levels of GABA are known to change in different parts of the brain and some nuclei of the auditory system. In our study, we attempted to estimate the age-related changes in the total number of GAD-immunoreactive neuronal population and have also correlated it to the total number of neurons in the human IC. After obtaining clearance from the Ethics Committee, thirty-one samples were obtained from the mortuary and divided into three age groups- young (10-29 years) middle (30-49 years) and old (50-79 years). The IC was cryoprotected, sectioned transversely at 50 µm thickness and then stained with either Cresyl violet (CV) or for immunohistochemical expression of GAD67 (Abcam ab26116, 1: 1000). The number of CV-stained and GAD67-immunoreactive (GAD67-ir) cells neurons were estimated by Optical Fractionator. The estimated total number of neurons in the IC was 1,269,085 ± 75,682 in young age group, while the number of GAD67-ir neurons was 184107 ± 46,811. A significant decline in the CV-stained and GAD67-ir neurons was observed during aging. The GAD67-ir neurons in the old group were significantly decreased approximately by 32% and 27% when compared to young ($p = 0.0068$) and middle ($p = 0.036$) age groups, while the CV-stained neurons old age decreased by 10% when compared to young age group ($p = 0.02$). Hence, our study provides neuroanatomical proof of the decline in quality of sound processing being affected due to not only a general but significant decline in the total number of neurons in the IC, but also due to a loss of GABAergic neurons, which would affect the inhibitory fields in the IC. Further, it may cause poorer ability to detect gaps in the auditory stimuli, therefore leading to poor speech discrimination.

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Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Program #/Poster #: 394.01/CC9

Topic: D.06. Auditory & Vestibular Systems

Support: The Japan Epilepsy Research Foundation
Non-linear Neuro-oscillology, MEXT, Japan

Title: Effects of deviance direction on frequency and duration mismatch fields: Hemispheric functional difference revealed by monaural presentation

Authors: *T. MATSUBARA[1], T. UEHARA[1], K. OGATA[1], T. MAEKAWA[2], S. TOBIMATSU[1]
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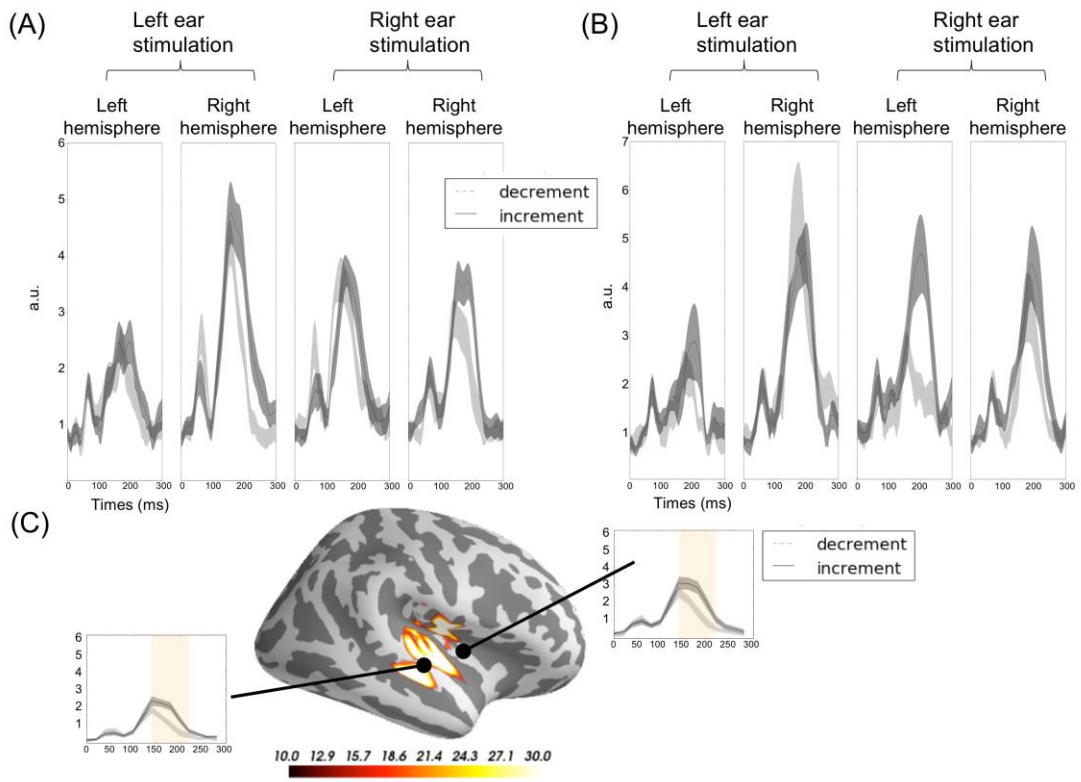
Abstract: The mismatch negativity (MMN) and its magnetic counterpart (MMF) reflect the process of change detection in the auditory system. Although behavioral study reported that listeners are more sensitive to increments (Inc) in frequency than they listen to decrements (Dec), little attention has been paid to the deviance direction on MMFs. The present study investigated the effects of deviance direction (increment (Inc) vs, decrement (Dec)) on frequency (Freq) and duration (Dur) MMFs to understand the hemispheric functional difference. Sixteen subjects were tested. Monaural pure tone stimulation was performed. For the Inc condition of Freq-MMF, the standard stimulus was the 500-Hz/100-ms tone while the deviant was the 550-Hz/100-ms tone. In Dur-MMF, the standard was the 50-ms/500-Hz tone whereas the deviant was the 100-ms/500-Hz tone. For the Dec condition, the standards and deviants were reversed in both MMFs. Source localization was performed followed by a non-parametric spatio-temporal clustering method and a two-way ANOVA with main factors of DIRECTON and EAR was carried out. MMFs were defined as a cluster containing the response occurring at around 200 ms in the auditory cortex. Thus, presence of the significant cluster directly implies the different origin of MMFs between the levels by the factor. Clear MMFs were obtained in all conditions in both Freq-MMFs (Fig. 1A) and Dur-MMFs (Fig. 1B). In Freq-MMF, right hemisphere was more activated in the Inc condition than the Dec condition (Fig. 1C). On the contrary, there was no significant effect of DIRECTION in the left hemisphere. In Dur-MMFs, there were also no significant clusters regarding DIRECTION. The current results were in accord with previous studies showing there was a bias to the right hemisphere for frequency activations, whereas duration activations were bilateral or biased to the left hemisphere. Our study also supports the behavioral evidence of better performance for identifying Inc in frequency. Our MMF paradigm focusing on the deviance direction may be useful for investigating the hemispheric functional difference.



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Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD Grant DC-013174

Title: Sequence sensitive processing in songbird auditory forebrain

Authors: *M. DONG, D. S. VICARIO
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Abstract: How stimulus sequence affects neural responses is important for understanding the neural mechanisms of speech processing because speech consists of a rapid sequence of sounds

governed by a set of transition probabilities (e.g. frequently occurring sound sequences may represent words). At the neural level, sequence processing has been widely studied using the "oddball" paradigm, in which one rare deviant sound (oddball) is presented in a series of frequent standard sounds. Neurons in the auditory cortex respond more strongly to the oddball than to the standard (oddball effect). However, these studies have typically used only this simple oddball paradigm and few have investigated neural responses to deviants in more complex sequences (e.g., alternation of two stimuli). Because the oddball effect can also be explained by synaptic habituation or violation of a constant auditory environment, the simple oddball paradigm cannot be used to test whether neural responses are sensitive to the stimulus sequence itself.

Using a more complex paradigm, the current study recorded extracellular neural activity in response to sound sequences, using 16 electrodes in the zebra finch auditory forebrain. Two stimuli were initially presented in either a random or alternating order at 1s intervals. Then a change was introduced: each stimulus was occasionally repeated at random times. When the preceding sequence before repetition was random, the 2nd stimulus in the repetition elicited smaller neural responses (lower single-unit firing rates) than the 1st stimulus, indicating that repetition has a suppressive effect. In contrast, when the preceding sequence was alternating, the suppressive effect was significantly reduced, indicating a neural response to the unexpected repetition. Neural responses were further analyzed at the population level using Linear Discriminant Analysis (LDA). LDA showed that the population responses the 1st and 2nd stimulus in the repeated pair were significantly different, confirming the suppression effect. However, LDA showed that the population responses to 1st and 2nd stimulus in the repeated pair were not distinguishable (reflecting reduced suppression). These results support the hypothesis that neural responses are sequence-sensitive and cannot be simply explained by synaptic habituation or violation of a constant auditory environment. We interpret this as evidence of predictive coding at the neural level that may have implications for the neural mechanisms of sequence sensitivity in speech processing.

Disclosures: M. Dong: None. **D.S. Vicario:** None.

Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Human Frontier in Science Young Investigator Award RGY0073/2014
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NIH R01DC015527

Title: Cortical excitatory and inhibitory neurons differentially affect collicular responses to sound

Authors: *J. BLACKWELL, W. RAO, D. RIDOLFI-STARR, M. GEFFEN
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Abstract: Within the auditory cortex, excitatory and inhibitory dynamics determine frequency selectivity in excitatory cells (Hamilton et al., 2013, Aizenberg et al., 2015; Seybold et al., 2015; Natan et al., 2015; Phillips et al., 2016). Auditory cortex sends an extensive descending pathway to the inferior colliculus, but how excitatory-inhibitory dynamics in the cortex affect this pathway remains unknown. Previous studies demonstrated that responses of neurons in the inferior colliculus are altered by focal electrical stimulation and pharmacological inactivation of auditory cortex (Jen et al., 1998; Yan et al., 2005; Zhang et al., 2005), but these methods lack the ability to manipulate specific cell-types. In this study, we use optogenetic techniques to modulate activity of excitatory cells, parvalbumin-positive (PV) interneurons, and somatostatin-positive (SOM) interneurons in the auditory cortex to test whether and how this pathway modulates frequency selectivity in the inferior colliculus in both anesthetized and awake mice. As expected from previous studies, activation of cortical excitatory cells increased spontaneous activity and decreased frequency selectivity, while activation of PV interneurons and SOM interneurons decreases spontaneous activity and increased frequency selectivity in putative excitatory cells in the auditory cortex. Furthermore, suppression of PV interneurons had the opposite effects on putative excitatory cell activity. However, whereas activation of excitatory cells decreased frequency selectivity and tone-evoked responses in the inferior colliculus, modulation of PV and SOM interneurons had weak effects on activity in the inferior colliculus. These findings suggest that modulation of frequency selectivity in auditory cortex by inhibitory neurons does not necessarily propagate to the inferior colliculus and that PV and SOM interneurons may differentially affect activity in the inferior colliculus.

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Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Program #/Poster #: 394.04/CC12

Topic: D.06. Auditory & Vestibular Systems

Support: Nebraska Tobacco Settlement Biomedical Reseach Foundation (BM)

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Title: Alpha7 nAChR knockout mice exhibit degraded auditory processing

Authors: R. A. FELIX, II[1], D. NOVICIO[1], V. CHAVEZ[1], C. V. PORTFORS[1], *B. J. MORLEY[2]
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Abstract: *CHRNA7*, the gene that encodes the alpha 7 subunit in the nicotinic receptor gene family, has been identified as a gene associated with some autism spectrum disorders and other neurodevelopmental conditions characterized, in part, by language impairment. Auditory processing deficits may underlie speech impairment. A reliable indicator of auditory processing disorders is impaired timing of neural activity despite normal ear function, which suggests that problems encoding temporal information arise within the brain. We examined the timing properties of sound-evoked activity following disruption of signaling mediated by the alpha 7 nicotinic acetylcholine receptor (α7 nAChR). Here, we measure auditory event-related potentials of α7 nAChR knockout mice of both sexes and age-matched colony controls. We find a significant timing delay in evoked neural signal that represents midbrain activity in knockouts, despite normal hearing thresholds. To further investigate the delay in midbrain timing, we examine single-neuron spiking activity in the inferior colliculus. We assess the temporal acuity of evoked activity by measuring the precision of first-spike latencies and response durations. In addition, we compare neural correlates of behavioral measures of temporal acuity including gap detection and forward masking in knockout and wild type animals. We find that temporal acuity is impaired in the midbrain of knockouts, whereas other responses properties such as spiking rates are no different than those of wild type controls. We also examine spiking responses of neurons in the superior paraolivary nucleus and the ventral nucleus of the lateral lemniscus, which are brainstem nuclei known to shape spike-timing properties of their synaptic targets in the inferior colliculus. Together, these areas represent a presumptive pathway for encoding timing information needed for identifying natural sounds. These areas also exhibit the highest levels of α7 nAChR expression in the developing auditory system. We find that, like the midbrain, the precision of first-spike latencies, gap detection thresholds, and masking abilities are impaired for brainstem neurons of knockout animals. We conclude that altered temporal processing at the level of the brainstem in α7 nAChR-deficient mice may contribute to degraded spike timing in the midbrain, which likely underlies the observed timing delay in the auditory event-related potentials. Our findings are consistent with a role for the α7 nAChR in auditory processing disorders and identify a neural circuit early in the central auditory pathway that represents a promising target for therapeutic intervention.

Disclosures: R.A. Felix: None. **D. Novicio:** None. **V. Chavez:** None. **C.V. Portfors:** None. **B.J. Morley:** None.

Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Program #/Poster #: 394.05/CC13

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant R01DC002260
REAC Grant from UCSF (2016)

Title: Enhanced ability of detecting rat vocalization-in-noise by sound exposure during a critical period

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Abstract: During critical periods, receptive fields develop to adapt to the sensory environment by forming neural circuits that optimally process relevant stimuli. In humans, this developmental stage is essential for acquiring language and better sound processing. It has been shown that sound exposure during a critical period dynamically altered the receptive field properties in the auditory cortex such as frequency tuning, tuning bandwidth, or temporal resolution (de Villers-Sadani and Merzenich, 2011). However, the relationship between altered neural coding and perceptual abilities is yet largely unknown. In this study, we tested the hypothesis that exposure to moderate levels of structured background noise during the critical period enhances the ability of adult animals to process vocalization-in-noise.
Sprague-Dawley rat pups were raised in moderate noises (~60 dB SPL) of different spectro-temporal statistics during their auditory critical period (P6-45). Once these animals reached adulthood, they were trained to detect vocalizations presented in these noises using a Go/No-go behavioral paradigm and compared to unexposed animals. The sensitivity index (*d'*) was calculated to evaluate the effect of different noise statistics on their ability of detecting vocalizations. Similarly, we investigated primary auditory cortical neuron responses to the same stimulus conditions in normal, exposed and trained animals. Neuronal discriminability was quantified using Euclidian distance-based spike train classifiers.
The noise exposure enhanced, in adult animals, their behavioral performance of detecting rat vocalizations in background noise. Improvement appeared to depend on stimulus statistics used for noise exposure. In addition, cortical signal encoding of vocalizations improved noise-exposed animals accompanied by specific shifts in receptive field properties compared to unexposed animals.
The results support the idea that maturational noise exposure can improve cortical receptive field properties best suited for information extraction in noisy environment thus reducing the impact

of background noise masking and helping the animals to perceptually segregate signals from noise background.

Disclosures: N.Y. Homma: None. **C.E. Schreiner:** None.

Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: SDCC Halls B-H

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Program #/Poster #: 394.06/CC14

Topic: D.06. Auditory & Vestibular Systems

Support: BBSRC New Investigator Award BB/M010929/1

Title: Two-photon imaging reveals “salt and pepper” tonotopy in ferret primary auditory cortex

Authors: *K. WALKER, Q. GAUCHER, M. PANNIELLO, A. IVANOV, J. DAHMEN, A. J. KING
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Abstract: Tonotopic organization has been a widely accepted property of the primary auditory cortex for over 50 years. It has been demonstrated in numerous electrophysiological studies across a wide range of mammals, including rodents, cats, ferrets, and primates. Nonetheless, recent studies in mice have challenged our understanding of tonotopy. Studies using 2-photon calcium imaging (2PI) to examine the activity of auditory cortical cells with unprecedented spatial resolution have shown that although tonotopic organization is visible at a large scale, neighboring neurons can have vastly different frequency preferences (Bandyopadhyay *et al.* 2010; Rothschild *et al.* 2012; Panniello *et al.*, submitted). As 2PI studies of auditory cortex to date have all been carried out in the mouse, it remains unclear whether this “salt and pepper” tonotopy is a general feature of mammalian auditory cortex, or a peculiarity of mice. To address this controversy, we have carried out 2PI in the auditory cortex of a carnivore - *Mustela putorius furo*. Each ferret (n=8) was injected with AAV1 expressing a genetically encoded calcium indicator (GCaMP6m or GCaMP6f) in the primary auditory cortex. After transfection (3-4 weeks), a craniotomy was performed above auditory cortex, and neural responses to sounds were imaged (200 x 200µm imaging fields; 100 - 400µm deep; corresponding to layers II/III) under medetomidine-ketamine anesthesia. Pure tones (1.2 - 41 kHz; 40 - 100 dB SPL) were presented to derive the Frequency Response Area of individual neurons. Our results show that although the classical tonotopic organization is visible across the cortical surface, neurons within an imaging field can widely vary in their frequency preferences. Quantitative analyses confirmed that the local variation in best frequency is similar in mice and ferrets. Therefore, the salt and pepper tonotopy described in the mouse is a common organizational feature of mammalian auditory cortex.

Disclosures: K. Walker: None. **Q. Gaucher:** None. **M. Panniello:** None. **A. Ivanov:** None. **J. Dahmen:** None. **A.J. King:** None.

Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Program #/Poster #: 394.07/CC15

Topic: D.06. Auditory & Vestibular Systems

Support: NIH R01 DC002260

Title: Receptive field characterization using pure tones and broadband stimuli in the mouse primary auditory cortex

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Abstract: The primary auditory cortex (A1) is a hub that communicates with subcortical and cortical regions. Sound information transferred from the medial geniculate body is further modified via intracortical horizontal pathways in A1. Although detailed anatomical pathway information is needed, functional descriptions of receptive field properties unambiguously illustrate spectral processing. Spectral integration in A1 can be shown via intracellular higher resolution recordings of the subthreshold receptive field. While subthreshold responses with intracellular recordings in A1 have been measured with pure tones, there is little evidence that compares the detailed properties of receptive fields with subthreshold and spiking responses using both pure tone and complex stimuli. We used anesthetized female C57BL/6 mice between 4 and 11 weeks of age and performed in vivo blind patch-clamp recordings in current clamp mode from A1 neurons located at a depth of 300-500 μm by applying pure tone (4-40 kHz, 0-70 dB) and broadband (0.5-40 kHz, 50dB relative to the pure tone threshold) dynamic moving ripple (DMR) stimuli. We constructed the tonal receptive field (TRF) from responses with pure tones and computed the spectrotemporal receptive field (STRF) using the spike-triggered average (STA) analysis for responses with the DMR stimulus. Sharpness of tuning (Q = characteristic frequency / bandwidth) for TRFs and STRFs was measured. TRFs from spiking responses showed narrower tuning than TRFs from subthreshold responses. STRFs in both subthreshold and spiking responses were more sharply tuned than TRFs. We conclude that single neurons in A1 perform spectral integration over a broad range of frequencies. The excitatory/inhibitory weighing for each neuron's spectral integration is modified by dynamic cortical activity resulting from inputs of spectrotemporally complex stimuli.

Disclosures: K.X. Kim: None. **C.A. Atencio:** None. **C.E. Schreiner:** None.

Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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ANR-10-IDEX-0001-02 PSL*
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Title: Multi-scale mapping along the auditory hierarchy using high-resolution functional ultrasound in the awake ferret

Authors: *Y. BOUBENEC[1], C. BIMBARD[1], C. DEMENÉ[2], S. A. SHAMMA[1], M. TANTER[3]
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Abstract: A major challenge in neuroscience is to longitudinally monitor whole brain activity across multiple spatial scales in the same animal. Functional UltraSound (fUS) is an emerging technology that offers images of cerebral blood volume over large brain portions. Here we show for the first time its capability to resolve the functional organization of sensory systems at multiple scales in awake animals, both within small structures by precisely mapping and differentiating sensory responses, and between structures by elucidating the connectivity scheme of top-down projections. We demonstrate that fUS provides stable (over days), yet rapid, highly-resolved 3D tonotopic maps in the auditory pathway of awake ferrets, with unprecedented physiological functional resolution (100µm). This was performed in four different brain regions, namely the auditory cortex, the medial geniculate body, the inferior colliculus, and the lateral lemniscus, this latter being a very small (1-2mm3 size), deeply situated subcortical (8mm deep) and previously undescribed structures in the ferret. Furthermore, we used fUS to map long-distance projections from frontal cortex, a key source of sensory response modulation, to the belt areas of the auditory cortex.

Disclosures: Y. Boubenec: None. **C. Bimbard:** None. **C. Demené:** None. **S.A. Shamma:** None. **M. Tanter:** None.

Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Program #/Poster #: 394.09/DD1

Topic: D.06. Auditory & Vestibular Systems

Support: Czech Science Foundation Grant 16-09086J

Title: Processing of acoustic signals in the presence of background noise in adult rats exposed to noise as juveniles

Authors: *D. SUTA[1], N. RYBALKO[2], J. POPELAR[2], T.-W. CHIU[3], J. SYKA[2]
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Abstract: Noise exposure in rat pups can serve as a model of early deprivation of the sensory input to the central auditory system that allow us to study developmental neuroplasticity of the auditory system. Our previous results demonstrated normal hearing thresholds, but abnormal intensity perception and frequency discrimination in adult rats briefly exposed to noise at the onset of hearing (on the 14th postnatal day, Rybalko et al., Physiol Behav, 2011, Šuta et al., Physiol Behav, 2015). In the present study, we assessed the gap-detection ability, tinnitus- and hyperacusis-like behavior using measurement of the acoustic startle response and its modulation by a gap in background noise or by background noise itself. A significant deficit in the gap detection ability was observed in exposed animals at a moderate level of the background broadband noise (55 dB SPL). An increase of the background noise intensity to 65-75 dB SPL led to strengthening of gap prepulse inhibition in exposed animals, which approached the gap prepulse inhibition values of control animals at those noise intensities. The signs of tinnitus - a deficit in detection of gap in 10 kHz narrow-band background noise but not in noise of other frequency content - were found in 2 of 8 exposed rats. An increased sensitivity to background noise as a typical sign of hyperacousis was manifested in exposed rats by suppression of the acoustic startle response at significantly lower background noise levels than in controls. Our results indicate that acoustic trauma producing only a transient threshold shift during critical developmental period may lead to a permanent impairment in processing of acoustic signals in the presence of background noise, which can be related to a deficit in temporal processing and/or development of tinnitus- and hyperacusis-like behavior.

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Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Program #/Poster #: 394.10/DD2

Topic: D.06. Auditory & Vestibular Systems

Title: Preclinical and clinical development of OTO-311, a sustained-exposure formulation of the NMDA receptor antagonist gacyclidine, for the treatment of tinnitus

Authors: *F. PIU, N. TSIVKOVSKAIA, R. FERNANDEZ, X. WANG, J. ANDERSON, K. BISHOP
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Abstract: Tinnitus, the perception of sounds without a correlated external auditory stimulus, is widely prevalent and often debilitating. Excessive activation of NMDA receptors within the cochlea may be particularly important in altering activity of the auditory nerve and generating tinnitus. OTO-311 is a thermosensitive, sustained-exposure formulation of the potent non-competitive NMDA receptor antagonist gacyclidine. Using a variety of techniques, the profile of gacyclidine/OTO-311 was characterized preclinically. Furthermore, a Phase 1 clinical study, designed to assess the safety and pharmacokinetics of intratympanic administration of OTO-311 was conducted in healthy volunteers. Gacyclidine was found to be a potent and selective NMDA receptor antagonist with low nM affinity, and a relatively rapid on-rate and slow off-rate. In cultured hippocampal or cochlear spiral ganglion neurons, gacyclidine dose-dependently blocked spontaneous activity that was NMDA receptor-dependent. The inner ear pharmacokinetic profile of gacyclidine, administered intratympanically as OTO-311, provided significant and sustained-exposure to the inner ear compartment. The Phase 1 clinical study was a single-center, randomized, placebo- and sham-controlled safety and pharmacokinetic study of OTO-311 administered by intratympanic injection in healthy volunteer subjects. OTO-311 (doses of 0.15, 0.3, and 0.6 mg gacyclidine) was evaluated in 12 healthy subjects per dose cohort (8 OTO-311; 2 placebo; 2 sham-injection). OTO-311 was found to be safe and well-tolerated, with no serious adverse events and no early discontinuations due to adverse events. Peak plasma concentrations of racemic gacyclidine occurred 1-2 hours post-dose and were dose-dependent. Extrapolating from preclinical data, local cochlear exposures were predicted to be in the therapeutic range. Overall, gacyclidine constitutes a potent, selective and attractive NMDA receptor antagonist for the treatment of tinnitus. A Phase 2 clinical study in tinnitus patients with an improved formulation of gacyclidine, OTO-313, is being developed.

Disclosures: F. Piu: A. Employment/Salary (full or part-time):; Otonomy Inc. **N. Tsivkovskaia:** A. Employment/Salary (full or part-time):; Otonomy Inc. **R. Fernandez:** A. Employment/Salary (full or part-time):; Otonomy Inc. **X. Wang:** A. Employment/Salary (full or

part-time):; Otonomy Inc. **J. Anderson:** A. Employment/Salary (full or part-time):; Otonomy Inc. **K. Bishop:** A. Employment/Salary (full or part-time):; Otonomy Inc.

Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Topic: D.06. Auditory & Vestibular Systems

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Pennsylvania Lions Club Hearing Research Fellowship Maria Neimark Geffen

Title: Neurons in auditory cortex are sensitive to frequency pattern violation

Authors: *L. GARAMI, C. ANGELONI, K. C. WOOD, M. N. GEFFEN
Univ. of Pennsylvania, Philadelphia, PA

Abstract: The human auditory system efficiently integrates spectral and temporal features of the environment, and auditory objects are often detected through regularities in their acoustical structure. Yet the underlying neural mechanisms remain unknown. Our goal was to identify the neuronal circuitry in auditory cortex (AC) that enables the auditory system to detect regularities in sounds.It has been shown that a network of auditory and frontal brain areas detects regular acoustic patterns. At a gross level, imaging studies showed that the AC and the prefrontal cortical regions exhibit sensitivity to violation of spectro-temporal acoustic regularities. Responses of individual neurons in AC also exhibit sensitivity to temporal regularities in sounds: Individual neurons exhibit stimulus-specific adaptation (SSA), a reduction in their response selective to frequently presented inputs. This adaptation may underlie the population sensitivity to more complex spectro-temporal acoustic regularities. Interneurons, such as Parvalbumin- or Somatostatin-positive neurons, through differential post-synaptic integration, can amplify adaptation to spectro-temporal patterns in excitatory neurons.Here, we adapted an oddball paradigm used for testing prediction errors in humans, to test the hypothesis that neurons in AC detect and encode pitch pattern violations. We used an oddball set up for probing the encoding of pitch structure. Tone pip frequencies of F1-4 were selected at 0.2 octave intervals, narrower than the typical tuning bandwidth of neurons in AC. The 4 different tone pips were arranged in pairs in a way that F1 was either preceded by a tone with an intermediate (standard) or with a large (deviant) difference in pitch. We recorded neuronal activity in AC of head-fixed mice using an

electrode array and compared the strength of responses to the deviant and standard to those in the equal probability condition.In our analysis for F1, we identified a group of neurons across 4 mice that showed stimulus-specific adaptation for this frequency. A subset of these neurons showed a firing rate increase when F1 was preceded by a rare tone (large pitch difference) compared with a standard tone (intermediate difference), suggesting that AC neurons are sensitive to spectro-temporal patterns and that the firing rate is modulated by the violation of the statistical pattern based on the frequency. These results advance our understanding of how an auditory figure would emerge based on adaptation to the spectro-temporal regularities.

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Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Topic: D.06. Auditory & Vestibular Systems

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Title: Dynamical properties of core vs. matrix thalamocortical networks in the auditory system

Authors: *M. N. O'CONNELL[1], A. BARCZAK[1], T. M. MCGINNIS[2], D. A. ROSS[4], J. SMILEY[3], P. LAKATOS[3]
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Abstract: It has been proposed that core regions of thalamic relay nuclei faithfully transfer specific sensory information, while matrix regions are responsible for orchestrating sensory input related modulatory responses (Jones, 1998). In the auditory system the thalamic matrix is dominant in the medial (MGBm) and dorsal (MGBd) subdivisions of the medial geniculate body (MGB), while in the ventral subdivision of the MGB (MGBv) core thalamocortical projections predominate. It has been speculated that inputs originating from the thalamic matrix, or non-specific thalamus (i.e. MGBm and MGBd), are responsible for the mechanism of oscillatory phase reset and entrainment in auditory cortical regions. To investigate this, we recorded neuroelectric activity from primary auditory cortex (A1) and MGB simultaneously using linear array multielectrodes in awake macaque monkeys while auditory (preferred modality) and visual (extramodal) stimulus streams were presented separately. MGB recordings were functionally classified as belonging to either the non-specific, matrix or the specific, core subdivisions of MGB. We found that, as expected, while auditory stimuli activated both core and matrix

subdivisions, the presentation of visual stimuli only resulted in matrix activation. Analyses of single unit responses, as well as functional and effective connectivity within and between MGB and A1, revealed fundamentally distinct cellular-network properties of the core vs. matrix thalamocortical networks, corroborating their proposed distinct functional roles in perceptual processes.

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Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant 2R01DC05660
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Title: Assessing evoked and oscillatory components in cortical synchronization to music using computational models

Authors: *K. DOELLING[1], M. F. ASSANEO[2], J. ROWLAND[3], D. BEVILACQUA[3], B. PESARAN[4], D. POEPPEL[5]
[1]Psychology, [2]New York Univ., New York, NY; [3]New York Univ., NYU, NY; [4]New York Univ. Ctr. for Neural Sci., New York, NY; [5]Neurosci., Max-Planck-Institute for Empirical Aesthetics, Frankfurt, Germany

Abstract: A body of research demonstrates convincingly a role for synchronization of auditory cortex to rhythms in environmental sounds such as speech and music. Some studies suggest that a hypothesized oscillator in auditory cortex could underlie important temporal processes such as segmentation and prediction. An important critique of these findings raises the question whether what is measured is in fact an oscillation or is instead a sequence of evoked responses. The two distinct mechanisms could look very similar in the case of rhythmic input, but an oscillator better provides the computational benefits mentioned above. We advance a new approach to adjudicate between the two models: analyzing the phase of synchrony at different stimulation rates. We use two kinds of computational models, evoked and oscillatory, to test this hypothesis. We show that in the evoked case, the phase of synchrony is heavily frequency dependent; the oscillatory model, in contrast, shows a much more consistent phase across frequencies. We compare these results to MEG data of participants listening to the music. The MEG data show a phase consistency that lies in between that of the pure oscillator and pure evoked model. Furthermore, the MEG signal can look more oscillatory (by this metric) depending on certain stimulus

features: the regularity of notes and the dampening of note attacks. The results support an auditory cortical signal that (i) contains components of both bottom-up evoked responses and internal oscillatory synchronization whose strengths are weighted by their appropriateness for particular stimulus types and (ii) cannot be explained by evoked responses alone.

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Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant R01DC015531
Office of the Vice President for Research & Innovation at the University of Oregon

Title: Contributions of distinct types of auditory cortical inhibitory interneurons to spectral surround suppression

Authors: *A. A. LAKUNINA[1], Y. AHMADIAN[2], S. JARAMILLO[1]
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Abstract: Spectral surround suppression in the auditory cortex is the phenomenon in which a neuron's response decreases when sound power increases in frequencies outside its classical receptive field. This process is potentially useful for filtering out broadband background acoustic noise. The role of different forms of cortical inhibition in generating spectral surround suppression remains unclear. Specifically, it is not known whether different types of inhibitory interneurons play distinct roles in shaping responses to broadband noise in surround suppressed neurons. The two most common inhibitory cell types in the cortex, parvalbumin-expressing (PV) and somatostatin-expressing (SOM) interneurons, differ in their morphology and connectivity, implying a difference in function. To test the hypothesis that PV and SOM cells contribute differently to spectral surround suppression, we used optogenetic tools to tag PV and SOM cells and identify them in awake adult mice during extracellular recordings. We then characterized the responses of PV, SOM, and putative pyramidal neurons to amplitude modulated bandpass filtered noise centered on the cells' preferred frequency. By varying the bandwidth of the stimulus, we determined the effect of adding power to the spectral surround on these cells' responses. We found that both PV and SOM cells exhibit little spectral surround suppression compared to pyramidal neurons. We also found that PV cells tend to respond strongly to the onset of sound stimuli, while their sustained responses to long duration sounds are weak. In contrast, most SOM cells respond over the entire duration of the sound, and have a greater

sustained response as compared to PV cells. These results suggest that SOM cells provide sustained, sound-driven inhibition that mediates spectral surround suppression in the auditory cortex.

Disclosures: A.A. Lakunina: None. **Y. Ahmadian:** None. **S. Jaramillo:** None.

Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Topic: D.06. Auditory & Vestibular Systems

Support: BBSRC New Investigator Award BB/M010929/1

Title: Investigation of pitch encoding neurons in the ferret auditory cortex

Authors: *Q. GAUCHER, A. Z. IVANOV, M. PANNIELLO, J. C. DAHMEN, B. WILLMORE, A. J. KING, K. M. M. WALKER
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Abstract: Pitch is one of the most salient and behaviourally relevant perceptual features of sound. It is the foundation of musical melody, and it plays a key role in both human and animal communication. Previous research in ferrets has helped to elucidate how the pitch of artificial vocal calls is encoded by auditory cortical neurons. We have shown that ferrets can classify the pitch of sounds as “low” or “high” (Walker *et al.*, 2009), that neurons which are sensitive to pitch cues are distributed widely across the auditory cortex (Bizley *et al.*, 2009, 2010; Walker *et al.*, 2011), and that auditory cortical neurons represent ferrets’ trial-to-trial pitch judgments (Bizley *et al.*, 2013). However, these studies did not examine whether the “pitch-sensitive” neurons neurons were able to maintain their tuning to a preferred fundamental frequency (F0) across a variety of stimuli (“pitch-selective” neurons), like those described in the marmoset pitch area (Bendor & Wang, 2005). In this study, we investigated whether pitch-selective neurons may also exist in non-primate auditory cortex. We recorded the responses of large populations of individual neurons to a variety of sounds that each varied in F0 (17 F0 values; 250 - 4000 Hz). The stimuli included click trains with varied temporal periodicity, pure tones, as well as sounds with filtering and phase manipulations to manipulate resolved harmonic and temporal envelope cues. Data were collected from ketamine and medetomidine anesthetized adult ferrets using high-channel-count multielectrodes (Neuropixels) and by imaging single neuron calcium dynamics with GCaMP6 under a 2-photon microscope. These experiments identified a subset of auditory cortical neurons with pitch selective responses, similar to those previously reported in marmoset (Bendor & Wang, 2005). Ferret pitch-selective neurons usually showed greater

sensitivity to temporal cues than resolved harmonic cues, in keeping with our recent behavioural findings (Walker et al., submitted).

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Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Topic: D.06. Auditory & Vestibular Systems

Support: Action on Hearing Loss
Royal Society
Medical Research Council

Title: Opposite abnormalities in gap-in-noise sensitivity in the auditory midbrain and thalamus of a mouse model of developmental disorder

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Abstract: The BXSB/MpJ-*Yaa* mouse is a powerful animal model for studying the neural mechanisms of gap-detection deficits. Around 30-50% of BXSB/MpJ-*Yaa* mice have small cortical malformations called ectopias. Previous work has shown that ectopic mice have more difficulty detecting very short gaps in noise than non-ectopic mice (Clark et al. 2000). Furthermore, minimum gap durations required to elicit significant changes in the activity of auditory thalamic neurons are longer in ectopic than non-ectopic mice (Anderson and Linden 2016). Moreover, sound-offset responses --- transient increases in firing at sound termination --- are less common in auditory thalamus of ectopic mice, suggesting the hypothesis that the abnormal thalamic gap-in-noise sensitivity might arise from a deficit in sound-offset responses (Anderson and Linden 2016).
To determine whether neural deficits in gap-in-noise sensitivity and sound-offset responses in the auditory thalamus of ectopic mice are inherited from the midbrain, we made extracellular recordings from the inferior colliculus (IC) in 10 ectopic and 10 non-ectopic BXSB/MpJ-*Yaa* mice. Mice were anaesthetised with urethane (as in the previous thalamic study), and neural responses recorded with 16-channel microelectrode arrays (Neuronexus). In contrast to the previous results from the lemniscal (primary) auditory thalamus, in IC we found that minimum gap durations for evoking responses to gap-in-noise stimuli were *shorter* in ectopic than non-ectopic mice, for neurons with V-shaped tuning curves typical of the lemniscal IC (rank-sum $p<0.001$; ectopic n=133 recordings, non-ectopic n=79). However, in agreement with previous

thalamic results, we found that the proportion of cells with sound-offset responses in IC was significantly reduced in ectopic mice (9% in ectopic, 15% in non-ectopic across all IC recordings; Fisher's exact test p=0.03). These results indicate that the offset-response deficit in auditory thalamus of ectopic BXSB/MpJ-*Yaa* mice may be inherited from the IC. Moreover, the observation that in ectopic mice, minimum gap duration thresholds are abnormally high in auditory thalamic neurons but abnormally low in IC neurons suggests a critical role for midbrain and/or thalamic circuitry in development of gap-detection deficits.

References:
Anderson LA, Linden JF (2016). Mind the gap: two dissociable mechanisms of temporal processing in the auditory system. J Neurosci 36:1977-95.
Clark MG, Sherman GF, Bimonte HA, Fitch RH (2000). Perceptual auditory gap detection deficits in male BXSB mice with cerebrocortical ectopias. Neuroreport 11:693-96.

Disclosures: J. Mattley: None. **L. Anderson:** None. **J.F. Linden:** None.

Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 394.17/DD9

Topic: D.06. Auditory & Vestibular Systems

Title: Auditory encoding of temporal and formal regularity in rhesus monkeys

Authors: *M. SCHWARTZE[1], H. MERCHANT[2], S. A. KOTZ[3]
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Abstract: Synchronization of motor behavior to events in the environment (entrainment) is readily observable in everyday activities such as sports, dance, or music performance. However, entrainment is also increasingly recognized as a hallmark of non-motor cognitive behavior, including speech processing, most likely serving the predictive allocation of neural and cognitive resources. This requires efficient encoding of recurrent regular stimulus relations as a potentially uniquely human precursor for predictive adaptation to dynamic sensory input. Here we used an experimental design previously tested in humans to assess auditory temporal (rate) and formal (pitch) regularity encoding in two rhesus monkeys (macaca mulatta). The monkeys listened repetitively to sequences of 512 standard (600 Hz) and 128 deviant (660 Hz) equidurational (300 ms, 10 ms rise and fall) sinusoidal tones (standard-to-deviant ratio 4:1). Pseudo-randomization ensured that each sequence started with four standard tones and that no more than two deviants were presented in a row. These sequences were presented with isochronous (900 ms; regular condition) or random (500-1300 ms; irregular condition) stimulus-onset-asynchronies.

Electroencephalographic (EEG) recordings were obtained from five scalp locations (Fz, F3, F4, Cz, Pz according to the 10-20 international system). Analyses focused on amplitude modulations (i.e., relative suppression effects) of the mid-latency P50 and N100 components of the event-related potential (ERP) and on evoked oscillatory responses at stimulation frequency taken as indices for the quality of temporal and formal regularity encoding in predictable and unpredictable contexts. The results confirm a similar pattern of ERP amplitude modulations for formal (amplitude suppression in response to standard relative to deviant tones) and temporal regularity (amplitude suppression for regular relative to irregular stimulus timing) in monkeys as in humans, suggesting that basic principles of regularity encoding also exist in monkeys and are comparable across both species.

Disclosures: M. Schwartze: None. **H. Merchant:** None. **S.A. Kotz:** None.

Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: SDCC Halls B-H

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Program #/Poster #: 394.18/DD10

Topic: D.06. Auditory & Vestibular Systems

Title: Human screams' roughness and pitch synergistically and simultaneously contribute to trigger efficient neural and behavioral responses

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Abstract: The ability to rapidly communicate danger using vocal signals (screams) is essential to warn conspecifics and promote our survival. Human screams constitute complex signals that exploit a combination of attributes (roughness, pitch) to convey a mixture of ecologically relevant information (about danger and emitter’s sex/age, respectively) to elicit adapted reactions by the receiver.
We previously identified roughness as the key acoustic features to selectively inform conspecifics about danger. Here, we consider another attribute, the pitch, as relevant to the receiver in a dangerous situation. For instance, it might be the case that a child’s high-pitched scream induces a distinct, faster reaction than an adult’s one. Whether these distinct features are concurrently or sequentially encoded in the brain and how they contribute to elicit adaptive behavioral responses is unknown.
Here, we used electroencephalographic (EEG) and intracranial recordings iEEG to investigate the neural encoding of pitch and roughness in the human brain. Sixteen participants (nine women) were required to spatially localize natural and synthetic vocalizations that varied along both pitch and roughness dimensions. We used a general linear model approach to measure the respective influence of these two fundamental features on neural and behavioral responses. We

identified that early neural signals (~P1, 50ms post stimulus onset) simultaneously represent these two features in a concurrent way, revealing a simultaneous encoding strategy. We further quantify the relative effect of these features on localization speed and show that these two features synergistically contribute to trigger rapid reactions to danger. These results show that human screams provide a sophisticated signaling system that rapidly elicits reactions optimally adapted to the ecological relevance of the situation.

Disclosures: L.H. Arnal: None. **P. Mégevand:** None. **A. Giraud:** None.

Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Program #/Poster #: 394.19/DD11

Topic: D.06. Auditory & Vestibular Systems

Support: Pfizer Collaboration Grant

Title: Auditory event related activity in children with Tuberous Sclerosis Complex

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Abstract: Tuberous Sclerosis Complex (TSC) is a genetic syndrome caused by a mutation in either the *TSC1* or *TSC2* gene characterized by benign growths throughout the body, including the brain. Within the brain, many individuals present with cortical tubers and alterations in white matter integrity, hypothesized to disrupt neural connectivity. Related to the brain pathology, many individuals with TSC are co-diagnosed with intellectual disabilities and/or an autism spectrum disorder (ASD) including deficits in language acquisition and development. We hypothesize that the deficits in neural connectivity in TSC will be evident through characterization of auditory event related potentials (ERP) in response to both basic auditory stimuli and, notably, in speech associated stimuli. As such, this study used high-density electroencephalography (EEG) to analyze auditory ERP in children with TSC during an auditory gating paradigm and a tone vs. speech mismatch negativity paradigm using both traditional methods of waveform analyses and novel multivariate pattern analyses (MVPA). Nine subjects with a diagnosis of TSC (ages of 4 to 14), and age-matched typically developing (TD) controls participated in the study. Comparison of amplitude and latency of the ERP to white noise stimuli across groups demonstrated children with TSC have significantly increased latency of the early P50 ERP component demonstrating altered basic auditory sensory processing. Deficits were also

seen in the mismatch response to tone stimuli in children with TSC relative to typically developing controls. Additionally, we utilized a novel analysis for EEG-based speech sound processing data, MVPA, to explore the functional specificity of neural processing for speech sounds compared to non-speech stimuli. A linear classifier was trained to decode neural activity between stimulus categories (e.g., standard speech stimuli vs. standard non-speech stimuli) over time by diagnosis. More reliable decoding of speech versus non-speech than of different speech or different non-speech stimuli was found for TD individuals but not for the TSC group in both early and late time windows of response. Using both traditional and novel analytical methods we have identified features of the auditory neural response that differentiate between children with TSC and TD controls. Based on findings in animal models of TSC, we believe the alterations in ERP responses are associated with deficits in axon formation and myelination and thus may be a biomarker of neural connectivity that could be used for both patient stratification and a measure of treatment response in future treatment trials.

Disclosures: A.M. O'Brien: None. **L. Bayet:** None. **K. Riley:** None. **C.A. Nelson:** None. **M. Sahin:** None. **M.E. Modi:** None.

Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: SDCC Halls B-H

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Program #/Poster #: 394.20/DD12

Topic: D.06. Auditory & Vestibular Systems

Support: Wellcome Trust DBT India Alliance Fellowship to SB
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Title: Spectral shape based adaptation unravels mechanisms underlying spectral contrast coding in the mouse auditory cortex (ACX)

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Abstract: Spectral contrast, a feature of auditory stimuli, plays an important role in recognition of sound objects in different situations for example in background noise. Further, spectral contrast plays a role in coding of complex stimuli with wideband characteristics especially in the presence of multiple sound sources and also in binding together auditory streams. Wideband

stimuli with varying spectral contrasts are more naturalistic as opposed to tonal sound stimuli, used generally to study tuning and coding properties of auditory cortical neurons. We study the mechanisms underlying spectral coding by excitatory (EX) and different classes of inhibitory (parvalbumin positive, PV and somatostatin positive, SOM) neurons (EXNs and INNs) in the mouse auditory cortex (ACX; primary ACX, AI and anterior auditory field, AAF) using streams of random spectral shape (RSS) stimuli (R) of varying spectral contrast with embedded deviant RSS stimuli (D; as RRR ⋯ RDRRR), also of different contrasts. Each of such stimulus token comprising the streams used, have a steady spectral shape throughout its duration and each stimulus have random spectral content (frequency resolution $1/8^{th}$ octave) drawn from a Gaussian distribution of different standard deviations, which defines the spectral contrast of the particular stimulus. Responses to RSS stimuli were found to be sparse. Further, using an RSS stimulus of a certain contrast as the repeating standard stimulus (R), neurons adapt based on each neuron's spectral tuning and contrast selectivity and depending on the particular spectral shape used as the deviant (D), each neuron responds weakly or strongly to the deviant. Using 164 such different streams of RSS stimuli with embedded deviants, we present organized estimated underlying synaptic spectral contrast selectivity providing input to supragranular cells in the ACX. Based on a model with synaptic adaptation in conjunction with our data, we conclude the differential roles played by synaptic adaptation and INNs in differential spectral coding by EXNs and PV and SOM neurons.

Disclosures: A. Mukherjee: None. **P. Patel:** None. **A. Mukesh:** None. **M. Mehra:** None. **S. Bandyopadhyay:** None.

Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant R01DC013412 (DM)
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Title: Validation of advanced cochlear nucleus auditory prosthesis with 3d penetrating microelectrodoe arrays

Authors: *M. HAN[1], D. B. MCCREERY[2]
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Abstract: For persons with deafness who cannot benefit from cochlear implants, some hearing can be restored by an array of stimulating electrodes implanted on the surface of or in their cochlear nucleus (CN). However, additional research is needed to show that the Auditory Brainstem Implants (ABIs) can restore hearing comparable to that provided by cochlear implants. In particular, the device has to be validated chronically and the encoding scheme has to be revealed. Previously, a clinical trial of a hybrid ABI containing macroelectrodes on the surface of the CN and also penetrating microwire-based microelectrodes showed that the surface electrodes best conveyed the loudness of the electrically-encoded sound but poorly conveyed its pitch, while the penetrating microelectrodes best conveyed the encoded sound's pitch, but with only limited range of loudness. In this study, we have chronically implanted arrays of penetrating silicon-based 3D microelectrode arrays into cats' ventral cochlear nucleus. These are three-dimensional, four-shanked devices that feature five independent electrode sites on each deep-reactive-ion-etched sturdy shank. This allows access to the tonotopic organization of the cochlear nucleus while also minimizing the number of penetrations into the brain (Han, 2012). The cochlear nucleus device also included a macroelectrode that reside on the surface of the nucleus. The neuronal activity induced by modulated electrical stimuli applied though both types of electrodes were recorded by another array of also silicon 3D microelectrode arrays implanted in the central nucleus of the inferior colliculus. Results showed that the temporal modulation of the neuronal activity induced by the modulated stimuli applied in (penetrating) or on (surface) the CN was very similar across a range of modulation frequencies and modulation depths, especially for transient modulation simulating the periodicity of speech. Our findings support the concept of a clinical ABI that employs surface stimulation and intranuclear microstimulation in an integrated manner.

Disclosures: M. Han: None. **D.B. McCreery:** None.

Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Topic: D.06. Auditory & Vestibular Systems

Support: DARPA NESD N66001-17-C-4013

Title: Exploring neural population dynamics in auditory processing from the macaque superior temporal gyrus

Authors: *J. LEE, L. LYNCH, D. M. BRANDMAN, A. NURMIKKO
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Abstract: The macaque superior temporal gyrus (STG) displays a robust tuning to the low-order auditory features, e.g. frequency, while it also shows a selectivity to high-order features of sound, such as species-specific communication call types and spatial information. Also, compared to the primary auditory cortex (A1), the auditory parabelt on STG is more accessible surgically, which offers an opportunity to explore broad principles of sound perception and possibilities in applying a functional intervention for neural prostheses. Our goal is to characterize neural states of the parabelt by performing intracortical microelectrode array (MEA) recordings in macaques. For study of neural population dynamics, we implanted two Blackrock 100-channel iridium oxide MEAs into the STG of one adult macaque. We also implanted titanium mesh to be ossiointegrated on the surface of the cortical bone to improve the stability of electrode pedestals during the experiment. Using the lateral sulcus and the superior temporal sulcus as a reference, electrode arrays were implanted on caudal parabelt regions above the medial lateral belt and caudal lateral belt. Those two areas are known to has a selectivity for the frequency of sound and the location of the sound source, respectively, and several researchers suggest that that information is also represented on the parabelt. Sinusoidal sound and temporally orthogonal ripple combination (TORC) was used to characterize the spectro-temporal receptive field (STRF) of neurons in STG during passive listening. More complex sound stimuli, such as macaque call and human speech were presented. Data processing including filtering, spike sorting and the cross-correlation with sounds and spikes was done in MATLAB. We report on the population recordings from our experiment and efforts to generate internal models for neurons. Based the model, our goal is to deliver patterned electrical stimuli into STG and assess the effects of the stimuli in the performance of the animal during the behavioral test, such as two-tone discrimination or sound recognition tasks.

Disclosures: J. Lee: None. **L. Lynch:** None. **D.M. Brandman:** None. **A. Nurmikko:** None.

Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Topic: D.06. Auditory & Vestibular Systems

Support: Grass Foundation Fellowship
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Title: Sex differences in frequency tuning in the inner ear of African clawed frogs (Xenopus)

Authors: *A. COBO-CUAN, P. M. NARINS
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Abstract: Reproductive success in anurans relies on their ability to detect, recognize, and localize sound. African clawed frogs, *Xenopus laevis*, use sex-specific vocalizations that convey sex identity and reproductive state. Recordings of auditory-evoked potentials in this species have demonstrated that auditory sensitivity to species-specific dominant frequencies in the advertisement calls is enhanced in females relative to males. In addition, this frequency sensitivity is affected by endocrine state. However, it is unclear where these sexual differences in spectral sensitivity arise. Here, we examine distortion product otoacoustic emissions (DPOAEs) to determine if the inner ear of this species exhibits a sexually dimorphic frequency tuning. DPOAEs were evaluated in adult individuals of *X. laevis* (5 males, 5 females). The frequency ratio of the species own dyad (1.14) was used as the f2/f1 ratio. A matrix of frequency-level combinations (37 frequencies × 15 levels) was randomly presented with f_2 values from 300 to 4000 Hz (100 Hz steps), and L_2 values from 60 to 90 dB SPL (2 dB steps). Input-output curves of DPOAEs from the two inner ear organs sensitive to airborne sound, the amphibian and basilar papillae, were also measured. Otoacoustic emissions appeared in a frequency range coincident with the spectral sensitivity previously characterized in behavioral and neurophysiological studies. DPOAE responses showed tuning differences between sexes. Males' ears are more sensitive to lower frequencies, while females' ears are tuned to the advertisement call frequencies. Consistent with anatomical adaptations for waterborne hearing, input-output DPOAE functions showed a saturating nonlinearity shifted toward high stimuli levels. We provide evidence that sexually dimorphic tuning in *X. laevis* occurs at the first stage in the auditory pathway. Since DPOAE generation is associated with the nonlinear mechanics in the hair cells, this could be the locus in the auditory periphery where sex differences in hearing emerge. Further studies are needed to determine if hair cells are the target of estrogens enhancing female auditory sensitivity.

Disclosures: A. Cobo-Cuan: None. **P.M. Narins:** None.

Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Topic: D.06. Auditory & Vestibular Systems

Support: National Science Foundation Award No. 1552946
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Title: Direct cortical localization of the meg auditory temporal response function: A non-convex optimization approach

Authors: *P. DAS[1], C. BRODBECK[2], J. Z. SIMON[1,2,3], B. BABADI[1]
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Abstract: Listening to continuous speech generates an ongoing auditory cortical response. When measured with magnetoencephalography (MEG), the functional relationship between the two can be characterized by the temporal response function (TRF). The TRF quantifies the relation between the acoustic input (speech envelope) and the auditory output (MEG responses) as the kernel of a linear convolution process. By capturing the temporal structure of the stimulus-response relationship, the TRF plays a key role in characterizing auditory information processing. For instance, the prominent negative peak observed in the TRF with a latency of ~100 ms has been shown to be modulated by the attentional state in a competing-speaker environment.

While functional roles of specific components of the TRF captured at the sensor level have been well characterized, the cortical distribution of the TRFs corresponding to underlying neural responses is not well-understood. The existing techniques for analyzing the cortical localization of these TRFs operate in a two-stage fashion: for instance, the raw MEG data are first mapped to the cortical surface using an anatomically constrained distributed source localization technique, followed by estimating TRF for each source location separately. The resulting estimates, therefore, are often highly biased and their quality can be heavily dependent on the correctness of the localization of the raw MEG data.

To address these shortcomings, we provide a unified framework for determining the cortical localization of the TRFs directly from the MEG data, by integrating the TRF and distributed forward source models into one, and casting the estimation task as a Bayesian optimization problem. Though the resulting problem emerges as non-convex, we show that solutions can nevertheless be obtained using an efficient algorithm that leverages recent advances in evidence maximization. We demonstrate the effectiveness of the resulting algorithm in both simulated and experimentally recorded MEG data from humans. Application of our algorithm to MEG responses to continuous speech provides new insight into the functional roles of the auditory and somatomotor cortices in the early and late stages of auditory processing.

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Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: SDCC Halls B-H

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Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD, DC014279

Pew Charitable Trusts

Title: Functional organization of human perisylvian cortex in response to speech

Authors: *B. KHALIGHINEJAD[1,2], S. V. NORMAN-HAIGNERE[3,4], J. L. HERRERO[5,6], A. D. MEHTA[5,6], N. MESGARANI[2,1]
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Abstract: Auditory cortex is thought to be organized into different cortical fields, each of which encodes multiple acoustic properties. Functional characterization of human cortical fields has largely depended on non-invasive brain imaging techniques such as fMRI. However, because fMRI indirectly measures neural responses via hemodynamic activity, it has limited temporal resolution, making it difficult to study the neural encoding of speech features which vary on the timescale of miliseconds. Thus, here we used direct intracranial recordings from 12 human subjects that were implanted with depth and grid electrodes.We recorded neural data in response to 40 minutes of natural speech and other commonly heard sounds. Using 350 electrodes in primary and secondary auditory cortices, we created seven cortical maps based on tuning for each of seven different acoustic features: frequency, latency, temporal modulation, spectral modulation, phonetic features, speaker features, and speech-specificity. The frequency and latency preferences were calculated from spectro-temporal receptive fields (STRF), estimated from each electrode. The temporal and spectral modulation maps were calculated by filtering the acoustic spectrograms through different cochlear filters and then by selecting the cochlear filters that best represent the temporal modulation preference and spectral modulation preference of the neural data.Consistent with prior fMRI studies, we found that topographic maps of frequency preference, latency, temporal modulation, and spectral modulation were dependent on two axes of medial-lateral and posterior-anterior direction in human auditory cortex. But contrary to prior fMRI studies, we did not find any specialized region for analyzing phonetic features, meaning that all of the four regions of medial Heschl's gyrus (HG), lateral Heschl's gyrus, planum temporale, and superior temporal gyrus (STG) encode the distinctive acoustic features of phonemes, and there was not a significant difference between their preferred phonetic feature. In addition, we found that information about speaker identity was better encoded in early auditory areas such as HG in comparison with secondary areas such as STG. Finally, we found that electrodes in STG selectively responded more to human speech compared to nonspeech sounds, such as animal vocalization, tones and music, confirming prior reports of speech-selectivity based on fMRI.Together, these findings advance our knowledge of the representational and functional organization of human auditory cortex and pave the way toward more complete models of cortical speech processing in the human brain.

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Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 394.26/DD18

Topic: D.06. Auditory & Vestibular Systems

Title: A computational model of the underlying mechanisms of temporal coding in auditory cortex

Authors: *J. LEE[1], X. WANG[1], D. A. BENDOR[2]
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Abstract: Neurons in the mammalian primary auditory cortex can temporally lock to individual acoustic events up to around 40-50Hz, covering the range of acoustic flutter. While modulation rates within the perceptual range of acoustic flutter are therefore represented temporally, primary auditory cortex neurons can also monotonically increase (Sync+) or decrease (Sync-) their discharge rates over this range of repetition rates that span flutter perception (Bendor and Wang 2007). Although neural mechanisms of stimulus-synchronized and non-synchronized responses have been studied using computational models of single neurons (Bendor 2015, Gao and Wang 2016), the integration of rate coding in stimulus-synchronized responses , to generate Sync+ and Sync- responses, has not yet been directly examined using such computational models. Here we investigated the underlying neural mechanisms responsible for Sync+ and Sync- responses in auditory cortex, and demonstrated that the addition of synaptic depression to a leaky integrate-and-fire excitation-inhibition model can reproduce these two response modes. Specifically, stronger synaptic depression of excitatory inputs relative to inhibitory inputs leads to Sync- responses while weaker synaptic depression of excitatory inputs relative to inhibitory inputs leads to Sync+ responses.

Disclosures: J. Lee: None. **X. Wang:** None. **D.A. Bendor:** None.

Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant R01 DC008983
NIH Grant R01 EY019049

Title: Synaptic mechanisms for bandwidth tuning in mouse primary auditory cortex

Authors: *H. LI[1], F. LIANG[2], W. ZHONG[3], L. YAN[5], L. MESIK[1], Z. XIAO[4], H. TAO[6], L. I. ZHANG[5]
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Abstract: Spatial size tuning in the visual cortex has been considered as an important neuronal functional property for sensory perception. However, an analogous mechanism in the auditory system has remained controversial. In the present study, cell-attached recordings in the primary auditory cortex (A1) of awake mice revealed that excitatory neurons can be categorized into three types according to their bandwidth tuning profiles in response to band-passed noise (BPN) stimuli: nonmonotonic, flat and monotonic, with the latter two considered as non-tuned for bandwidth. The prevalence of bandwidth-tuned (i.e. nonmonotonic) neurons increases significantly from layer 4 to layer 2/3. With sequential cell-attached and whole-cell voltage-clamp recordings from the same neurons, we found that the bandwidth preference of excitatory neurons is largely determined by the excitatory synaptic input they receive, and that the bandwidth selectivity is further enhanced by flatly tuned inhibition observed in all cells. The latter can be attributed partially to the flat tuning of parvalbumin (PV) inhibitory neurons. The tuning of auditory cortical neurons for bandwidth of BPN may contribute to the processing of complex sounds.

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Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

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Topic: D.06. Auditory & Vestibular Systems

Title: Attention increases information flow between auditory cortex and subcortex

Authors: *D. PRETE[1], M. BAIN[2], L. J. TRAINOR[2]
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Abstract: Within the auditory neural pathway there are bidirectional projections between the auditory cortex (AC) and subcortical regions, such as the inferior colliculus (IC). Yet, how these

connections influence auditory processing in humans is unclear. To estimate the activity and information flow between the AC and IC we measured EEG while presenting tones to elicit activity from both regions. The tones consisted of a pure tone carrier frequency that was amplitude modulated at 37 Hz. The amplitude modulation was chosen to index AC activity (the classic 40 Hz response), and the carrier to elicited subcortical activity via the frequency following response (FFR). Tones were presented to the right ear in an oddball sequence with a 500 Hz carrier frequency for 80% of trials and a 600 Hz carrier frequency for 20% of trials (these carrier frequencies ensure a subcortical source). Only standard tones are analyzed here. Participants either attended to the sequence by counting the number of deviant tones or passively listened while watching a silent movie. Information flow between the AC and IC was computed using normalized symbolic transfer entropy (NSTE). NSTE measures how much more one signal can predict the future of a second signal, specified by a delay, than past of the second signal. NSTE for each standard tone was calculated using 300 delays from 1 to 299 ms between the tone onset and offset. NSTE was then averaged across trials per participant per delay per direction of information flow and NSTEs at delays of a priori interest (8 ms, the IC to AC delay time; 17ms), as well as the maximum NSTEs across all delays, were extracted. A repeated measures ANOVAs on predictability, attention, direction of information flow and delay (8 and 17 ms) found that, overall, NSTE was greater from IC to AC (bottom up) than vice versa ($p < 0.001$, Cohen's $f = 0.373$), attention increased NSTE overall ($p = 0.005$, Cohen's $f = 0.380$) and the attentional effect was marginally greater from AC to IC (top down) than vice versa ($p < 0.066$, Cohen's $f = 0.115$). Interestingly, the maximum NSTE across all delays was consistently around 230 ms across participants for both directions. A repeated measures ANOVA reveal greater information flow from AC to IC (top down) at this delay in contrast to the shorter delays. A duration around 200ms is interesting as it is around the syllables rate in speech, a time at which sound events are clearly individuated, and features such as pitch and loudness are clearly integrated into auditory objects. We are currently conducting follow up studies to investigate the robustness of this effect.

Disclosures: D. Prete: None. **M. Bain:** None. **L.J. Trainor:** None.

Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 394.29/EE3

Topic: D.06. Auditory & Vestibular Systems

Title: Cricket song temporal pattern recognition in two populations of ormia ochracea

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Abstract: The parasitoid fly *Ormia ochracea* relies on host crickets for reproduction. Finding host crickets depends on the auditory system to recognize and localize cricket songs. These songs are species-specific and differ primarily in the temporal patterning of sound pulses. *O. ochracea* occur in several geographically distinct regions in the United States and each population targets a different cricket species. In Florida, *Gryllus rubens* is the primary host, which produces a ~50 pulses/sec calling song. In California, the primary host *Gryllus lineaticeps* produce songs at a higher pulse rate. Whether different populations of *O. ochracea* evaluate the same temporal parameter for song recognition is unknown. In this study, we use a high-speed treadmill system to record tethered walking responses to song models that vary in pulse duration and interpulse intervals. We describe song recognition 'maps' for Floridian and Californian *O. ochracea* to test the hypothesis that both populations evaluate pulse periods for recognition and song preferences are based on species-specific pulse periods. These results will guide our investigation of the neural correlates of song recognition in *O. ochracea*.

Disclosures: A.T. Kirtley: None. **N. Lee:** None. **J. Hao:** None. **M.C. Mason:** None.

Poster

394. Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 394.30/EE4

Topic: D.06. Auditory & Vestibular Systems

Support: Rita L. Atkinson Graduate Fellowship

Title: Composite receptive fields across neuronal populations in songbird auditory cortex

Authors: *N. W. VAHIDI[1], M. THEILK[1], T. GENTNER[2]
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Abstract: How the brain encodes complex stimuli such as communication signals in a population of neurons and how this encoded information is combined to shape response outputs are important questions in sensory neuroscience. In addition to a diversity of response characteristics across neurons in a population, recent work points to similar response diversity within single neurons. This within-neuron response diversity is observed throughout the secondary auditory cortical regions NCM (caudo-medial nidopallium) and CM (caudal mesopallium) in European starlings, a species of songbird, where increases and decreases in spiking activity of a single neuron are tied to many (typically >10) unique acoustic features. We term these acoustic feature sets the neuron's 'composite receptive field' (CRF). Here we examine auditory CRFs in more detail, asking how these feature sets vary within and between neurons, how they relate to the anatomical positions of neurons along laminar tracts spanning starling auditory cortex, and how natural stimuli drive CRFs in single neurons and populations across

time. Using silicon arrays, we recorded extracellular spiking responses from large neuronal populations comprising single and multi-unit activity in the NCM and CM of lightly anesthetized starlings, while presenting large libraries (> 1 h) of conspecific songs. We then computed the CRF for each unit using the Maximum Noise Entropy model, which allows for identification of relevant receptive field features akin to the spike-triggered covariance but unbiased by the non-Gaussian structure of song. This yielded a pool of acoustic features tied to either significant increases or decreases in spiking activity in each unit. We created a coarse linear map of the population 'spatial tuning' by assigning the CRFs for each unit to the array site with the largest magnitude spike waveform for that unit. We create a 'temporal tuning' map, by aligning the CRFs for each unit to the time varying acoustics of songs using cross correlation. The resulting spatio-temporal organization of CRFs reveals no discernable low-dimensional structure (e.g, frequency range, spectral modulation, etc) within a single unit, or between units across either anatomical space or time. Instead, the CRF feature sets express a rich heterogeneity of spectro-temporal characteristics, that collectively provide a detailed representation of many conspecific songs stable across epochs lasting seconds. Extrapolating from the coverage of the observed spatio-temporal CRF maps, we estimate the approximate number of neurons and CRF features needed to encode an entire conspecific song bout, and the representational capacity of NCM.

Disclosures: N.W. Vahidi: None. **M. Theilk:** None. **T. Gentner:** None.

Poster

395. Retinal Circuitry

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 395.01/EE5

Topic: D.07. Vision

Support: NIH Grant EY15573
NSERC Grant 194640

Title: Reconstitution of horizontal cell inhibitory feedback to cones in mouse retina with PSAM-GlyR, an orthogonal chemogenetic ligand-gated anion channel

Authors: J. C. GROVE[1], A. A. HIRANO[2], J. DE LOS SANTOS[2], S. C. PUROHIT[3], G. D. FIELD[4], N. C. BRECHA[2], *S. A. BARNES[5,6,7]
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Abstract: Feedback signaling by horizontal cells regulates the gain of photoreceptor synaptic output and contributes to the center-surround receptive field properties of downstream neurons in the retina. Many studies have shown that horizontal cell membrane potential drives actions leading to the modulation of presynaptic Ca channels in photoreceptors. Emerging evidence suggests that GABA is responsible for this feedback and that it acts not at cones but rather on horizontal cells, the same cells that release it, producing interstitial pH shifts that modulate photoreceptor Ca channels. In support of this hypothesis, the GABA agonist muscimol inhibits cone Ca channels when horizontal cells are depolarized, and blocking GABA receptors produces disinhibition of cone Ca channels. Both of these are pH-dependent actions. That the GABA responsible for this action is released by horizontal cells is shown by using a conditional and cell-type specific knockout of VGAT (Cx57-VGAT) in horizontal cells. VGAT is the transporter that loads GABA into synaptic vesicles and without VGAT, horizontal cells do not release GABA and there is no feedback. Since the reversal potential for current in GABA activated channels, permeable to Cl^- and HCO_3^-, is close to -30 mV, their activation depolarizes the horizontal cell. Depolarization of horizontal cells reduces the driving force on HCO_3^- efflux, allowing normal acidifying influences to dominate cleft pH, and block of NKCCs with bumetanide reduces inhibition of cone Ca channels, similar to the actions of amiloride and cariporide, which block NHEs. Acidification of the synaptic cleft inhibits cone Ca channels, reducing the gain of synaptic signaling to postsynaptic neurons, including bipolar cells. To confirm the site-to-site nature of this pathway, we reproduced the core mechanism that mediates feedback with targeted expression to horizontal cells of an orthogonal chemogenetic anion channel (PSAM-GlyR), an anion permeable GlyR complex. Horizontal cells transduced with AAV-7m8 virus containing a PSAM-GlyR-IRES-GFP construct showed a high degree of expression in horizontal cells and in no other cell types. Activation of PSAM-GlyR with the ligand, $PSEM^{308}$ caused inhibition of cone Ca_v channels, reproducing the site-specific actions of GABA on cone Ca_v channels. $PSEM^{308}$ had no effect in untransduced eyes, indicating that the expression of this engineered GlyR anion pore in horizontal cells is sufficient to reconstitute the Ca channel inhibition in cone photoreceptors produced under normal physiological conditions by autaptic GABA activity in horizontal cells.

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Poster

395. Retinal Circuitry

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 395.02/EE6

Topic: D.07. Vision

Support: NSF 1557820

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Title: Characteristics of ATP-induced extracellular acidification from retinal Müller (glial) cells

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Abstract: Within the retina, acidification of synaptic environments has been shown to significantly attenuate synaptic transmission. This study characterizes a novel non-neuronal pathway in the retina that evokes an extracellular acidification. In this pathway, Müller glia, upon activation by the signaling molecule ATP, acidify the extracellular environment. Measurements of proton fluxes from the apical (photoreceptor) end of isolated salamander Müller cells were performed using H^+ sensitive self-referencing microelectrodes, an effective method for measuring relative ion fluxes from cells (see Kreitzer et al., 2007). Calcium imaging experiments using Oregon Green implicate that ATP binds to a $P2Y_1$ receptor, and in combination with self-referencing experiments point toward the ATP-dependent acidification requiring Ca^{2+} release through an IP_3 pathway. We report here a dependency of the acidification on the presence of extracellular Na^+. Removal of Na^+ was shown to significantly attenuate the ATP-induced extracellular acidification. Attenuation of the acidification by the sodium transport blocker amiloride and the sodium-hydrogen exchanger blocker cariporide were also observed. Surprisingly, the ATP-induced extracellular acidification was sensitive to extracellular K^+. Reintroduction of K^+ from a nominally 0 K^+ solution significantly potentiated the ATP-induced acidification. However, this K^+ sensitivity was dependent upon the presence of Na^+. We hypothesize that Müller glia are potent acidifiers of the extracellular space. This extracellular acidification induced by ATP is mediated by a $P2Y_1$ receptor calcium-dependent pathway and is highly sensitive to the presence of Na^+ and K^+ outside of the cell. Our findings also point to a potential role for Na^+/H^+ exchange in driving this acidification. We hypothesize glial cell based extracellular acidification may be an important regulator of signaling throughout the nervous system.

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Poster

395. Retinal Circuitry

Location: SDCC Halls B-H

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Topic: D.07. Vision

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PRESTO JST

Title: Differential infection pattern of AAVs in mouse retin

Authors: *T. HORI[1,4], M. FUKUTOME[4,2], C. MAEJIMA[3,4], S. MORITOH[4,5], K. KOBAYASHI[6], C. KOIKE[3,4,7,8]
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Abstract: With an increasing number of causative genes identified, the widespread use of gene therapy becomes feasible. For gene therapy, it is desirable to have a delivery method with both high cell type specificity and high efficiency. The need for cell type specificity and high efficiency is particularly important for treating retinal degenerations, which may result from defects in the pigmented epithelium, in rods, or in cones. Viruses are potent gene delivery vehicles for the nervous system, but they suffer from non-specific infection. To address specificity and efficiency in targeting retinal photoreceptors, we have screened various adeno-associated virus (AAV) serotypes for infection patterns in the mouse retina following subretinal injection. We have identified a serotype that specifically and efficiently infects cone photoreceptors. Or study may provide a useful tool for delivering new genetic information to cones for the purpose of restoring genes involving in degenerations.

Disclosures: T. Hori: None. **M. Fukutome:** None. **C. Maejima:** None. **S. Moritoh:** None. **K. Kobayashi:** None. **C. Koike:** None.

Poster

395. Retinal Circuitry

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 395.04/EE8

Topic: D.07. Vision

Title: How feedback at the first visual synapse shapes functional diversity in the retina

Authors: *A. DRINNENBERG[1,2], F. FRANKE[3], R. K. MORIKAWA[1,2], J. JÜTTNER[1,2], A. HIERLEMANN[3], R. A. D. SILVEIRA[4], B. ROSKA[1,2]
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Abstract: Horizontal cells reside at a strategic position within the visual system, since they act at the first visual synapse before the signal is split into parallel channels that, ultimately, gives rise to the responses of ~30 types of retinal output neurons. It is known that horizontal cells deliver feedback inhibition to photoreceptors via a sign-inverting synapse. How does horizontal cell feedback shape the dynamics of the retinal output? Are individual retinal output channels differentially affected? Here we specifically and reversibly perturbed the activity of horizontal cells across the entire retina using chemogenetics and combined the perturbation with a system-level and cell-type specific readout of the retinal output. We injected mice expressing Cre recombinase in horizontal cells with adeno-associated virus (AAV) that conditionally express a chemogenetic channel. Intravenous injection of virus coated with the PHP.B capsid led to strong and retina-wide expression of the channel exclusively in horizontal cells. In the same retinas, we targeted the calcium indicator GCaMP6s to cones by injecting AAV that expressed GCaMP6s under a cone-specific promoter. By two-photon calcium imaging of cone photoreceptors in whole-mount retinas, we demonstrate that the perturbation effectively and reversibly blocked the light-modulation of horizontal cell feedback. To monitor the effects in the retinal output, we recorded the light-driven spiking activity in thousands of ganglion cells using high-density microelectrode arrays. We uncovered six effects on the response dynamics and response range of ganglion cells. Unexpectedly, perturbing horizontal cells suppressed or enhanced the responses of ganglion cells of the same polarity at different epochs of the response, even within the same ganglion cell. We then used chemogenetic identification of ganglion cell types on the microelectrode array, functional classification of ganglion cells using naturalistic visual stimuli, and laser-targeted single-cell recordings of genetically identified ganglion cell types to show that the horizontal cell perturbation differentially affects individual ganglion cell types. How can the perturbation of a single interneuron type result in such a variety of effects? We developed a computational model of the retinal circuitry. The model reproduced all observed effects, thus providing insights into how feedback at the first visual synapse can affect the retinal output in

diverse ways. Our combined experimental and theoretical work reveals how a single interneuron type can differentially shape the dynamical properties of distinct output channels of a brain region.

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Poster

395. Retinal Circuitry

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 395.05/EE9

Topic: D.07. Vision

Support: NIH Grant EY028633
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HHMI
KCO

Title: Molecular specification of cells in the primate fovea and peripheral retina

Authors: *Y.-R. PENG[1], K. SHEKHAR[2], W. YAN[1], D. HERRMANN[1], G. S. BRYMAN[3], A. LIU[3], T. VAN ZYL[4], M. T. H. DO[3], A. REGEV[2,5], J. R. SANES[1]
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Abstract: The retina, like other parts of the central nervous system, uses complex neuronal networks to perform sophisticated computations. Its genetic accessibility has made the mouse retina a valuable model for analyzing the structure and function of neural circuits. As a model for primate vision, however, it suffers from a severe drawback. High acuity vision in primates is mediated largely by a specialized, central region of the retina called the fovea or macula (with the macula extending into parafoveal and perifoveal zones). Indeed, even though the fovea comprises <1% of the retina, loss of foveal function leads to functional blindness. However, among mammals, only primates have a fovea. Although the same cell classes (horizontal, bipolar, amacrine, and retinal ganglion cells [RGCs], photoreceptors, and Müller glia) are found in fovea and peripheral retina, structural and functional differences abound. For example, cones and rods are the dominant photoreceptors in the fovea and periphery, respectively; and the ratio of photoreceptors to RGCs is many-fold higher in periphery than fovea. Yet, little is known about genes expressed by foveal cell types, and few markers of specific types have been

identified. Here, as a step toward generating a primate retinal cell atlas, we used a high-throughput single-cell RNA sequencing (scRNAseq) platform (10X Genomics) to profile 77,368 foveal and 56,264 peripheral cells from the retina of the macaque monkey, *macaca fascicularis*. Bioinformatic analysis of the data allowed us to identify ~65 foveal and ~80 peripheral subsets of cells. We used *in situ* hybridization and immunohistochemistry to validate selectively expressed genes, and combined molecular with biolistic or viral labeling to characterize morphological features of cell types defined molecularly. We also showed that some of the foveal specializations in macaque are conserved in marmoset and human. Key findings include: (1) molecular difference between foveal and peripheral cones; (2) distinct types of Müller glia in the fovea and periphery; (3) correspondence between 12 bipolar cell types in fovea and periphery but with differences in frequency; (4) 11 types of foveal RGCs with peripheral counterparts, plus 6 types found only in the periphery; (5) markers for the four major RGC types, ON and OFF midgets and parasols; (6) molecular differences between foveal and peripheral RGCs; and (7) selective expression by specific cell types of genes conferring susceptibility to macular degeneration or glaucoma. These results provide a foundation for analyzing gene expression patterns in primate retina that underlie visual function and dysfunction.

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Poster

395. Retinal Circuitry

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 395.06/EE10

Topic: D.07. Vision

Title: An approaching motion selective RGC in the mouse retina

Authors: *F. WANG, Y. ZHANG
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Abstract: Classification and functional study of every type of neurons are the first steps to decipher complex neural circuits such as the retina. There are at least 30 subtypes of retinal ganglion cells (RGCs) in the mouse retina, each of them extracts different features from the visual inputs. Motion is one of the most prominent visual features that the retina extract. RGCs that integrate spatial information nonlinearly are more sensitive to motion within their receptive fields (RFs). And this nonlinear spatial integration is generally thought to reflect how RGCs integrate excitatory inputs from bipolar cells.
We have characterized one type of RGC that integrates spatial information linearly. This RGC does not respond to high spatial frequency stimuli and is insensitive to lateral motion within its

RF, yet it can be strongly activated by approaching motion. Whole cell patch clamp experiments were performed to study how this linear RGC respond to motion in the RF center, and the synaptic mechanisms underlying its selectivity for approaching motion. We found that even though the spiking response of the RGC shows linear spatial integration, its excitatory inputs are non-linear. If we pharmacologically remove the inhibition, the RGC becomes non-linear, and responds to all types of motion without selectivity. This suggests that the non-linear excitation to the RGC is masked by a tonic inhibition, making the RGC insensitive to motion. Further study showed that the approaching motion is the only type of motion that can temporarily disinhibit the RGC under normal stimulus conditions, so the approaching motion selectivity originates from the selectivity of disinhibition. Thus, we present a RGC whose linear spatial integration property depends on the inhibitory inputs from amacrine cells, not on the excitatory inputs from bipolar cells. And this RGC uses a different mechanism to generate motion selectivity than generally observed in the retina.

Disclosures: F. Wang: None. **Y. Zhang:** None.

Poster

395. Retinal Circuitry

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Topic: D.07. Vision

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NIH Grant EY026878
NSF Graduate Research Fellowship
Gruber Science Fellowship

Title: Heterotypic electrical synapses retrogradely couple melanopsin signaling in retinal ganglion cells to a neuropeptidergic amacrine cell population

Authors: *J. POTTACKAL[1,2], P. RAHMANI[1], J. B. DEMB[1,2,3]
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Abstract: Intrinsically-photosensitive retinal ganglion cells (ipRGCs) constitute a population of inner retinal photoreceptors, composed of five subtypes (M1–M5) in mice, that express melanopsin and detect irradiance. In addition to signaling anterogradely to several brain targets, ipRGCs also apparently signal retrogradely via heterotypic electrical synapses with amacrine cells, a large class of inner retinal interneurons. Despite recent physiological description of a subset of ipRGC-coupled amacrine cells, both the function of ipRGC-amacrine cell coupling and

the genetic identities of these amacrine cells have yet to be completely elucidated. Here, we report that corticotropin-releasing hormone-expressing (CRH^+) amacrine cells, a neuropeptidergic amacrine cell population, form functional electrical synapses with non-M1 ipRGCs in mice. Current-clamp recordings from CRH^+ amacrine cells during glutamate receptor (GluR) blockade revealed slow depolarizing responses to visual stimulation that persisted for >20 seconds. GluR-independent depolarization was sensitive to the gap junction blocker meclofenamic acid. Additionally, voltage-clamp recordings showed that the amplitude of this response was invariant across holding potentials. We also observed fast inward currents in non-M1 ipRGCs during optogenetic stimulation of CRH^+ amacrine cells. Finally, Neurobiotin injection into non-M1 ipRGCs demonstrated tracer coupling to CRH^+ amacrine cells. Together, these experiments suggest gap junction-mediated electrical synaptic input from non-M1 ipRGCs as the source of GluR-independent visual responses in CRH^+ amacrine cells. During retinal development, ipRGC-CRH^+ amacrine cell coupling is first physiologically detectable at ~P10 (postnatal day 10), achieves maturity shortly after eye opening (P13-P16), and parallels the concurrent elaboration of CRH^+ amacrine cell neurites. These data identify an anatomical circuit linking melanopsin signaling to a neuropeptidergic cell population, suggesting a potentially novel function for retrograde ipRGC signaling in the irradiance-dependent modulation of intraretinal neuropeptide release.

Disclosures: J. Pottackal: None. **P. Rahmani:** None. **J.B. Demb:** None.

Poster

395. Retinal Circuitry

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Topic: D.07. Vision

Support: NIH Grant R01EY025087
Stanford University Discovery Innovation Grant 1195339

Title: Functional diversity of wide-field amacrine cells with a common molecular marker

Authors: *J. KIM[1], M. INOUE[2], C. RAMAKRISHNAN[2], K. DEISSEROTH[2,3,4], S. A. BACCUS[1]
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Abstract: Although the retina is a central place for understanding neural computations, visual processing in the inner retina still remains largely unexplored due to the large diversity of inhibitory amacrine cells. Molecular markers provide a way to study specific types of neurons such as amacrine cells. The specificity of such markers embodies a tradeoff - markers specific

for just a single cell type provide unambiguous access to one type but not others, and those markers present in too diverse a population require extensive further physiological or molecular tests to classify each cell. Here we studied the physiological diversity of amacrine cells that expressed a transcription factor Bhlhb5 (b5), which stains a set of GABAergic inhibitory neurons in the adult mouse with sparsely-branched, long processes. Cells stratify narrowly at different levels of the inner plexiform layer, indicating the potential of a few different cell types labeled by this one marker. Using a b5-cre knockin mouse line (Ross et al, 2010), we expressed a red calcium (Ca) indicator on b5 amacrine cells using AAV in adult retina and performed two-photon imaging under a set of visual stimuli including a uniform field flash, spatiotemporal white noise, and natural movies. Visual stimuli were delivered using a digital light projector with UV (385 nm) and blue (460 nm) LEDs and Ca responses in the ganglion cell layer (GCL) were imaged at a frame rate of 30 Hz using a custom resonant-scanning two-photon microscope. The typical scanning area was a ~300 µm square, allowing up to 80 b5 cells to be imaged within a single field-of-view. In response to a uniform flash stimulus, the molecularly-identified amacrine cells in the GCL showed heterogeneous Ca responses. The time courses of the simultaneously imaged population of cells formed approximately six distinguishable clusters. The spatial distributions of cells within these clusters appeared homogeneous and cells of different clusters were spaced at a distance smaller than those within single clusters. These results indicated that b5 positive cells were comprised of cells with different functional types that were distributed evenly over the retina. We conclude that rather than identifying a single cell type, the Bhlhb5 molecular marker provides a way to study a select population of a few amacrine cell types that can be quickly identified using simple visual stimuli.

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Poster

395. Retinal Circuitry

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Program #/Poster #: 395.09/EE13

Topic: D.07. Vision

Support: NIH R01 EY021372

Title: Contrast adaptation in the rod bipolar pathway

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Abstract: Through a process called contrast adaptation, the retina encodes visual stimuli of wide temporal variability into a relatively narrow range of ganglion cell firing rates. This work examines the circuit level mechanisms (gain controls) behind contrast adaptation in a well-characterized circuit within the inner retina: the rod bipolar pathway. In this pathway, rod bipolar cells (RBs) respond with graded potentials to light stimuli. Depolarization of RBs drives glutamatergic release via ribbon synapses onto AII amacrine cells (AIIs). AIIs then depolarize the terminals of Type-6 cone bipolar cells (T6 CBs) via gap junction coupling. This drives excitatory release from T6 CB ribbon terminals onto ON-α ganglion cells (ON-αs). To determine the circuit loci of gain controls, we pharmacologically ablate photoreceptor responses in the outer retina and use Cre-mediated expression of channelrhodopsin-2 (ChR2) to drive either RBs or T6 CBs directly. Using paired pulse and temporally modulated white noise stimuli, we evoke ChR2-driven responses from either RBs or T6 CBs. Excitatory postsynaptic currents (EPSCs) are recorded from ON-αs, and Linear-Nonlinear (LN) cascade models are generated from the data. These methods allow us to analyze gain changes within the inner retina using naturalistic stimuli, without the limitations of paired patch recordings. Our results show that RB-driven responses exhibit a greater level of synaptic depression as compared to T6 CB-driven responses, and that the majority of contrast adaptation occurs when driving RBs, rather than T6 CBs. In conjunction with previous findings, these results suggest a prominent role for [A] synaptic release from RB terminals, and [B] the activity of AII amacrine cells in contrast adaptation within the rod bipolar pathway.

Disclosures: G.E. Perrin: None. **J.B. Demb:** None. **J.H. Singer:** None.

Poster

395. Retinal Circuitry

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 395.10/EE14

Topic: D.07. Vision

Support: WRF UWIN postdoctoral fellowship

Title: Info in a bottleneck: The compression of information in neural circuits

Authors: *G. J. GUTIERREZ, F. M. RIEKE, E. T. SHEA-BROWN
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Abstract: FR and ES-B are joint senior author
Abstract:
Neural circuits can be organized in layers that converge and diverge. In particular, inputs to the retina converge on Retinal Ganglion cells that process information from a subpopulation of bipolar cells. This subunit structure produces an information bottleneck because information is

compressed from tens of bipolar cells that tile the visual field. Additionally, this arrangement enables different ganglion cell types to perform distinct computations using information from the same subset of photoreceptor inputs. ON and OFF ganglion cell types are sensitive to contrast increments and decrements, respectively. However, their response sensitivities are not symmetrical. Likewise, there is an asymmetry in the distribution of contrasts in natural scenes. While the asymmetries in ganglion cell response properties and natural image statistics have been studied, it is not known whether the corresponding asymmetries are optimal for the preservation of visual information.
Using computational models of convergent neural circuits, we simulate datasets of neural responses under different input conditions as well as for different circuit configurations. Our computational circuits have a convergent subunit structure in which input from several subunits is summed by an output ganglion cell. The results are analyzed using information theory methods to determine how much visual information is preserved or lost as it exits the bottleneck of the circuit. We compare results given a generic, symmetric, gaussian distribution of inputs and a natural distribution which is asymmetric. Additionally, different circuit configurations are simulated so that all combinations of linear and nonlinear subunits among the ON and OFF pathways are tested.
Our comparisons indicate that an asymmetric configuration of ON and OFF pathways may preserve more information than the symmetric circuit configuration for natural image distributions, however, this may be dependent on the amount of subunit noise and the placement of the noise in the circuit. In summary, our preliminary results support our hypothesis that the asymmetric configuration of the retina is driven by a general demand of maximizing available stimulus information through a compressive circuit.

Disclosures: F.M. Rieke: None. **E.T. Shea-Brown:** None.

Poster

395. Retinal Circuitry

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Program #/Poster #: 395.11/FF1

Topic: D.07. Vision

Support: EC Horizon 2020 Grant 696656

Title: Graphene based electrodes for retinal implants: An *in vivo* study on biocompatibility and functionality

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Abstract: Blindness affects up to 36M people worldwide and has a major impact on the quality of life. In developing rehabilitation therapies, visual prosthetics are capable of stimulating neurons at different levels of the visual pathway using an array of electrodes. However, only few conductive materials are available for neural interfacing, and their evaluation have shown drawbacks. Material must exhibit not only high electrochemical performance, but also long-term stability and biocompatibility. In addition, complementing with soft material technology is required to achieve an intimate contact with tissue without inducing inflammation. Even with known compatible materials, a difference in mechanical properties can damage neurons at the interface and induce a glial scar. In this study, we propose graphene as suitable electrode material for retinal implants. First, we evaluated bidirectional capabilities of graphene on *ex vivo* pigmented Long-Evans rat retina. Custom microelectrode arrays (MEAs) with graphene electrodes were used to record spontaneous and elicited (via light and electric current) activity of the retina. The results confirmed the functionality of graphene electrodes. Biocompatibility was evaluated with implants having graphene which were placed in the subretinal space of adult P23H rats (retinitis pigmentosa). A follow-up using OCT and fundus of were done to monitor the tissue-implant interface up to 2 months. Afterwards, a biopsy containing the implant was stained for cell nuclei, bipolar cells, microglia, and macroglia. Confocal imaging and image reconstruction reported cell species on the contact area of the implant. Count of microglia and their location in the retinal tissue above the implants suggests a high biocompatibility of the GRM-based implants as compared to the control devices. Additionally, we present preliminary results of implant functionality *in vivo* using blind P23H and pigmented Long-Evans rats under anesthesia. Connected implants were acutely placed in the subretinal space. Stimulation via light or current controlled electrical pulse at the implant were delivered to the eye. Ultrasound recording in the visual cortex measured cerebral blood volume (CBV) in higher visual levels. Results showed an increase in CBV at the superior colliculus and visual cortex correlating to stimulation parameters which indicate implant functionality. The results demonstrate that implants with graphene are compatible and capable of stimulating rat retina. The elicited activity can be correlated to CBV changes found in ultrasound recordings which supports that graphene based implants can be a candidate for visual prosthetics.

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Poster

395. Retinal Circuitry

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Topic: D.07. Vision

Support: NIH K99 EY028625-01

Title: Molecular survey of the vertebrate retina at single-cell resolution

Authors: *K. SHEKHAR[1], Y. PENG[2], I. E. WHITNEY[3], I. BENHAR[6], W. YAN[4], N. M. TRAN[3], A. JACOBI[7], M. LABOULAYE[2], E. MARTERSTECK[5], T. VAN ZYL[8], G. S. BRYMAN[9], H. BAIER[10], Z. HE[11], M. H. DO[9], A. REGEV[6], Y. KOELSCH[10], J. R. SANES[2]
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Abstract: Neurons in the brain can be subdivided into transcriptomically distinct types, which correspond well to classical distinctions based on morphology, physiology and connectivity. Knowing the full compendium of neuronal types in a given brain region, and having reliable genetic markers for each type will enable neural circuits to be studied at unprecedented molecular resolution in health, development, disease and across different species. We are using large-scale single-cell transcriptomic analysis of the vertebrate retina to address these issues. Building on initial "proof-of-principle" studies (Macosko et al., Cell, 2015 ; Shekhar et al., Cell, 2016), we are completing a comprehensive neuronal atlas of the mouse retina, which will be among the first of its kind for any brain region. We have defined molecular signatures for a total of >120 neuronal types, including 3 photoreceptor, 1 horizontal, 15 bipolar, 45 ganglion, >60 amacrine and ~10 non-neuronal cell types, confirming a large number of them histologically. Using the mouse "retinome" as a foundation, we are pursuing two directions. **First** we are performing large-scale single-cell surveys of two other vertebrate retinas that differ from that of the mouse in key aspects: (a) macaque retina, which contains the fovea, a structure responsible for high acuity vision in primates that is absent in mice, and (b) zebrafish retina, which unlike mice and primates, can regenerate following injury. Using molecular signatures, we have been able to compare the taxonomy of retinal neurons across species, and identify "homologous" neuronal types. In the primate, we have also identified key molecular differences between foveal and peripheral counterparts of each neuronal type, some of which might underlie diseases of the central retina like macular degeneration. **Second**, we are transcriptiomically profiling neuronal and non-neuronal cells of the mouse retina following physical injury to the optic nerve. We showed previously that retinal ganglion cell types differ dramatically in their ability to survive following axonal transection (Duan et al., Neuron, 2015). By comparing changes in gene expression among types following injury, we can identify early transcriptional signatures that correlate with, and may underlie, selective resilience. In addition, we are identifying injury responses of interneurons and non-neuronal cells (glia and immune cells), all of which have been shown to influence survival.

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Poster

395. Retinal Circuitry

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KT Corporation

Title: Digital museum of retinal ganglion cells with dense anatomy and physiology

Authors: *J. A. BAE[1,2], S. MU[1], J. S. KIM[1], N. L. TURNER[1,3], I. TARTAVULL[1], N. KEMNITZ[1], C. S. JORDAN[4], A. D. NORTON[4], W. M. SILVERSMITH[4], R. PRENTKI[4], M. SOREK[4], C. DAVID[4], D. L. JONES[4], D. BLAND[4], A. L. R. STERLING[4], J. PARK[5], K. L. BRIGGMAN[6,7], H. S. SEUNG[1,3], T. EYEWIRERS[8]
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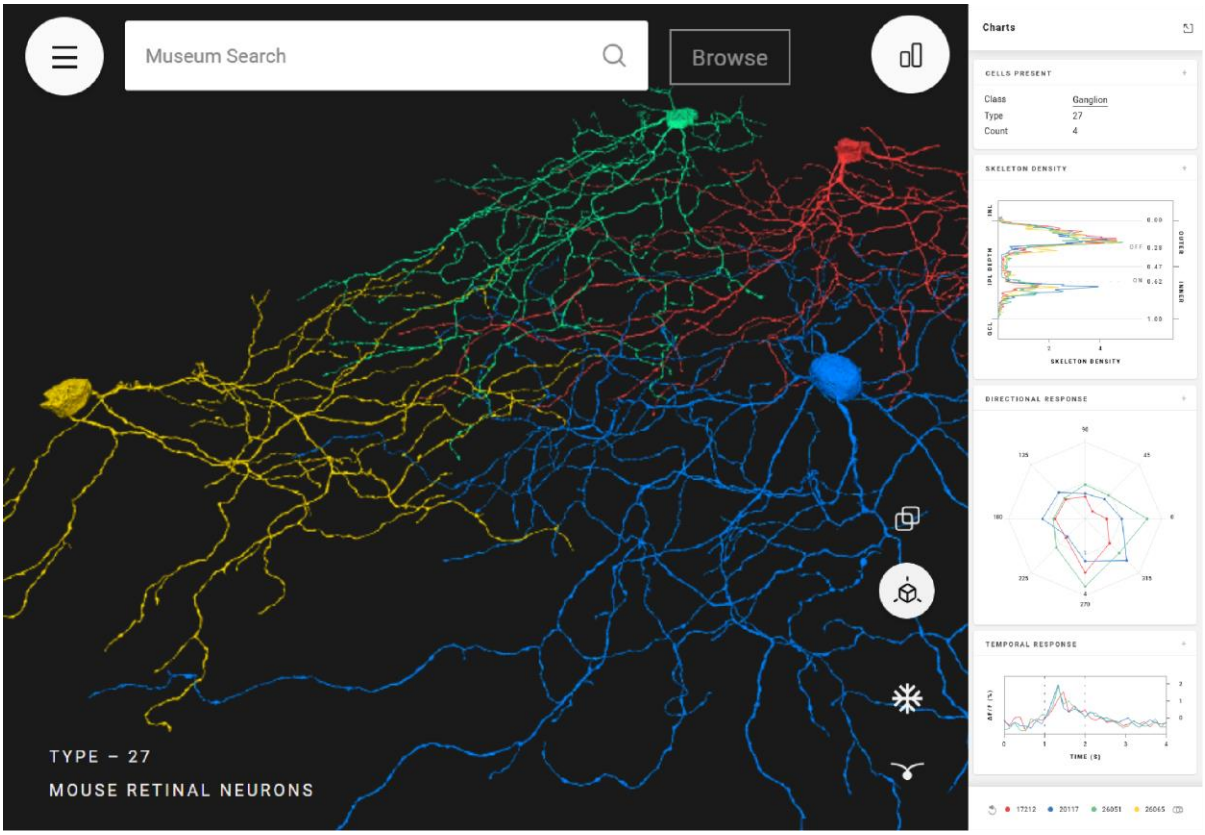
Abstract: Traditional brain atlases in macroscopic resolution (Lein et al., 2007, Amunts et al., 2013, Zingg et al., 2014) typically divide the brain into macroscopic regions so it is not feasible to view individual cells. Other brain atlases such as neuromorpho.org (Ascoli et al., 2007) and wormatlas.org (Hall et al., 2007) provide only the morphology. Here we present a new kind of resource that combines dense maps of both anatomy and physiology at cellular resolution. An online "museum" (http://museum.eyewire.org) provides a 3D interactive view of each cell's anatomy as well as graphs of its anatomical and functional properties (Figure).
3D electron microscopy (EM) after calcium imaging has become established as a powerful approach for obtaining anatomical and physiological information about the same neurons. The resource encompasses exhaustive set of all the ganglion cells, almost 400 cells, that have cell bodies within the 0.3 x 0.35 mm^2 patch of retina. These ganglion cells have been reconstructed from 3D EM by 30,000 members of online community from the crowdsourcing game known as Eyewire. In addition, calcium signal was extracted for individual cells, providing dense sample of physiological data. Previously, dense EM reconstructions in the mouse retina (Helmstaedter et

al., 2013) and larval zebrafish olfactory bulb (Wanner et al., 2016) were only limited to anatomy only. A recent large-scale calcium image study had dense sample of visual responses but included only limited amount of cells' morphology (Baden et al., 2016).
To demonstrate the utility of the resource, we have discovered several organizing principles of the inner plexiform layer (IPL). The resource reveals two aspects of the retina's IPL: an arbor segregation principle governing structure along the light axis, and a density conservation principle governing structure in the tangential plane. Lastly, we relate structure to visual function: ganglion cells with arbors near the layer of ganglion cell somas are more sustained in their visual responses on average.



Disclosures: J.A. Bae: None. **S. Mu:** None. **J.S. Kim:** None. **N.L. Turner:** None. **I. Tartavull:** None. **N. Kemnitz:** None. **C.S. Jordan:** None. **A.D. Norton:** None. **W.M. Silversmith:** None. **R. Prentki:** None. **M. Sorek:** None. **C. David:** None. **D.L. Jones:** None. **D. Bland:** None. **A.L.R. Sterling:** None. **J. Park:** None. **K.L. Briggman:** None. **H.S. Seung:** None. **T. Eyewirers:** None.

Poster

395. Retinal Circuitry

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Program #/Poster #: 395.14/FF4

Topic: D.07. Vision

Support: SERB, (SR/SO/AS-027/2012,TCN)

Title: Study of horizontal cells following light-induced outer retinal cell damage in Sprague-Dawley rats

Authors: *S. GUPTA, T. C. NAG, T. S. ROY
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Abstract: Light-induced damage to the retina resembles retinal degenerative diseases that are observed in humans, particularly age related macular degeneration. It is conceivable that after photoreceptor death, there is a likely secondary degeneration of the neurons of the inner retina, where one of the principal
retinal neurons are horizontal cells (HC). We examined the modifications in the HC after light induced damage to the outer retina. Adult Sprague-Dawley rats (N= 40) of both sexes were kept in an ambient light of 300 lux at normal photoperiod (12 hour light [L] : 12 hour dark [D]) for 7 days and then at 3000 lux
at normal photoperiod and constant light (24 L : 0D) for 2days. After that, rats were brought to normal photoperiod and reared for a period of 15 days. They were sacrificed at different time points to find out how the HC remodeled to the photic insult. Cytoarchitecture of HC was examined by light and transmission
electron microscopy. Localization and expression of GABA were studied by immunohistochemistry and immunoblotting to see the status of HC. We found that light induction caused significant damage to HC, after photoreceptor death, especially in their perikarya, dendritic compartments and axons. There were
swellings in the HC perikarya and alterations in their organelles and cytoskeleton. Immunohistochemically, there was an increased expression of GABA in HC of the retina exposed to continuous light. After reversal to normal photoperiod, there was downregulation of GABA in HC, in the rats reared up to
15 days. The present results show that continuous light induces damage to HC, and remodeling happens with the reversal of the photic insult, implying that HC after light insult can re-establish connectivity with the extant healthy photoreceptors.

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Poster

395. Retinal Circuitry

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CAPES
FAPERJ

Title: Deletion of GD3 synthase modifies retinal structure and impairs visual function in adult mice

Authors: *C. A. SANTANA[1], L. C. T. PINHEIRO[2], A. JORDÃO[2], R. LANI[2], G. NASCIMENTO-DOS-SANTOS[2], J. F. VASQUES[2], R. MENDEZ-OTERO[2], M. F. SANTIAGO[2]
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Abstract: Gangliosides are found ubiquitously in several tissues and fluids, being especially abundant in the nervous system. The expression of gangliosides in the brain is highly specific for each region. The ganglioside 9-O-acetyl GD3 is space-temporally correlated with cell migration in the retina, superior colliculus, and to the axonal growth in the optic tract in rodents. However, biological roles of this ganglioside in retinal postnatal development are unclear. Here, we used adult mice lacking GD3 synthase (Siat3a KO), an enzyme that converts GM3 to GD3, which can be further converted to 9-O-acetyl GD3. Using optomotor system we measured visual acuity at spatial frequencies ranging between 0.03 and 0.272 cyc/deg. We observed a significant reduction in adult GD3s -/- mice performance compared to wild-type (WT) mice. Immunohistochemistry analyzes for TUJ1, marker of retinal ganglion cells (RGC), were performed and showed a significant 40% decrease in number of cells in GD3s -/- mice. Also, electrophysiological evaluation of retinal function was performed by two variants of ERG technique. Under particular luminance-stimulus conditions, the Flash-ERG displays components that reflect electrical activity originating from neurons in proximal retina. Responses were averaged per light intensity. For analysis, amplitudes of a-wave were measured in relation to the baseline, whereas the b-wave amplitudes were estimated in relation to a-wave. Oscillatory potentials were sorted by 30-400 Hz bandpass filtering, and the peak-to-peak amplitudes were assessed. We found reduced amplitudes of the a and b-wave in GD3s -/- animals. Pattern Electroretinogram (PERG) analyzes response to contrast reversing gratings or checkerboards, which selectively depends on the presence of functional RGCs, and was also significantly reduced by 75% in GD3s -/- mice, thus

demonstrating significant visual impairment in those animals. Together, these results suggest that GD3 synthase absence alters retinal structure and visual function.

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Poster

395. Retinal Circuitry

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Title: Gap junction circuitry of aii and a8 amacrine cells in the mouse retina

Authors: *S. YADAV, S. TETENBORG, K. DEDEK
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Abstract: AII and A8 cells represent the majority of narrow-field glycinergic amacrine cells in the mammalian retina [1]. Both are bi-stratified and partially share a common stratification profile [4]. Gap junctions of AII amacrine cells are known to undergo remarkable light-dependent and dopamine-modulated plasticity and represent a crucial node in the primary rod pathway [3]. In contrast, the function of A8 cells is poorly understood.
Previous studies indicate that A8 cells form homocellular (A8-A8) and heterocellular (A8-ON cone bipolar cells) gap junctions [2, 3]. Furthermore, A8 cells are likely to receive dopaminergic input [2]. These features make A8 cells very similar to AII amacrine cells. In addition, both cells are likely to receive glutamatergic input from the same bipolar cell they form gap junctions with [3]. However, A8 cells are primarily cone-driven. Therefore, we hypothesized that A8 gap junctions could be dopamine- and/or light-dependent and thus may work in concert with AII cells to amplify/suppress rod signals or facilitate cone signals.
To test this, we injected tracers into whole-mount retinas of wild-type and ier5-EGFP mice and subsequently used a set of somatic and synaptic markers to (1) reveal the type of connexin expressed by A8 cells and their potential gap junction partners; (2) determine whether A8 cells express D1-receptors; and (3) discern the relative location of chemical and electrical synapses on A8 cells. Also, the coupling profile of A8 and AII cells was compared under light-adapted conditions, with and without application of D1-receptor antagonists.
Our data shows that A8 dendrites express less Cx36 puncta than AII dendrites. Interestingly, A8

cells expressed Cx36 on both ON and OFF dendrites, unlike AII cells; however, both amacrine cells couple to secretagogin-positive and -negative ON bipolar cell terminals. Importantly, both AII and A8 cells express D1 receptors; however, A8 cells were coupled to less bipolar cells and did not undergo an increase in coupling with D1 receptor blockade. This points to a different mechanism of gap junction modulation in A8 cells. As the total number of gap junctions on A8 cells is small and the few gap junctions occur close to chemical synapses, we suggest that gap junctions aid A8 cells in facilitating cone bipolar cells signals.

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Poster

395. Retinal Circuitry

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Title: CaBP5 is essential for normal synaptic transmission from rod bipolar cells to AII amacrine cells

Authors: *M. B. KOZHEMYAKIN[1,2], R. HEIDELBERGER[2]
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Abstract: CaBP5, a member of the calmodulin (CaM)-like family of Ca^{2+} binding proteins, is required for normal rod-mediated light sensitivity in the mammalian retina. The rod bipolar cell (RBC) expresses CaBP5, however little is known about the role that CaBP5 plays in the regulation of neurotransmitter release from RBC synaptic terminals. To provide this information, we examined miniature-like events (**ml**-EPSCs) and light-evoked synaptic events (**le**-EPSCs) in a neuron postsynaptic to RBCs, the AII amacrine cell (All). Results were compared across retinal slices obtained from $CaBP5^{-/-}$ mice or wild-type (WT) mice. We found that the mean amplitude of **ml**-EPSCs recorded in AIIs in $CaBP5^{-/-}$ slices was significantly larger than that of WT. This was largely accounted for by the emergence of a second peak in the frequency distribution of amplitudes of AIIs in $CaBP5^{-/-}$ retinal slices that was not apparent in WT AIIs. This new peak was of larger amplitude (≈11pA) than the first peak (≈6pA). The frequency of the AII **ml**-EPSCs was also altered in the absence of CaBP5; events had a shorter mean inter-event interval in $CaBP5^{-/-}$ retinal slices than in WT. This was attributed to a significant reduction in events with an inter-event interval greater than ≈75ms that were present in WT AIIs but essentially absent in

AIIs in CaBP5$^{-/-}$ slices. Light-evoked synaptic transmission, generated by a brief light flash, was also significantly altered in retinal slices from CaBP5$^{-/-}$ mice. The mean amplitude of AII **le**-EPSC was decreased by more than 50% in Alls in CaBP5$^{-/-}$ slices, while the **le**-EPSC was longer in duration relative to WT. Together, the results provide a strong indication that CaBP5 regulates RBC exocytosis. Furthermore, the data raise the possibility that CaBP5, via its ability to decrease spontaneous release and augment evoked release amplitude, supports rod-mediated light-responses by improving the signal to noise ratio at the RBC-AII amacrine cell synapse.

Disclosures: M.B. Kozhemyakin: None. **R. Heidelberger:** None.

Poster

395. Retinal Circuitry

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Program #/Poster #: 395.18/FF8

Topic: D.07. Vision

Title: Effects of Bmal1 gene deletion in GLAST positive cells on retinal morphology and physiology

Authors: F. BOI[1], S. RICCITELLI[2], D. LONARDONI[1], S. BISTI[1], O. BARCA-MAYO[1], D. DE PIETRI TONELLI[1], S. DI MARCO[2], *L. BERDONDINI[1]
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Abstract: Mammalian physiological functions are modulated with a circadian rhythm both at cellular and tissue level. Light is the main driver of this rhythmicity modulating a wide range of endogenous clock genes that are present in almost all cell populations.
Recent studies by Barca-Mayo et al. have shown that the selective deletion of the clock gene Bmal1 in GLAST positive cells alters locomotor activity and cognition in mice. We wonder whether such a selective deletion in GLAST positive cells of the retina might impinge on retinal function. Interestingly, Storch et al. found that adult mice lacking Bmal1 in all retinal cells modify their retinal information processing.
Here, we studied whether conditional deletion in adult life of the gene Bmal1 in astrocytes and Muller cells interferes with retinal function and morphology. We recorded retinal light responses in Bmal1cKO and control subjects both in vivo (flash-Electroretinogram) and ex-vivo (high-density CMOS multi electrode array). In addition, we collected retinal tissue for morphological and immunohistochemical analyses.
In our experimental condition preliminary results show no major differences between control and Bmal1cKO, suggesting that Bmal1 deletion in GLAST positive cells does not impact on retinal physiology and morphology.

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Poster

395. Retinal Circuitry

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Topic: D.07. Vision

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Title: Motion anticipation of inhibitory transmission from amacrine cells

Authors: *D. LEE[1], S. A. BACCUS[2]
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Abstract: Understanding how neurons in a network act together to perform a computation is a central goal of neuroscience, but it is difficult to assess the contribution of individual neurons in the face of diverse dynamic and nonlinear properties and other parallel neural pathways. In the retina, amacrine cells are a diverse population of primarily inhibitory neurons whose dynamic effects on retinal output are poorly understood, especially for ethologically relevant stimuli such as visual motion. Retinal ganglion cell activity anticipates the motion of an object by positioning its activity near the leading edge of a stimulus, but the specific interneurons that contribute to this process are unknown. Here we take an approach to directly measure the contribution of individual amacrine cells to the processing of moving stimuli using simultaneous intracellular recording from a single amacrine cell and multielectrode extracellular recording from a population of ganglion cells in the salamander retina. We manipulated the gain of individual sustained Off-type amacrine cells using simultaneous intracellular and multielectrode recording in the presence of a moving visual stimulus. After recording the response of the amacrine cell to a moving bar, we repeated the presentation of the stimulus while injecting into the amacrine cell timed current calculated to amplify or reduce the response of the amacrine cell to the moving stimulus, while recording ganglion cell responses. We found that although the peak of Off-type ganglion cell responses occurs near the leading edge of the moving bar, amacrine cell responses were more delayed than that of ganglion cells. By changing the gain of the amacrine response, we found unexpectedly that suppression of ganglion cell activity caused by the amacrine response to the moving bar occurs only on the rising phase of amacrine cell depolarization. As a consequence, although the peak of the amacrine cell response lags the moving bar, the peak of inhibitory transmission is shifted toward the leading edge of the bar. These studies show a general approach to directly measure the contribution of an interneuron to a computation, and

that inhibition from sustained Off-type amacrine cells exhibits a computation similar to one seen in the ganglion cell population, motion anticipation that compensates for visual delays.

Disclosures: D. Lee: None. **S.A. Baccus:** None.

Poster

395. Retinal Circuitry

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 395.20/FF10

Topic: D.07. Vision

Title: Connectivity patterns of starburst amacrine cells in the mouse retina

Authors: *S. MU[1], N. L. TURNER[1], W. M. SILVERSMITH[1], J. S. KIM[1], H. SEUNG[2], . EYEWIRERS[3]
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Abstract: It is well believed that starburst amacrine cells (SACs) contribute significantly to the direction selective (DS) circuits in the mammalian retina. The extent of this contribution, specific mechanisms and effects on innervated neurons, remain unknown. We anatomically reconstructed roughly 400 GCs and 200 SACs from serial electron microscope (EM) images of a $(0.3\ mm)^2$ patch of the inner plexiform layer of the mouse retina. In Bae et al. 2018, we clustered the GCs and identified 47 clusters, 35 out of which were verified to be types. Here, we studied the contacts among the cells by their types, and found intriguing contact preferences among SACs, and between GC and SACs.
It is known that an On-Off direction selective ganglion cell (ooDSGC) prefers to receive synapses from SAC dendrites oriented along its null direction (Briggman et al. 2011), and the same is thought to be true for On DSGCs (Yonehara et al. 2010). We found several other GC types (63, 2aw, 73 in nomenclature of Bae et al. 2018) that receive directionally biased SAC contacts.
We discovered a novel class of contacts between a dendritic termination of one Off SAC and the soma and/or proximal dendrites of another Off SAC. In many cases, the terminating dendrite veers towards the inner nuclear layer (INL), traveling almost parallel to the light axis. This is unexpected because Off SAC dendrites normally stratify at a particular depth in the inner plexiform layer (IPL) and also terminate at that depth. The terminating dendrite often travels in contact with a proximal dendrite, and if it reaches the soma of the other Off SAC, it typically spreads to a lump as it terminates. Most Off SAC cells in our dataset display this type of outbound and/or inbound contacts with one or more other Off SAC cells, with inbound contact patches from up to 4 partner cells observed on any given soma.
We also discovered a novel class of contacts between somas of On SACs. In studying SAC populations, Whitney et al. (2009) reported higher number of “close-neighbor pairs” in the

ganglion cell layer (On SACs) than in the inner nuclear layer (Off SACs). Not only have we found On SACs often pairing up next to each other in the ganglion cell layer, the pairs often form intertwined short twigs in between the two somas. In our specimen, 35 out of the 103 On SACs formed 18 adjoining pairs, with 13 pairs judged to have directly abutting somas, and the rest having dedicated short branch(es) reaching between the two somas from within the ganglion cell layer.

Disclosures: S. Mu: None. **N.L. Turner:** None. **W.M. Silversmith:** None. **J.S. Kim:** None. **H. Seung:** None. **. Eyewirers:** None.

Poster

395. Retinal Circuitry

Location: SDCC Halls B-H

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Program #/Poster #: 395.21/FF11

Topic: D.07. Vision

Support: NSF Grant IOS1556388
NSF Grant IIS1724421
NEI Grants
2016 Salk W&S Awards

Title: Why threshold modulation leads to sparse responses in the brain

Authors: *W.-M. HSU[1,2], D. B. KASTNER[3], S. A. BACCUS[4], T. O. SHARPEE[1,2]
[1]CNL-T, Salk Inst., La Jolla, CA; [2]Physics, UCSD, La Jolla, CA; [3]EPFL - LCN, Lausanne, Switzerland; [4]Neurobio., Stanford Univ., Stanford, CA

Abstract: Neurons typically have low response rates. Although this limits information transmission, this can reduce metabolic costs that would otherwise rise steeply. Here we report an additional strict limit on the response rate above which information decreases with response rate. This phenomenon occurs in the presence of threshold modulation that happens ubiquitously in the brain. The rate limit also applies to pairs or groups of neurons that jointly encode the same type stimulus feature. This is exemplified by pairs of adapting and sensitizing cells in the retina ganglionic layer. The observed rates for adapting-sensitizing cell pairs are below the predicted rate limit, as are response rates of adapting cells that experience strong threshold modulation. Analysis of data using the two-pathway model that includes threshold modulation identifies amacrine cells as the source for threshold modulation, with minimal input from these cells to sensitizing cells. Overall, these results reveal a novel constraint on neural computation.

Disclosures: W. Hsu: None. **D.B. Kastner:** None. **S.A. Baccus:** None. **T.O. Sharpee:** None.

Poster

395. Retinal Circuitry

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 395.22/FF12

Topic: D.07. Vision

Title: Short-term plasticity and upregulation of biopolar-ganglion synapses can resolve the neural states both in the normal and the rd1 retinas

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Abstract: While the retina of the retinal degeneration rd1 mouse fails to respond to light stimuli due to loss of photoreceptors, the rd1 retina exhibits spontaneous rhythmic activity at a low frequency (<10 Hz) even without light stimuli that is not observed in the normal retina. Experimental results thus far suggest that the spontaneous rhythmic activity could be attributed to the upstream neuronal network including bipolar cells (BCs) and AII amacrine cells (AII-ACs) (Euler and Schubert, 2015). Based on the results, two potential mechanisms have been proposed; one arises from the property of a gap junction network between BCs and AII-ACs and between AII-ACs (Trenholm et al., 2012) whereas the other does from the intrinsic property of AII-ACs (Choi et al., 2014). In either case, the oscillatory activity is enhanced when the AII-ACs are hyperpolarized, suggesting that both AII-ACs and BCs would be more hyperpolarized in the rd1 retina than in the normal retina. Depolarization of presynaptic neuron (BC) would cause activation of the synapse from the BC to the postsynaptic neuron, ganglion cells (GCs), which implies that GC should be more activated in the normal retina than in the rd1 retina. Therefore, it should be solved why the normal retina does not show such an activity as well as how such spontaneous rhythmic activity is generated in the rd1 retina. In the present study, we studied the mechanism for the spontaneous rhythmic activity using a computational model of AII-AC, BC, and GC network. In particular, to solve the paradoxical phenomenon mentioned above, we incorporated a modified short-term plasticity model as the BC-GC synapse (Sagdullaev et al., 2011) because BCs do not generate action potentials. Even at a depolarized state, the synapse model was not activated sufficiently because of short-term depression. If we assume upregulation of the synapses in the inner plexiform layer of the rd1 retina (Dagar et al., 2014), the model could reproduce both the normal and abnormal neural states in the absence of light stimuli: no response in the normal retina and spontaneous rhythmic activity in the abnormal retina.

Disclosures: K. Kitano: None. **K. Taniguchi:** None.

Poster

395. Retinal Circuitry

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Program #/Poster #: 395.23/FF13

Topic: D.07. Vision

Support: NIH Grant EY026027-01

Title: Retinal light adaptation and dopamine D4 receptor sensitivity is impaired at the ganglion cell level after six weeks of diabetes

Authors: *M. FLOOD[1], E. D. EGGERS[2]
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Abstract: *Purpose:* There is growing evidence to suggest that normal retinal signaling is disrupted early on in diabetes, long before the onset of the vascular pathologies associated with diabetic retinopathy. Previously, we have shown that after six weeks of diabetes in a mouse model, inhibitory inputs to rod bipolar cells are significantly reduced. The purpose of this study was to determine whether these upstream changes have a significant impact at the ganglion cell level, in terms of excitability and adaptation to changing light conditions. *Methods:* Diabetes was induced in C57BL/6J mice at 5 weeks of age by i.p. injections of streptozotocin (STZ, 75 mg/kg) dissolved in citrate buffer. Age-matched controls were given sham injections with citrate buffer alone. Diabetes was confirmed by blood glucose levels > 200 mg/dL. Six weeks post injections, whole-cell voltage clamp recordings of light-evoked (L) and spontaneous (s) excitatory post synaptic currents (EPSCs) were made from ON ganglion cells by holding at -60 mV, the reversal potential for chloride ions. µM). Light responses were elicited at multiple intensities by a 30ms full field LED stimulus (λ = 525 nm). Light adaptation was performed by exposing retinal slices to a 5 minute rod-saturating background. D4 receptors were agonized with the selective agonist PD-168077 maleate (500 nM). Responses were normalized on a cell by cell basis to the maximum response recorded under dark-adapted conditions. All experiments were performed under infrared illumination to preserve retinal sensitivity. The peak and charge transfer (Q) were measured for all evoked responses. All light response data was analyzed by 2- or 3-way repeated measures ANOVA. *Results:* Raw L-EPSC peak amplitudes ($p=0.013$), but not Q ($p=0.631$) were significantly larger for diabetic vs control animals. For the light adapted responses, no significant differences in peak amplitude were found ($p=0.354$), but Q was significantly larger for diabetic animals at 9500, 95000, and 950000 photons/μm^2/s ($p<0.001$ at each intensity). For D4 receptor-activated responses, both Q ($p=0.024$) and peak amplitude ($p<0.001$) were significantly larger in diabetic animals compared to controls, and these differences were most apparent at 95000 and 950000 photons/μm^2/s, cone photoreceptor dominated light intensities. *Conclusions:* After 6 weeks of diabetes, the overall sensitivity of ON ganglion cells is significantly increased, and

their ability to adapt to increased light levels may be compromised. This impairment seems to be due to changes in D4 receptor signaling, suggesting a causative role for dopamine deficiency in diabetic retinopathy.

Disclosures: M. Flood: None. **E.D. Eggers:** None.

Poster

395. Retinal Circuitry

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 395.24/FF14

Topic: D.07. Vision

Support: National Research Foundation of Korea (NRF) 2016R1D1A1A09918427

Title: A much higher density of melanopsin-IR cells in two nocturnal microbat retinas than that in diurnal animals

Authors: M.-J. JEONG[1], E.-B. PARK[1], H.-G. KIM[1], H.-R. SONG[1], *C.-J. JEON[2]
[1]Sch. of Life Sci., BK21 Plus KNU Creative Bioresearch Group, Kyungpoo, Daegu, Korea, Republic of; [2]Kyungpook Nat'l Univ., Daegu, Korea, Republic of

Abstract: Intrinsically photosensitive retinal ganglion cells (ipRGCs) respond to light and play roles in non-image forming vision, such as circadian rhythms, pupil responses, and sleep regulation, or image forming vision, such as processing visual information and directing eye movements in response to visual clues. To investigate the organization of the ipRGCs in the retinas of a nocturnal animal, quantitative analysis of melanopsin-immunoreactive (IR) cells was conducted on the retinas of two microbats, *Rhinolophus ferrumequinum* and *Eptesicus serotinus*. The mean melanopsin-IR cell density was 227.12 ± 11.41 cells/mm^2, and the total number of melanopsin-IR cells was 819.74 ± 52.03. In the *R. ferrumequinum* retina, the total number of the neurons in the ganglion cell layer (GCL) was 12,254.17 ± 660.39 and that of the optic nerve axons was 5,179.04 ± 208.00. Thus, the ipRGCs constituted approximately 15.83% of the total RGC population in microbat *R. ferrumequinum* retina. Another microbat *E. serotinus* retinas also had high melanopsin-IR cell densities: the mean melanopsin-IR cell density was 382.61 ± 13.04 cells/mm^2, and the total number of melanopsin-IR cells was 943.29 ± 42.55. In the *E. serotinus* retina, the total number of neurons in the GCL was 15,672.79 ± 605.02. The proportion of melanopsin-IR cells in microbat *E. serotinus* was estimated to be approximately 10.03% to 15.05%. In addition, the present study identified the types and distribution of melanopsin-IR cells in *R. ferrumequinum* retina. Three types of melanopsin-IR cells were observed in the present study: M1 type in the GCL (M1c; 21.00%) or in the inner nuclear layer (INL, M1d; 5.15%), M2 type (M2; 5.79%), and M3 type in the GCL (M3c; 26.66%) or INL (M3d; 4.69%). Additionally, some M3c cells had curved dendrites leading up towards the OFF sublayer of the

IPL and down to the ON sublayer of the IPL (M3c-crv; 7.67%). Melanopsin-IR cells displayed a medium soma size and medium dendritic field diameters. There were 2-5 primary dendrites and sparsely branched dendrites with varicosities. The present study demonstrates that the nocturnal microbat has a much higher density of melanopsin-IR cells than documented in diurnal animals.

Disclosures: M. Jeong: None. **E. Park:** None. **H. Kim:** None. **H. Song:** None. **C. Jeon:** None.

Poster

395. Retinal Circuitry

Location: SDCC Halls B-H

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Program #/Poster #: 395.25/FF15

Topic: D.07. Vision

Support: National Research Foundation of Korea (NRF) 2016R1D1A1A09918427

Title: Identification of rod bipolar and dopaminergic cells in the bat retina

Authors: *E.-B. PARK, J.-Y. JEON, E.-S. LEE, C.-J. JEON
Sch. of Life Sci., BK21 Plus KNU Creative Bioresearch Group, Kyungpoo, Daegu, Korea, Republic of

Abstract: A growing number of studies have revealed the functional neuroarchitecture of the microbat retina and suggested that microbats can see using their eyes. To better understand the oranization of the microbat retina, quantitative analysis of protein kinase C alpha (PKCα)- and tyrosine hydroxylase (TH)-immunoreactive (IR) cells was conducted on the greater horseshoe bat (*Rhinolophus ferrumequinum*) retina. As a result, PKCα immunoreactivity was observed in rod bipolar cells, consistent with previous studies on other mammalian retinas. PKCα-IR cell distribution in the inner nuclear layer showed regional differences in density, with the highest density found in the nasal retina. The average density of PKCα-IR cells was 10,487 ± 441 cells/mm^2 (mean ± S.D.; n = 4), with a total of 43,077 ± 1,843 cells/retina. TH-IR cells in the *Rhinolophus ferrumequinum* retina could be classified into four types based on soma location and ramification in the inner plexiform layer: conventional amacrine, displaced amacrine, interplexiform, and intercalated cells. The majority of TH-IR cells were conventional amacrine cells. TH-IR cells were nonrandomly distributed at low density over the retina. The average density was 29.7 ± 3.1 cells/mm^2 (mean ± S.D.; n = 3), with a total of 124.0 ± 11.3 cells/retina. TH-IR processes showed varicosities and formed ring-like structures encircling AII amacrine cells. Our study provides the foundation for understanding the neurochemical architecture of the microbat retina and supports the notion that the eyes do play a role in the visual system of microbats.

Disclosures: E. Park: None. **J. Jeon:** None. **E. Lee:** None. **C. Jeon:** None.

Poster

395. Retinal Circuitry

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 395.26/FF16

Topic: D.07. Vision

Support: NEI-EY020895

Title: Characterization of neuroinflammatory mechanisms associated with diabetic retinopathy using human transcriptomic

Authors: *P. E. FORT[1], Y. SHAN[2], J. E. ROGER[3]
[1]Ophthalmology and Visual Sci., [2]Univ. of Michigan, Ann Arbor, MI; [3]CERTO, Orsay, France

Abstract: Diabetic retinopathy, the major ocular complication associated with diabetes, remains the primary cause of vision loss in the working age population. Using non-targeted transcriptome analysis approaches, several groups have identified specific regulatory pathways affected in diabetic rodents. Use of those animal models has yielded critical discoveries relative to the general changes affecting the retinal transcriptome, but those are limited by the absence of a macula and the limited difference of peripheral versus central retina in rodents. These limitations are critical as diabetic retinopathy is a regional disease that affects the retina heterogeneously, as demonstrated by macular edema, peripheral non-perfusion and regional loss of receptor fields in diabetic patients.Using non-fixed, freshly isolated retinal tissues from human donors, with and without diabetes and with or without retinopathy, we used RNA deep sequencing to assess the transcriptional changes affecting the retina. In this study, we independently analyzed the transcriptome of the macular, perimacular and peripheral regions of the retina (n=6 per tissue and per group) in order to identify the regional impact of diabetes. Results were validated by quantitative pcr analysis using the same samples (n=6) and an independent set of samples (n=12). Pathways identified were then further analyzed using biochemical methods including immunoblot and ELISA. Over 800 genes were statistically significantly affected ($p>0.05$) with region specific patterns as a function of the disease state. Principal component analysis confirmed the clustering of the samples while pathway analysis using the GeneGo/MetaCore integrated software identified specific inflammatory, metabolic and neuroglial regulatory pathway. Using qRT-PCR, consistent significant alterations of the expression of genes associated with inflammation (including the alternative pathway of the complement) and neuroglial regulatory pathways (growth factor signaling) were dissected and demonstrated a regional alteration with a primary diabetes component in the central retina and a primary retinopathy component in the peripheral retina.This study offers the first regional analysis of the pathophysiological mechanisms of diabetic retinopathy with a high potential of identification of

specific therapeutic targets including specific regulators of the inflammatory response and regulation of the neuroglial tissue homeostasis.

Disclosures: P.E. Fort: None. **Y. Shan:** None. **J.E. Roger:** None.

Poster

395. Retinal Circuitry

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

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Topic: D.07. Vision

Support: NIH Grant EY024567
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Title: The impact of light adaptation and cell type on correlated spiking and retinal population codes

Authors: *K. RUDA[1], J. W. PILLOW[2], G. D. FIELD[1]
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Abstract: Retinal ganglion cell (RGC) types encode and transmit distinct visual signals to the brain. RGCs can exhibit precisely correlated spiking with nearby RGCs of the same and different types. The extent to which these signal and noise correlations need to be accounted for to understand retinal coding has been actively debated. At stake are the complexities of both encoding and decoding models that are needed to describe early visual processing. Two key features of retinal function are likely to shape the role of correlations in sensory processing: cell type and adaptation state. Different RGC types exhibit distinct amounts of correlated activity with neighboring cells, and changes in light level alter the strength of correlated activity among RGCs. Thus, we hypothesize that the role of correlated activity in encoding and decoding of visual information depends on cell type and light level. To test this hypothesis, we measured the spiking activity from hundreds of RGCs from rat retina on a large scale multielectrode array. RGCs were functionally classified into >6 distinct cell types according to their response properties to white noise and drifting gratings. We fit recorded responses with a generalized linear model (GLM). This model extends a basic linear-nonlinear-Poisson model by adding a feedback term that depends on spike history and a coupling term that depends on the spiking of other RGCs. This model accurately predicted the spike trains of individual RGCs and pairwise correlations among RGCs. We first tested the significance of correlations for describing RGC activity across light levels and cell types. We compared the encoding performance of the full, coupled GLM to an independent GLM that does not include coupling between cells. At cone light levels, the coupled GLM captures 400% more correlations than the independent model. The

necessity of the coupling term increases at rod light levels, where the coupled GLM captures 500% more of the correlation structure. This result holds for various RGC types. Next, we determined whether correlated activity is necessary for conveying visual information by performing model-based decoding on RGC responses. We found that decoding performance across the independent and coupled GLMs was similarly impacted by light adaptation. These results reveal that accounting for correlations is necessary to fully capture RGC activity and suggest that the role of correlations in retinal processing is altered by light adaptation. Ultimately, this work will constrain how regions downstream of the retina may optimally process RGC output, as well as provide an example of how adaptation can alter the impact of correlations on sensory coding.

Disclosures: K. Ruda: None. **J.W. Pillow:** None. **G.D. Field:** None.

Poster

395. Retinal Circuitry

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 395.28/GG1

Topic: D.07. Vision

Support: NEI R01EY024334
NEI R24EY023937
NEI P30EY003176

Title: Retinoic acid is the trigger for retinal ganglion cells pathophysiological remodeling during retinal degeneration

Authors: *M. TELIAS, Z. HELFT, B. DENLINGER, R. KRAMER
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Abstract: Retinitis Pigmentosa (RP) and Age-related Macular Degeneration (AMD) are common forms of blindness, caused by the death of photoreceptor cells (PRs) in the retina. In health, light responses are initiated in rod and cone photoreceptors, processed by interneurons, and synaptically transmitted to retinal ganglion cells (RGCs), which generate action potentials that carry visual information to the brain. In RP and AMD, upon death of PRs, RGCs survive but undergo significant changes in gene expression, morphology and electrophysiological properties, collectively known as ‘RGCs-remodeling’. We have previously shown that two early-on, critical aspects of remodeling involve RGCs becoming hyper-permeable and hyper-excitable, exhibiting a high spontaneous firing rate that obscures responses to dim light from surviving PRs. Therefore, understanding RGCs-remodeling is key for curing blindness, because any form of vision restoration strategy will rely on RGCs response to renewed or super-imposed visual signaling in the degenerated retina. Yet, no study so far has been able to determine the

mechanism responsible for RGCs-remodeling. Here we show that Retinoic Acid (RA), signaling through the retinoic acid receptor (RAR), is the trigger that initiates RGCs-remodeling. Using rodent models for blindness (i.e., rd1 mice, rd10 mice and S334ter rats), we show that loss of PRs leads to an increase in RGCs' RAR-signaling, as measured by a novel genetically encoded double RA-reporter virus. In-vivo and ex-vivo pharmacological and genetic inhibition of RA and RAR-signaling reverses remodeling in rd1 mice, as measured through dye-loading assays and multiple electrode arrays recordings. Conversely, pharmacological and genetic agonism of RA and RAR-signaling in WT mice, induces hyper-permeability and hyper-excitability, mimicking remodeling. Furthermore, in partially blind rd10 mice, we show that inhibition of RAR-signaling dramatically improves RGCs' firing upon residual light-responses of surviving PRs. In this study we identify RA and RAR-signaling as the molecular trigger behind pathophysiological remodeling of RGCs in-vivo. RA and RAR are necessary and sufficient for remodeling to take place, and can be used as drug/gene therapy targets for reversing it. This study presents new therapeutic opportunities for mitigating vision impairment in retinal degenerative disease, as well as for improving signal fidelity in vision restoration techniques.

Disclosures: M. Telias: None. **Z. Helft:** None. **B. Denlinger:** None. **R. Kramer:** None.

Poster

396. Visual Cortical Streams: Rodentia, Primate, and Carnivora

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 396.01/GG2

Topic: D.07. Vision

Support: Israeli Science Foundation #157-16
Israeli Ministry of Science

Title: Retinal stimulation through a DLP based projection system for studying visual cortex responses with voltage sensitive dye imaging

Authors: *A. GROSS, N. IVZAN, N. FARAH, Y. MANDEL
Bar Ilan Univ., Ramat Gan, Israel

Abstract: High-resolution recording of visual cortex in response to patterned stimuli in rodents is an important tool for studying various retinal diseases and vision restoration techniques. Towards this end, we constructed a unique slit-lamp based projection system for localized retinal stimulation based on a DMD projector (912x1140 pixels). The system uses visible light (LED 532nm) and NIR (910nm) sources, for the study of combined visible and prosthetic vision, yielding a retinal image size of 1mm wide and 3mm high. The position of the projected pattern on the desired retinal location is monitored through the slit lamp lens and a camera. The DLP system offers great flexibility and facilitates the generation and projection of visual stimuli at

different intensities, temporal frequencies, pulse durations and light-wavelength. More importantly, the system has the advantage of direct control of retinal stimuli location, with no need to perform back-projection, as is the case in currently available set-ups where a computer monitor is used.
Using this system, we obtained robust VSDI responses (dF/F up to 0.1%) to pulsed retinal stimuli of 3mm in diameter with the response increasing with stimuli irradiance. Moreover, retinotopic mapping of the visual cortex was obtained in response to projection of 8 squares at the size of 0.5mm. The retinotopic map demonstrated an amplification factor of about 30 μm/deg, comparable to previous studies.
This system is a useful tool for studying the cortical response to localized retinal stimulation and may shed light on cortical processes occurring during outer retinal degeneration and on the cortical integration of prosthetic and natural vision in rodents.

Disclosures: A. Gross: None. **N. Ivzan:** None. **N. Farah:** None. **Y. Mandel:** None.

Poster

396. Visual Cortical Streams: Rodentia, Primate, and Carnivora

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 396.02/GG3

Topic: D.07. Vision

Support: HFSP - LT

Title: A cortical visual area for processing collicular information

Authors: *R. BELTRAMO, M. SCANZIANI
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Abstract: Two distinct anatomical pathways carry visual signals from the retina to the visual cortex: the “geniculo-striate” and the “collicular” pathways. The geniculo-striate pathway runs through the dorsal lateral geniculate nucleus (dLGN) of the thalamus to the primary visual cortex (V1). From V1, visual information is broadcasted to retinotopically organized higher cortical areas that surround V1 and are targeted by V1 axonal projections. The other route, the collicular pathway, passes through the superior colliculus (SC) and reaches the visual cortex via the pulvinar nucleus of the thalamus (or latero-posterior nucleus, in rodents). While much is known about the role that the geniculo-striate pathway plays in the cortical response to visual stimuli, the role of the collicular pathway is not well understood. In fact, lesions of the SC have either no impact or only a relatively minor role on visual responses in the cortex of primates, carnivores and rodents. Here, we discover that visual responses in the mouse postrhinal cortex (POR), a higher visual area located in the caudo-lateral portion of the visual cortex, are abolished upon silencing the SC. By contrast, despite the axonal projections that V1 send to POR, silencing V1

has only a minor impact on visual responses in POR. Furthermore, we show that the SC connects to POR through a disynaptic input via a region of the latero-posterior nucleus of the thalamus that doesn't rely on V1 activity. Finally, we show that POR neurons greatly outperform V1 neurons at discriminating small objects moving along a linear trajectory. The ability of POR to extract stimulus features poorly discriminated by V1 indicates that the two visual pathways to the cortex capture different aspects of the visual world. These results demonstrate that in at least one visual cortical area, namely POR, the main visual drive is relayed from the periphery through the SC and not via V1. Because of its reliance on the ascending visual input through the collicular pathway, POR could be considered a cortical visual area specialized in the processing of collicular information.

Disclosures: R. Beltramo: None. **M. Scanziani:** None.

Poster

396. Visual Cortical Streams: Rodentia, Primate, and Carnivora

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NIH Director's New Innovator Award DP2 EY024505-01 (SPG)
Searle Scholars Award (SPG)
Klingenstein Fellowship (SPG)

Title: Functional segregation of eye-specific visual pathways in higher visual cortex

Authors: *K. J. SALINAS, J. H. ZEITOUN, H. KIM, S. P. GANDHI
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Abstract: The mammalian visual system processes visual information via multiple parallel streams. In primates, the dorsal and ventral streams are central to spatial navigation and object recognition. Evidence suggests that stream segregation is also present in the mouse visual system. In the classical hierarchical model, there are distinct circuits within primary visual cortex (V1) that serve the dorsal and ventral stream. We have recently discovered eye-specific functional specificity of binocular, low spatial frequency and monocular, high spatial frequency cardinal information processing in V1, suggesting that the mouse visual system has an eye-specific organization to its dorsal and ventral streams (Salinas et al., 2017). We asked whether this functional eye specificity is preserved outside of V1, into higher visual areas, and if these differences relate to stream segregation. Widefield retinotopic mapping of CaMK2a-tTa-tetO-GCaMP6s mice (P70-P200, both sexes) were used to delineate visual areas. Awake mice were shown drifting sinusoidal gratings of various spatial frequencies, ranging from 0.03 c/d to 0.96

c/d at 1-2 Hz through the contralateral eye and ipsilateral eye. Recordings were positioned in binocular V1 within layer 2/3 as well as in two higher visual areas, lateromedial (LM) and posteromedial (PM), which have been grouped into ventral and dorsal streams. Here we report eye-specific functional segregation at multiple levels of the hierarchy. Interestingly, we find that areas LM and PM contain similar proportions of binocular cells as compared to V1 (V1: 31%, LM: 34%, PM: 36%). However, ipsilaterally dominated monocular neurons represent a smaller proportion in PM compared to V1 (V1: 28%, LM: 23%, PM: 12%). We find that contralateral-eye dominated monocular neurons formed the majority in all three areas. Contralateral-eye dominated neurons in PM and V1 preferred higher spatial frequencies than those in LM (median preferred spatial frequency in V1: 0.14 c/d, LM: 0.11 c/d, PM: 0.17 c/d). Similar to a previous study, PM neurons were found to be cardinally biased. Our data further revealed that PM neurons preferred cardinal directions regardless of contralateral- vs. ipsilateral-eye dominance. These results demonstrate functional segregation of eye-specific responses in higher visual cortex. The organization of mouse visual cortex into distinct streams may be mediated by eye-specific developmental mechanisms.

Disclosures: K.J. Salinas: None. **J.H. Zeitoun:** None. **H. Kim:** None. **S.P. Gandhi:** None.

Poster

396. Visual Cortical Streams: Rodentia, Primate, and Carnivora

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 396.04/GG5

Topic: D.07. Vision

Support: FCT
Champalimaud Foundation
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Title: Layer-specific cortico-cortical loops in mouse visual cortex

Authors: H. YOUNG, B. BELBUT, *L. T. PETREANU
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Abstract: The mammalian neocortex consists of multiple functionally specialized areas, with each area composed of different layers and different cell types within those layers. These areas are hierarchically organized such that all inter-areal cortical connections may be divided into two types: feedforward (FF) connections propagating information from lower to higher areas, and feedback (FB) connections carrying information in the opposite direction. Finding fundamental rules of connectivity for FF and FB cortico-cortical projections is necessary to constrain and inspire theories of hierarchical cortical computation. Here we use subcellular channelrhodopsin-2(ChR2)-assisted circuit mapping (sCRACM) in combination with retrograde tracers to dissect

the connectional specificity of FF and FB projections for different projection neuron populations in mouse visual cortex. We recorded from pairs of neighboring neurons in the same cortical layer (L) in primary visual cortex (V1) or the higher order lateromedial (LM) visual area in acute brain slices containing FB (LM-->V1) or FF (V1-->LM) ChR2-expressing axons, respectively. For each pair, one cell projected to the source of the ChR2-expressing FF or FB inputs, and one cell projected to a different cortical or subcortical area. For each cell, we measured both the strength and dendritic location of FF or FB monosynaptic inputs.FF and FB innervated projection neurons in L2/3, L5 and L6. Inputs from both FF and FB projections to L5 cortico-cortical projection neurons were, on average, twice as strong as those to neighboring neurons projecting to the superior colliculus. However, the total input strength of FF and FB axons was similar in different populations of L5 or L2/3 cortico-cortical projection neurons, regardless of whether they projected to the source of FF or FB inputs or to other visual areas. By contrast, in L6, the total connectional strength of FF and FB projections was twice as strong in cortico-cortical neurons projecting back to the source of the FF or FB inputs when compared to those projecting to other visual areas. Our results show that, in specific layers but not all, cortico-cortical connections preferentially target specific projection neurons. This specificity results in reciprocal cortico-cortical loops in deep cortical layers, consistent with several theories of hierarchical processing.

Disclosures: H. Young: None. **B. Belbut:** None. **L.T. Petreanu:** None.

Poster

396. Visual Cortical Streams: Rodentia, Primate, and Carnivora

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Topic: D.07. Vision

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Title: Visual scene segmentation in the mouse

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Abstract: Mechanistic studies of the rodent visual system have exploded in the past few years owing to the wealth of molecular, genetic, and chemical tools available for neural circuit perturbations. Although mice have lower spatial acuity than primates, responses of neurons in mouse visual cortex show a marked resemblance in their tuning to elementary image features (e.g. spatiotemporal frequency, direction and orientation) when differences in acuity are taken into account. An outstanding question in the field concerns how these elementary visual feature

representations are further transformed by the rodent visual system. For example, does the mouse visual system explicitly extract object boundaries from visual scenes to facilitate object and scene perception? In an attempt to answer this question, we trained mice on a figure-ground segmentation task where figures were defined by either iso oriented gratings in the foreground/background, cross oriented gratings, or naturalistic textures, moving in counterphase to the background. Mice were readily able to report the side of the stimulus containing a texture-defined figure, doing so most readily and with highest accuracy for figures defined by cross oriented gratings. These animals, were able to generalize reports of a visual figure when presented with novel orientations, suggesting that they were able to learn abstract rules about the visual stimulus. Similar, albeit reduced performance could be measured using iso oriented gratings and performance was further reduced for naturalistic textures. We also recorded visual responses when presenting the same iso/cross oriented gratings and naturalistic textures in V1 and extrastriate visual areas RL and LM using both 2-photon calcium imaging and electrophysiology revealing, diverse contextual surround effects. Coherent spatial preference across all texture conditions presented, indicative of a texture-invariant figure response, was never observed. Taken together, these findings serve to inform the limits of the rodent as a model system in the study of scene segmentation.

Disclosures: F. Luongo: None. **L. Liu:** None. **D.Y. Tsao:** None.

Poster

396. Visual Cortical Streams: Rodentia, Primate, and Carnivora

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 396.06/GG7

Topic: D.07. Vision

Support: NIH R01 EY023756

Title: Distinct roles of cortical interneuron subtypes in contrast-dependent surround suppression

Authors: *D. P. MOSSING[1], J. VEIT[3], H. ADESNIK[2]
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Abstract: Spatial context powerfully influences how sensory cortical neurons encode external input, pooling congruent evidence when signals are weak and reducing redundancy when signals are strong. In primary visual cortex, this manifests as "surround suppression," in which stimuli beyond the classical receptive field can weaken visual responses to stimuli within it. The magnitude of suppression is feature dependent, increasing with higher contrast and more uniform textures; yet the neural circuit basis of this feature dependence is poorly understood. We use a combination of cell-type specific calcium imaging, optogenetics and whole cell recording in V1

of awake, locomoting mice to determine the inhibitory circuit basis for this fundamental neural computation. Our data demonstrate differential roles for somatostatin (SST) and two functional subclasses of vasoactive intestinal peptide (VIP) interneurons in mediating the feature dependence of surround suppression in layer 2/3. The competitive inhibitory dynamics between cortical SST and VIP neurons may represent a conserved neural circuit to mediate flexible modulation of neural coding depending on context.

Disclosures: D.P. Mossing: None. **J. Veit:** None. **H. Adesnik:** None.

Poster

396. Visual Cortical Streams: Rodentia, Primate, and Carnivora

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Program #/Poster #: 396.07/GG8

Topic: D.07. Vision

Support: 1R01MH109954-01
1F32HD087028-01

Title: Neuronal population responses in the human ventral temporal and lateral parietal cortex during arithmetic processing with digits and number words

Authors: *S. BAEK[1], A. L. DAITCH[2], P. PINHEIRO-CHAGAS[2], S. SAHA[2], J. PARVIZI[2]
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Abstract: Past research has identified anatomically specific sites within the posterior inferior temporal gyrus (PITG) in the ventral temporal cortex (VTC) and the intraparietal sulcus (IPS) in the lateral parietal cortex (LPC) that are engaged during arithmetic processing. While a small region of the PITG known as the number form area (NFA) is selectively engaged in the processing of numerals, its surrounding area is activated during both digit and number word processing. We compared the timing and selectivity of electrophysiological responses during arithmetic processing with digits and number words in the brain regions surrounding the PITG and IPS. We obtained intracranial recording from 38 electrodes near the PITG and 33 electrodes near the IPS implanted across 8 (6 males; 2 females) subjects during two math tasks. In the first task, subjects performed arithmetic trials in the form of calculations presented in digit numbers and control trials in the form of autobiographical memory questions. This task revealed 9 selective electrodes in the PITG and 11 in the IPS that were considered for further analyses. In the second task, subjects answered true-or-false math questions that were presented in the form of digits (e.g. “2+2=4”) or number words (e.g. “two plus two equals four”). Our recordings revealed early (200 to 400ms after stimulus onset) activations in the PITG, during which stronger high frequency broadband (HFB) responses were observed in response to digits than to number words ($p = 0.015$, $n = 9$, permutations test), and weaker but still significant responses to number

words ($p < 0.001$, $n = 9$, permutations test). Conversely, much slower responses were observed in the IPS region. Specifically, stronger responses to digits than to number words were found late at 500 to 700ms after stimulus onset ($p < 0.001$, $n = 11$, permutations test) in this region. The heterogeneity in the selectivity and temporal signature of the neuronal responses to the two visual formats in the anatomically specific mathematical processing regions in the human brain illustrate the specificity of the brain functions during mathematical cognition.

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Poster

396. Visual Cortical Streams: Rodentia, Primate, and Carnivora

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Title: Visual cortical area MT is crucial for development of the dorsal stream and normal reach and grasp behaviour

Authors: *J. A. BOURNE, C.-K. CHANG, I. C. MUNDINANO, M. J. DE SOUZA, W. C. KWAN
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Abstract: The primate visual cortex comprises a multitude of areas which emerge and mature at different stages of development. It has previously been demonstrated that the primary visual cortex (V1) and the middle temporal area (MT) mature at the same time, leading to the hypothesis that area MT is a “primary-like” area. Apart from its involvement in the perception of motion, MT is a constituent area of the dorsal stream; a network proposed to be involved in the guidance of actions and recognising where objects are in space.
Considering the central position of MT in the dorsal stream and its direct connectivity with component areas, we hypothesise that MT is critical to the establishment of the dorsal stream network and that perturbation in early life results in anatomical, connectional and behavioural changes associated with the stream. To address this, marmoset monkeys (*Callithrix jacchus*) received a unilateral mechanical ablation of left hemisphere area MT at postnatal day 14 (n=3). Animals were allowed to recover from the ablative surgery for 12 months, until adult. During this period, animals underwent longitudinal diffusion tensor imaging (6 and 18 weeks post-lesion) to examine structural changes in the ventral and dorsal streams.

Following 12 months of recovery, animals underwent training to perform visually-guided behavioural tasks, whereby the animal had to reach and grasp static and moving objects. Our results show that the animals showed no perturbation in proficiency in reaching and grasping static objects when compared to non-lesioned controls (n=2). However, when performing a moving task, lesioned animals exhibited abnormal reach-to-grasp behaviour. Lesioned animals showed larger maximum grip aperture when grasping, suggesting a deficit in prehension. When attempting to reach and grasp moving objects, lesioned animals showed reduced performance compared to controls. Specifically, lesioned animals required more attempts in a trial compared to controls, suggesting a perturbation in motion-related reach and grasp behaviour. Diffusion MRI/ tracer studies revealed changes to dorsal stream anatomy and connectivity.
These results suggest that the absence of MT early in life affects numerous dorsal stream areas and additionally leads to perturbations in visually-guided motor behaviours.

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Poster

396. Visual Cortical Streams: Rodentia, Primate, and Carnivora

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Topic: D.07. Vision

Support: Medical Research Council: Research Grant [MR/K014382/1]
The Wellcome Trust Strategic Award [101092/Z/13/Z]

Title: V1/V4 intercortical correlations evolve alongside binocular disparity selectivity at different time scales

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Abstract: Many visual cortical areas respond to binocular disparity, and successive stages of processing are thought to be required. In particular, the transformation from V1 to V4 may help to solve the correspondence problem. To gain insight on this process, we investigated the timescales of correlation between V1 and V4 while both areas responded to stereo images. We recorded from two alert, behaving *Macaca mulatta* that performed an odd-one-out task in which four random-dot stereograms (RDS) were presented. The animals fixated while all RDS were presented with a baseline disparity for 1 or 2 seconds. A step change in the target's disparity would then occur, which the animal had to locate with a saccade. Both animals' reaction times and accuracies improved with increasing step size. Each animal was implanted with two Utah

arrays, one in V1, another in V4. One of the four RDS was positioned to stimulate both V1 and V4 receptive fields (RF) simultaneously, although the RFs did not overlap (V1 RF distance < 1° of fixation, V4 RFs > 3°). V4 responses to the baseline disparity suggested three epochs: an initial peak response, a middle response of decreasing activity, and a late response of increasing activity. By comparison, the ability of units to distinguish different disparities decreased from an initial peak in V1 (mean disparity discrimination index ; initial = 0.288 ; middle = 0.276 ; late = 0.272 ; init vs mid paired Wilcoxon signed rank p = 2.163e-04) and V4 (init = 0.251 ; mid = 0.232 ; late = 0.238 ; p = 0.008). Noise correlations between and within cortical areas were quantified at different timescales using r_{CCG}. All types of correlation rapidly increased within tens of milliseconds and then rose more slowly, similar to earlier studies in V1, V4, and V5. The V1/V4 correlations were also dynamic. The initial responses had stronger correlations at short lags (up to 32 ms) compared with later in the trial (mean r_{CCG}[10ms] ; init = 0.016 ; mid = 0.013 ; late = 0.014 ; one-way ANOVA p = 0.043 ; init vs late paired signed rank p = 2.058e-22), while late correlations were stronger than middle responses for lags up to 190ms (mean r_{CCG}[80ms] ; mid = 0.017 ; late = 0.019 ; signed rank p = 1.580e-14). There was a weak tendency for the initial V1/V4 correlations at short lags to decrease as the similarity in disparity tuning curves increased (r_{CCG}[10ms] vs r_signal Spearman rho = -0.049, p = 9.928e-04), which may serve to reduce redundancy in neural coding. The low latency and dynamic changes of V1/V4 correlations thus may reflect the ongoing process between those areas of detecting and encoding disparity.

Disclosures: J.E. Smith: None. **A.J. Parker:** None.

Poster

396. Visual Cortical Streams: Rodentia, Primate, and Carnivora

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Topic: D.07. Vision

Support: NSF Award 1734887 to SLM and SMC

Title: A novel pressure regulating brain imaging implant for ultra-large field-of view mesoscopic imaging in nhps

Authors: *O. CABALLERO[1], M. LEDO[2], S. KOLLA[3], S. MARTINEZ-CONDE[2], S. TANG[4], A. NANDY[5], A. YAZDAN-SHAHMORAD[6], E. CALLAWAY[7], J. REYNOLDS[7], M. AVERY[7], P. LI[7], E. SEIDEMANN[8], Y. Y. CHEN[8], S. MACKNIK[2]
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Abstract: Several problems challenge mesoscopic imaging in the brain: 1) Difficulty with positioning high-NA objectives near the brain; 2) Creating a flat imaging window against the surface of the brain; 3) Adjusting the imaging window to changes in swelling and pressure in the brain, such as those that may occur due to hydration changes and other physiological factors; 4) Preventing growth of dura and biofilms that cloud the imaging window; 5) Follow-on MRI imaging of the animal post-implantation. We propose here a large-windowed radiolucent implant to address these issues. Our approach provides a 3cm diameter window for non-human primates that regulates pressure and employs a stable, strong, and thin design—that is mechanically modeled and stress-tested—to achieve access to the brain by large objectives, with design features that allow for manual repositioning of the imaging lens. To optimize the distance between the objective and the brain, we prioritize a thin implant design. A strong radiolucent implant is created using PEEK plastic, a radiolucent, strong, thermoresistant biostable material. We heighten strength of the chamber's attachment to the skull by positioning screws normal to the surface of the bone, to optimize the bond. The implant design has several parts and contemplates two different potential methods to maintain pressure on the brain. The first method uses springs to maintain even pressure of the imaging window on the brain's surface, irrespective of brain motion relative to the skull. The second method uses a hydrogel plastic cushion to achieve the same goal. Either method allows for the manual repositioning of the cover slip to create a flat imaging window, which can be moved to target electrode penetration slots in the glass, if needed. Lastly, our approach is designed to prevent dural growth by blocking the migration of migratory biofilm-forming cells; we hypothesize that use of dynamic pressure maintenance on the brain is key to the long-term patency of recordings implants that have been reported previously though occasionally. We have also implemented these design elements human prosthetics.

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Poster

397. Vision: Extrastriate Cortex

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Title: Vertical occipital fiber tract in the common marmoset

Authors: *H. TAKEMURA[1,2], T. KANEKO[3], F. PESTILLI[4], A. C. SILVA[5], F. Q. YE[6], D. A. LEOPOLD[7]
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Abstract: Comparative diffusion MRI (dMRI) approach for cerebral white matter can shed light on the complex organization of the human brain and the evolution of major fiber tracts. A recent study demonstrated that there are commonalities between human and macaque vertical occipital fasciculus (VOF), which is an important white matter tract to understand communication between dorsal and ventral visual cortex (Takemura et al., 2017). Here we aim to expand this knowledge by performing dMRI on the common marmoset (Callithrix jacchus), a New World monkey that diverged from humans and macaques approximately 35 million years ago. We collected high-resolution dMRI datasets from two post-mortem marmoset brains using a 7T Bruker MRI scanner (0.15 mm isotropic; 126 directions; b= 4800 s/mm^2). First, we found that a tract seemingly homologous to VOF is clearly visible in marmoset as a principal diffusion signal toward superior-inferior axis. This putative marmoset VOF was located laterally adjacent to optic radiation, similar to humans and macaques. Second, we evaluated the microstructural properties of this tract by collecting Magnetization Transfer Ratio (MTR) map which can approximate myelin volume in white matter. We found that the putative VOF in the marmoset has significantly lower MTR than the optic radiations, suggesting that the degree of myelination of these tracts differs in a consistent manner from classical histological study in human (Vogt, 1904). Third, in order to estimate cortical endpoint of putative marmoset VOF, we performed tractography and estimated which cortical maps are near putative VOF endpoint using histology-based marmoset brain atlas (Hashikawa et al., 2015). We found that the dorsal endpoints of the putative VOF were near cortical areas DM (V6), DA (V3A) and PPm (V6A), and ventral endpoints were near VLA (V4) and ITc (TEO), broadly consistent with findings in the human and macaque. These observations support the idea that the vertical occipital fasciculus serves to transmit visual information across relatively early stages of the dorsal and ventral visual streams. However, one notable difference is that the putative marmoset VOF is a continuous structure spanning a large area of parietal and temporal cortex, whereas human tracts are manifest as multiple bundles (VOF, posterior arcuate, middle longitudinal fasciculus). In sum, this study

demonstrates the key organization principle of VOF may be present at common anthropoid primate ancestor. However, the vertically oriented tracts in marmoset brain have more spatial contiguity and less branching than in humans, differences that stem from the much smaller brain and relative absence of cortical folding.

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Poster

397. Vision: Extrastriate Cortex

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Topic: D.07. Vision

Support: NEI/NIH EY-008128 (YMC)
EY-003611 (ELS)
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Title: Spiking noise in visual area V2 of infant monkeys shortly after experiencing monocular defocus

Authors: *Y. WANG[1], B. ZHANG[2], X. TAO[3], E. L. SMITH, 3rd[1], Y. M. CHINO[1]
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Abstract: Visual information is encoded and processed by a network of cortical neurons that depends on precisely timed spiking. We previously found that experiencing chronic monocular defocus during early infancy leads to abnormal dynamics of spike counts and the noisy and variable firing of V2 neurons in macaque monkeys that are tightly correlated with their perceptual loss (Wang et al, 2017). To understand how abnormal spiking noise (increased spiking irregularity and higher trial-to-trial fluctuation) and dynamics of spike counts developmentally emerge in V2 of amblyopic monkeys, it is necessary to know how noisy the spiking of V2 neurons is in normal infant monkeys around the time when the monocular defocus was introduced in the above mentioned study (3-4 weeks of age). We previously reported that both the magnitude of spiking irregularity and the trial-to-trial fluctuation in V2 neurons of infant monkeys of comparable ages were much lower than spiking noise in normal adult monkeys (Neurosci abstr. 2017). To gain insights into the neural mechanisms underlying the remarkable 'transformation' from very low spiking noise without experiencing defocus (*normal infants*) to abnormally high noise after experiencing monocular defocus during the critical period (*amblyopic adults*), here we analyzed the spiking noise of V2 neurons *immediately* after rearing infants with a defocus lens (*defocus infants*). Macaque monkeys were reared with a light-weight

helmet containing a defocus lens (-10 diopter) for one eye and a plano lens for the fellow eye between 4 and 8-10 weeks of age. At the end of rearing (without recovery), we recorded from individual V2 neurons under anesthesia. We quantified the spiking noise of V2 neurons in response to brief (640 ms) sine wave gratings that were optimized for orientation and spatial frequency for each neuron and repeated for 25 times. We calculated the square of the coefficient of variation in inter-spike intervals (CV^2) and mean-matched fano factor (m-FF) for stimulus contrast of 0%, 1%, 2.5%, 5%, 10%, 25%, 50% and 80%. Compared to the age-matched normal monkey, we found that: 1) the maintained (spontaneous) firing was much higher, 2) the mean firing rate was also elevated, 3) the onset latency was shorter, and 4) the spiking noise (mean CV^2 and m-FF) was significantly elevated, including the noise during spontaneous firing. However, the overall contrast sensitivity (C_{50}) was unchanged. The results suggest that the developmental alterations of V2 circuitry responsible for the increased spiking noise in adult amblyopic monkeys emerge rapidly after experiencing monocular defocus.

Disclosures: Y. Wang: A. Employment/Salary (full or part-time):; University of Houston. 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.; NEI/NIH EY-008128 (YMC), EY-003611 (ELS), CORE Grant P30-EY 007551. **B. Zhang:** A. Employment/Salary (full or part-time):; Nova Southeastern University College of Optometry. **X. Tao:** None. **E.L. Smith:** A. Employment/Salary (full or part-time):; University of Houston college of optometry. **Y.M. Chino:** A. Employment/Salary (full or part-time):; University of Houston college of optometry.

Poster

397. Vision: Extrastriate Cortex

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Topic: D.07. Vision

Support: NSERC

Title: Focal reversible inactivation of macaque inferior temporal (IT) cortex reveals a topographically selective causal role in primate core object recognition behavior

Authors: *R. RAJALINGHAM[1,2], J. J. DICARLO[1,2,3]
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Abstract: Primate core visual object recognition --- the ability to rapidly discriminate among objects near the center of gaze in spite of naturally occurring identity-preserving image variability --- is thought to rely on the ventral visual stream, a hierarchy of cortical areas

culminating in inferior temporal (IT) cortex. Previous work has shown that a particular read-out of particular IT population response patterns (a.k.a. neural codes) accurately predicts primate core object recognition behavior for each and every tested core object recognition task.. While this strongly suggests that these IT population codes underlie these behaviors, direct causal evidence for that hypothesis has been equivocal at best, especially beyond the specific case of "face-selective" sub-regions of IT supporting invariant face detection and discrimination behaviors. Here, we aimed to test the general causal role of IT in core object recognition by reversibly inactivating individual, millimeter-scale regions of IT via injection of muscimol while monkeys performed several binary object discrimination tasks, interleaved trial-by-trial. Our results show that inactivation of even single, millimeter-scale subregions of IT resulted in reliable contralateral-biased behavioral deficits and, importantly, that these deficits were highly selective over tasks. Inactivating different millimeter-scale subregions of primate IT resulted in different patterns of task deficits, each significantly predicted by that subregion's neuronal selectivity. Moreover, the effect of inactivation was topographically organized in that the pattern of behavioral deficit was most similar at anatomically neighboring subregions. Taken together, these results provide direct causal evidence that the proposed IT population codes (above) do indeed underlie primate core object recognition behavior, and that those codes are topographically organized.

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Poster

397. Vision: Extrastriate Cortex

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Topic: D.07. Vision

Support: NIH Grant EY006821-06

Title: Stripe-like organization of secondary visual cortex in tree shrew

Authors: *K.-S. LEE, M. SEDIGH-SARVESTANI, N. C. SHULTZ, R. SATTERFIELD, J. W. SCHUMACHER, D. FITZPATRICK
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Abstract: The tree shrew has long been recognized as a model for studying functional architecture of primary visual cortex (V1) due to its columnar-organized representation of multiple visual properties and close evolutionary relationship to primates. However, the functional organization of the secondary visual cortex (V2) remains unexplored. Whether V2 of the tree shrew exhibits an interleaved mapping organization like that found in primate V2 (functional stripes) or a single visuotopic organization like that found in the mouse homologue of

area V2 (lateral medial area) is unknown. In this study, we used both anatomical tracing and physiological mapping methods to examine this issue. To characterize V1-V2 projections, tree shrews (Tupaia belangeri, n=25) were injected with adeno-associated viral vectors (AAVs expressing Ruby2sm-Flag, GFPsm-myc, or tdTomato) in V1, followed by a postsurgical survival period of 2-3 weeks. *Post hoc* immunohistochemistry was performed on the flattened brain sections to enhance fluorescence. Consistent with previous results from the Kaas lab (Lyon et al., 1998), we found three distinct areas that receive direct V1 inputs: secondary visual cortex (V2), temporal dorsal area (TD), and temporal posterior area (TP). Unexpectedly, single large injections in V1 (~ 2 mm) produced a stripe-like organization in V2, with multiple bands separated by unlabeled regions, which are spaced periodically and consistently. The intensity profile of fluorescence parallel to the V1-V2 border displays cycles of densely labeled stripes (medium 0.91 mm) interrupted by weakly labeled stripes (thick 1.15 mm, thin 0.34 mm), while fluorescence intensity is relatively uniform along the orthogonal axis. The periodicity and relative bandwidth of this organization are reminiscent of V2 stripes labeled by cytochrome oxidase staining in the primate. To probe the functional properties of neurons in V2 compartments, V2 neurons were labeled with GCaMP6s calcium indicator through AAV injection, and *in vivo* two-photon calcium imaging was applied to measure the tuning properties, such as spatial frequency and color. We found that neurons located in the thin stripes prefer low spatial frequency and are responsive to isoluminant chromatic drifting gratings. In conclusion, both anatomical and physiological results suggest that there is a stripe-like functional specialization in tree shrew V2, which could serve as a model to test the evolutionary significance of the functional stripes and their transformation in higher-level visual processing.

Disclosures: K. Lee: None. **M. Sedigh-Sarvestani:** None. **N.C. Shultz:** None. **R. Satterfield:** None. **J.W. Schumacher:** None. **D. Fitzpatrick:** None.

Poster

397. Vision: Extrastriate Cortex

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FWO Vlaanderen G0A8516N

Title: Effective connectivity reveals an interconnected inferotemporal network for three-dimensional structure processing

Authors: *E. PREMEREUR, P. JANSSEN
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Abstract: Previous single-cell and monkey fMRI studies have shown that disparity-defined three-dimensional shape is processed in both the ventral (inferior temporal cortex, ITC) and the dorsal visual stream. The network of cortical areas that is activated more by curved surfaces than by flat surfaces at different disparities includes, besides parietal areas CIP, PIP, AIP and premotor area F5a, three clearly distinct regions in the ventral stream: area TEO in the Superior Temporal Sulcus (STS); a region in posterior TE on the convexity (pTE); and a region in anterior TE in the STS (TEs). To fully understand an area's role in behavior we have to investigate not only its neuronal properties but also the connectivity of the area under study. The latter can be achieved by combining fMRI with electrical microstimulation. We previously showed that microstimulation of anterior AIP activates a somatomotor network comprising PFG, SII, MIP and ventral premotor cortex (F5), whereas microstimulation of posterior AIP activates areas involved in object processing, including CIP, TEO and TE (Premereur et al, 2015). Here, we investigated the effective connectivity of the inferotemporal 3D-structure nodes. Three macaque monkeys were scanned at a 3T magnet (Siemens Prisma; voxel size: 1.2 mm3 isotropic) during sedation. 40-secs blocks of electrical stimulation were interleaved with 40 secs blocks of no stimulation, for a total of 480 secs. During stimulation blocks, 250 ms trains of stimulation (pulse width: 0.48 ms, freq: 200 Hz, amplitude: 1 mA) were delivered on average every 2.5 secs. We found that electrical stimulation of each of the 3D-structure nodes in ITC elicited increased fMRI activation in the other 3D-structure nodes in the ITC. Importantly, no increased activation was found in parietal areas, nor in prefrontal cortex. Our results indicate that 3D-structure nodes in ITC form a strongly interconnected network, receiving input from parietal areas in the dorsal stream implicated in 3D-structure-processing. The output of this ventral stream 3D-structure network remains elusive.

Disclosures: E. Premereur: None. **P. Janssen:** None.

Poster

397. Vision: Extrastriate Cortex

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Program #/Poster #: 397.06/GG17

Topic: D.07. Vision

Support: Wellcome Trust
BBSRC
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Title: Asymmetries in the intrinsic 3D connectivity within dorsal and ventral parts of area LIP of macaque

Authors: *B. AHMED, M. RUESSELER, J. SMITH, A. J. PARKER, K. KRUG
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Abstract: Lateral intraparietal (LIP) plays a central role in visuo-motor information processing, saccade planning and decision-making, but its intracortical wiring is not well understood. We examined the 3D pattern of LIP's intrinsic connectivity in 5 Rhesus Macaques (macaca mulatta) after focally injecting either the retrograde tracer Chloro-Toxin beta (CTb, ~80 nL) or Fluorogold (FG, ~160 nL). Injections were placed with a posterior approach. In parasagittal sections (1:5 series, 50 µm), we marked cell bodies under Neurolucida (Microbrightfield) and quantified the pattern of labelled cells. For different layers in each section, we reconstructed the density of labelled cells along the dorso-ventral axis of LIP. Consecutive parasagittal sections were aligned based on nearest neighbour density distributions to yield 2D spatial maps of connectivity patterns in LIP, separately for layers 1, 2-4, 5 and 6.
Anatomically, LIP can be divided into dorsal (LIPd) and ventral (LIPv) regions based on myelination density. In 3 macaques, tracer was injected into LIPd, layers 3 to 5, back-labelling cells across LIPd. The highest density of labelled cells was near the injection site forming a longitudinal run along the dorso-ventral extent of LIPd, with the densest areas on sections just medial to the injection site. In 2 animals, a single, well defined cluster of labelled cells in LIPv was also aligned in the dorso-ventral axis; in the third, a cluster was nearby but slightly offset medially. This pattern was aligned across superficial and deep layers. Also, in LIPd, there were a number of dense patches of labelled cells medio-laterally further away from the injection site, but again aligned across layers. In one macaque, tracer was injected at the border of LIPd/LIPv in Layers 1 to 3a and in another in layers 5 and 6 of LIPv. In these animals, there was a more widespread pattern of label in LIPv, but highest density label was also predominantly running in the dorso-ventral axis.
Apart from distinct point-to-point input from LIPv to LIPd, we show that there is an asymmetry in connections within LIP: a high density of inputs to LIP cells emanate from cells aligned along the dorso-ventral axis across all layers and weighted towards inputs from close-by medial LIP sites.

Disclosures: B. Ahmed: None. **M. Ruesseler:** None. **J. Smith:** None. **A.J. Parker:** None. **K. Krug:** None.

Poster

397. Vision: Extrastriate Cortex

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Program #/Poster #: 397.07/HH1

Topic: D.07. Vision

Support: NIH Grant EY000404

Title: Macaque MT neurons show a different specialization for horizontal disparity than V1 neurons

Authors: I. KANG, *B. G. CUMMING
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Abstract: A systematic mapping of disparity tuning along both horizontal and vertical axis has shown that macaque V1 neurons are specialized for horizontal disparity (Cumming, 2002): the response surface of a neuron on the 2-dimensional disparity space is elongated along the horizontal disparity axis, covering a wider range of horizontal disparity than vertical, regardless of preferred orientation. In area MT, odd-symmetric tuning curves (with distinct peaks and troughs) are more common than in V1. Depending on how these are constructed (starting from V1), it is possible that the orientation of the peak-trough axis may behave quite differently than the elongations seen in V1. We recorded responses of 112 MT neurons in one monkey to random dot stereogram (RDS) with combinations of horizontal and vertical disparity. Preferred motion directions were estimated from the response to an RDS or random line stimulus moving coherently in one direction. We fit the 2-dimensional disparity response with a mixture of 2 or 3 Gaussian functions to identify the peak and trough of the response surface. The orientations of the peak-trough axis were non-uniformly distributed ($p < 10^{-25}$, Rayleigh test), centering around 0 deg (mean = 0.4 deg, 95% CI = -4.5 ~ 5.4 deg), despite that the preferred directions were distributed uniformly ($p = 0.65$, Rayleigh test). This indicates that responses are typically an odd-symmetric function of horizontal disparity, regardless of preferred direction. Odd-symmetric functions of vertical disparity were seen much less frequently. MT disparity signals seem therefore to be organized in a way that produces more rapid changes in firing in response to horizontal than to vertical disparity - the opposite of the pattern seen in V1. One simple explanation for this pattern is to suggest that MT combines inputs from two (or more) V1 neurons that systematically differ in their preferred horizontal disparity, while having similar preferred vertical disparities, regardless of preferred direction within the subunits.

Disclosures: I. Kang: None. **B.G. Cumming:** None.

Poster

397. Vision: Extrastriate Cortex

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Topic: D.07. Vision

Support: NIH RO1 EY024912
NIH P50 MH103204

Title: Spatial generalization of repetition suppression in macaque inferotemporal cortex

Authors: *N. P. WILLIAMS, C. R. OLSON
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Abstract: Neurons in macaque inferotemporal cortex (ITC) exhibit repetition suppression. When an image is presented twice at the same location, first as prime and then as probe, the neuronal response to the probe is reduced relative to the response to the prime. This effect is identity-specific as indicated by the fact that suppression is greatest when the probe is identical to the prime (McMahon and Olson, 2009; Sawamura and Vogels, 2013). It is also location-specific as indicated by the fact that suppression is greatest when the probe is at the same location as the prime (De Baene and Vogels, 2010). The aim of the present experiment was to determine how identity-specific and location-specific effects combine to determine neuronal response strength in ITC. We recorded neuronal responses to displays consisting of a prime, a delay and a probe, each 300 ms in duration. The stimuli were 5° images of background-free objects presented in either the upper or lower contralateral visual field at horizontal and vertical eccentricity of 6°. We varied independently across trials the relation of the probe to the prime with respect to identity (same or different) and with respect to location (same or different). Upon analyzing data from 108 neurons (57 in monkey S and 51 in monkey O), we found that the probe response was reduced relative to the prime response under all conditions including the condition in which the probe differed from the prime in both identity and location. However the degree of suppression varied across conditions. To analyze the pattern of variation, we carried out an ANOVA with identity (same or different) and location (same or different) as factors and with firing rate, mean normalized across the four conditions, as the dependent variable. This analysis revealed a significant main effect of identity (greater suppression when identity was the same, $p < 0.0001$, effect size = 7.0 Hz), a significant main effect of location (greater suppression when location was the same, $p = 0.0045$, effect size = 1.5 Hz) and a significant interaction effect (greater identity-based suppression when location was the same, $p = 0.0001$, effect size = 2.4 Hz). The fact that suppression generalized across visual field quadrants suggests that it arises in part at the level of ITC because ITC is the first ventral stream processing stage at which the receptive fields of individual neurons typically span both visual field quadrants. The absence of complete spatial generalization suggests a role for low-level visual areas upstream from ITC. Repetition suppression is likely the culmination at the level of ITC of adaptive processes occurring at multiple stages of the visual processing hierarchy.

Disclosures: N.P. Williams: None. **C.R. Olson:** None.

Poster

397. Vision: Extrastriate Cortex

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Program #/Poster #: 397.09/HH3

Topic: D.07. Vision

Support: 1R01EY027853-01

Title: Developmental changes in parvalbumin expression in ferret primary and higher visual cortex

Authors: A. KAVUTURU[1], A. A. LEMPEL[2], *K. J. NIELSEN[2]
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Abstract: Changes in inhibitory circuits are considered a critical factor in the development of the visual system. However, while the timeline of inhibitory neuron development has been well investigated in primary visual cortex (V1), data for higher visual areas are lacking. Here, we make use of the ferret's early birth to provide a first systematic comparison of inhibitory neuron development in V1 and higher visual cortex. Our experiments focus on parvalbumin (PV) expressing inhibitory neurons in V1 and PSS, a recently identified higher order motion area in the ferret. More precisely, we investigate developmental changes in the laminar distribution of PV expression in V1 and PSS around eye opening, which marks the onset of direction selectivity development in both areas. PV neurons were identified in brain slices from young animals (age postnatal day (P) 28 - P47) and adults using DAB-peroxidase immunohistochemistry. A Nissl stain was used to identify cortical layers. We then determined the density of PV neurons for each layer in both V1 and PSS. Our data show significant changes in the laminar distribution of PV expression following eye opening (P30 - P32) in both areas. Before visual experience, layers 5 and 6 contain the largest density of PV neurons in V1 and PSS. After eye opening, this laminar profile shifts to the homogenous laminar distribution observed in adults. Intriguingly, our data suggest that the adult profile is reached sooner in PSS than V1, contrary to what would be expected from a strictly hierarchical development. Additionally, we also analyzed tissue from animals before natural eye opening that had been exposed to drifting gratings for 8h. Our results suggest that this premature visual stimulation was sufficient to accelerate development of PV expression in both areas.

Disclosures: A. Kavuturu: None. **A.A. Lempel:** None. **K.J. Nielsen:** None.

Poster

397. Vision: Extrastriate Cortex

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Program #/Poster #: 397.10/HH4

Topic: D.07. Vision

Title: A V1-recipient cortical area in the tree shrew contains direction selective neurons

Authors: *M. SEDIGH-SARVESTANI, J. SCHUMACHER, K. MAXIMOV, R. SATTERFIELD, N. SHULTZ, D. FITZPATRICK
Max Planck Florida Inst. of Neurosci., Jupiter, FL

Abstract: Comparing cortical circuits across species reveals similarities and differences in their functional architecture. We studied the sensitivity to motion direction in the tree shrew, a small diurnal animal with relatively high visual acuity and columnar cortical architecture. The phylogenetic classification of tree shrews is a subject of debate, but some evidence suggests a close evolutionary relationship to primates. Although the tree shrew has long served as a model system for studies of the striate cortex (V1), little is known about the function of its extrastriate regions.

Area TD is one of three V1-recipient extrastriate regions rostral to V2 in the tree shrew. Based on its anatomical connectivity patterns, TD has been hypothesized to be the homologue of MT in the macaque (Lyon and Kaas, 1998). To determine whether TD contains direction selective neurons, a hallmark of MT, we combined anatomical tracing with functional imaging of single-cells in the awake head-fixed animal.

We found that as predicted, TD contains cells highly tuned for the direction of motion. Nearby cells shared a preferred direction, giving rise to a columnar map of direction in TD. Nearby cells also shared a preferred point in the visual field, giving rise to a retinotopic map in TD. This suggests that tree shrews and macaques share a common extrastriate architecture dedicated to motion direction. To determine whether area TD in the tree shrew is a true functional homologue of area MT in the macaque, we also characterized single-cell tuning for the disparity in depth cues as well as sensitivity to component and pattern motion.

In the macaque, inputs to MT are already direction selective. To determine whether inputs to TD are also direction selective, we have begun to characterize the functional properties of V1, and V2, which provide dense projections to TD. We found that cells in the superficial layers (L2/3) of V1 are not sensitive to the direction of motion, consistent with previous electrophysiology-based findings (Van Hooser et al., 2013). We are in the process of identifying the functional properties of TD projection neurons across various layers of V1 and V2 so that we can understand how direction selectivity arises in TD. This work will clarify the functional similarities in the tree shrew and macaque visual system and will contribute to our understanding of how various diverged cortical circuits can provide solutions to the same computational problem.

Van Hooser SD, Roy A, Rhodes HJ, Culp JH, Fitzpatrick D (2013). J Neurosci 33(28):11494-505. Lyon DC, Jain N, Kaas JH (1998). J Comp Neurol 401(11):109-28

Disclosures: M. Sedigh-Sarvestani: None. **J. Schumacher:** None. **K. Maximov:** None. **R. Satterfield:** None. **N. Shultz:** None. **D. Fitzpatrick:** None.

Poster

397. Vision: Extrastriate Cortex

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Topic: D.07. Vision

Support: Whitehall 2015-12-120

Title: Clustering of 3D and 2D shape information in area V4

Authors: *R. SRINATH[1], K. J. NIELSEN[2], C. E. CONNOR[3]
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Abstract: Coding transformations in ventral visual cortex convert image information into object and scene understanding. Area V4, an intermediate ventral pathway stage, is known to represent 2D contour shape. Our recent results show that a substantial fraction of V4 neurons are more responsive to 3D volumetric shape (shape-in-depth) than to 2D shape in the image plane. Here, using 2-photon functional microscopy, we observed that 2D and 3D shape tuning cluster separately in area V4.
We used realistic shading cues to render simple volumetric shapes (Cs and Vs with cylindrical cross-sections and smoothly curved joints and endcaps). These shapes were presented at a wide range of 3D orientations so that their constituent fragments spanned the space of surface orientations and curvatures. We contrasted responses to these 3D stimuli with responses to 2D silhouette stimuli with the same outline in the image plane, filled with a single color lighter or darker than the background. Each 3D/2D pair of stimuli shared the same 2D contours, but in the 3D case these appeared as self-occlusion boundaries of volumetric objects, while in the 2D case they appeared as sharp edge boundaries of planar shapes. The 2D boundaries spanned the space of contour orientations and curvatures.
We imaged in anesthetized macaque monkeys using Oregon Green BAPTA-1AM (OGB) to measure neuronal responses. A substantial fraction of neurons (on the order of half) responded more strongly to 3D volumetric shapes compared to congruent 2D shapes. There was strong local clustering of 3D- and 2D-responsive neurons in separate patches on the order of several hundred microns. In addition, however, neighboring 3D and 2D patches were most responsive to congruent 3D and 2D shapes. These results suggest that derivation of 3D volumetric shape from 2D image information is a major constraint on microorganization in area V4.

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Poster

397. Vision: Extrastriate Cortex

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Title: Monotonic responses to numerosity and second-order contrast in early visual cortex

Authors: *J. M. PAUL, T. C. TEN CATE, B. M. HARVEY
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Abstract: Introduction: Humans and many animals have neurons tuned to specific numerosities, the number of visual items in a set. Numerosity tuned neurons respond selectively, decreasing their response amplitude with distance from their preferred numerosity. However, it remains unclear how such tuned responses are derived from visual images. Computational models suggest an initial monotonic stage where response amplitude increases with numerosity. Recent evidence suggests this monotonic stage may occur in early visual cortex. Here, we extend this finding, utilizing the superior spatial resolution of fMRI and pRF visual field mapping to characterize the location and nature of these responses. Method: We acquired ultra-high-field (7T) fMRI data while presenting visual stimuli whose numerosity changed gradually between 1 and 7, with 20 as a baseline. We used various stimulus configurations with different relationships between numerosity and (other) low-level visual features such as luminance, edge length and item size. We quantified the recorded responses using a response model that increases response amplitude proportionally to log(numerosity). Results: In all stimulus configurations, this monotonic response model closely matched responses in early visual areas (V1, V2, V3). The model's explanatory power was highest in V1 and progressively decreased up the visual hierarchy to LO1. Monotonic response model fit depended critically on the visual field position of the voxel's pRF, with goodness of fit decreasing when pRFs fell outside the area where the numerosity pattern was displayed. Responses were affected by stimulus configuration; stimulus configurations featuring very different dot sizes or a smaller, more densely filled pattern area were less well described by a monotonic response. However, numerosity explains responses better than low-level stimulus features which sometimes co-vary with numerosity and have been suggested to underlie numerosity discrimination abilities. To account for visual field stimulation caused variations in contrast energy across the visual field, we modeled second-order contrast from the visual images we presented. Conclusion: Our findings indicate monotonic responses to second-order contrast in our stimuli as a simple, biologically plausible mechanism linking established low-level response properties of early visual cortex with higher-level numerosity-tuned responses and perception.

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Poster

397. Vision: Extrastriate Cortex

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Program #/Poster #: 397.13/HH7

Topic: D.07. Vision

Support: ERC 637638 “OptoVision”
SUAG/002/RG91365

Title: Theta rhythmic neuronal activation in primate areas V1, V4 and TE and negative BOLD responses in the presence of multiple nearby visual stimuli

Authors: *B. AGAYBY[1], M. AINSWORTH[2], R. KIENITZ[1,3,4], J. T. SCHMIEDT[4], M. ORTIZ-RIOS[1], A. BELL[2,5], M. C. SCHMID[1]
[1]Inst. of Neurosci., Newcastle Univ., Newcastle upon Tyne, United Kingdom; [2]MRC Cognition and Brain Sci. Unit, Univ. of Cambridge, Cambridge, United Kingdom; [3]Epilepsy Ctr. Frankfurt Rhine-Main, Goethe Univ. Frankfurt, Frankfurt, Germany; [4]Ernst Strüngmann Inst. for Neurosci., Frankfurt, Germany; [5]Dept. of Exptl. Psychology, Univ. of Oxford, Oxford, United Kingdom

Abstract: Growing evidence indicates that reaction times and behavioral performance can become rhythmically modulated at 3-6 Hz (theta) when attention is distributed in the presence of multiple objects [1–3]. The underlying brain mechanisms for this rhythmic behavioral fluctuation remain little understood. Evidence for theta-rhythmic spiking at the neuronal level has been reported under different task conditions in area V4 of the extrastriate visual cortex and in area TE of macaques [4,5]. It is thought that this neural rhythm might arise from a balance between excitatory and inhibitory processes [6] that could be brought about by receptive field interactions [7] that can also be observed on a larger scale in fMRI [8].
Here we report on experiments in which we recorded multi-unit activity (MUA) using multi-electrode arrays implanted into V1 (monkey K), V4 and TE (monkey V), and in which we measured during separate runs (monkey D) the BOLD activity in visual cortex using fMRI at 4.7T. In all experiments, monkeys maintained passive fixation while either a single visual stimulus alone or together with a second stimulus was presented in the periphery. The stimuli were black disks, rings or rectangles in electrophysiology and rotating checkerboard ring patterns in fMRI. For V1 recordings, stimulus sizes were adjusted to the RF while a larger size was used for V4 and TE recordings. At the neural level, responses to a single stimulus inside the RF tended to be not rhythmic. When, however, a second stimulus was added near the RF, responses became rhythmic at 3-6 Hz in all three sampled cortical areas. fMRI analysis confirmed the emergence, most pronounced in V1, of a negative (compared to baseline) BOLD region between the two stimulus representations associated with a positive BOLD signal nearby.
Taken together, interactions between the excitatory RF center and its inhibitory surround account for the emergence of theta rhythmic activation in electrophysiology and appear to relate to a positive/negative BOLD activation pattern in fMRI across the visual cortical hierarchy. It seems that visual cortex might use this balance of patterned excitation- inhibition during situations that require flexible spatial filtering, such as during distributed attention in the presence of multiple objects.

1.Landau, A.N., and Fries, P. (2012). Curr. Biol.
2.Fiebelkorn, I.C. *et al.* (2013). Curr. Biol.
3.Dugué, L. *et al.* (2015). Proc. Natl. Acad. Sci. U. S. A.
4.Lee, H. *et al*. (2005). Neuron

5.Rollenhagen, J.E., and Olson, C.R. (2005). J Neurophysiol.
6.Moldakarimov, S. (2005). J. Neurophysiol.
7.Kienitz, R. *et al.* (2017). bioRvix
8.Bles, M. *et al.* (2006). Neuroimage

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Poster

397. Vision: Extrastriate Cortex

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Title: Saccade-related activity in V4 varies with behavioral outcome in a change-detection task

Authors: *K. ACAR[1,4], M. A. SMITH[2,4,3]
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Abstract: Primates explore the visual world by making a series of rapid, serial eye movements called saccades. When a behaviorally relevant object appears, it is thought that a decision to saccade arises through an interaction between visual cortical neurons representing the stimulus and neurons in oculomotor areas that encode saccade plans. Previous studies have observed an influence of saccade preparation signals on visual information processing in visual cortical neurons, and psychophysical work has shown an improvement in visual perception at the intended target of saccades. Furthermore, neurophysiological studies demonstrate a pre-saccadic enhancement of stimulus-evoked responses in visual area V4 neurons with receptive fields at the saccade target. However, our understanding of the contribution of the saccade-related V4 activity to saccade generation remains incomplete. V4 is an intermediate visual area in the ventral stream that is reciprocally connected with oculomotor control areas such as the lateral intraparietal area (LIP) and frontal eye fields (FEF). Therefore, the pre-saccadic enhancement in V4 activity could have a non-causal role in saccade decisions, simply reflecting a motor signal about an impending saccade. Alternatively, saccade-related V4 activity could be a source of sensory evidence that influences the decision to generate a saccade.
To understand the significance of the saccade-related modulation of V4 activity, we recorded simultaneously from many V4 neurons while rhesus macaques performed an orientation change-

detection task. Importantly, our task introduced a degree of uncertainty as to where and when the change would occur trial to trial, with two potential target locations placed in the same hemifield as the V4 neuronal receptive fields. For analysis, we divided trials by saccade direction and behavioral outcome. We found that saccade-aligned population activity in V4 predicts the direction of saccades in both false alarm and correct trials. Interestingly, the magnitude of the pre-saccadic modulation was greater before “correct” saccades than “false alarm” saccades. We also found that we could predict the direction of the eventual saccade from V4 activity earlier in the trials. A naïve Bayes classifier trained on V4 population activity during the penultimate stimulus flashes (stimuli preceding the eye movement behavior) was able to decode the eventual saccade direction with ~70% accuracy on correct trials and ~60% accuracy on false alarms (cross-validated, 50% chance). Together, these results highlight the confluence of sensory and saccade-related signals present in visual cortex during saccade decision making.

Disclosures: K. Acar: None. **M.A. Smith:** None.

Poster

397. Vision: Extrastriate Cortex

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Topic: D.07. Vision

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Harvard Mind, Brain, Behavior Initiative

Title: Object representations in rodent visual cortex

Authors: *J. Y. RHEE, C. E. ECHAVARRIA, D. D. COX
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Abstract: The brain's visual system translates ambiguous patterns of light falling on the retina into a coherent representation of the external world that can be used to guide behavior. The hierarchical organization of sensory cortex is thought to play a key role in this process. However, the nature of transformations that occur from one level of the hierarchy to the next remains poorly understood. An increasing amount of evidence shows that rats have the capacity for sophisticated visual behaviors. Moreover, both anatomical and functional studies have revealed signatures of a hierarchically organized rodent visual cortex. Building on these findings, we have undertaken a systematic investigation of visually-evoked responses in rat extrastriate cortex. Specifically, we test whether neuronal responses across distinct visual areas differ in their selectivity for object stimuli and tolerance to identity-preserving object transformations. We

conduct widefield imaging to characterize the retinotopic organization of rat visual cortex and identify distinct visual areas thought to be involved in object recognition behavior in rodents (V1, LM, LI, and LL). Using acute electrophysiological recordings, we find that selectivity and tolerance both increase from early visual cortex (i.e. V1) to higher-level, extrastriate areas, such as areas LL and LI. To better understand how these visual areas represent information at the population level, we use two-photon imaging to simultaneously record from large populations of neurons. These methods allow us to ask previously intractable questions about the transformation of visual information across cortical populations. We find that there are systematic differences in the representation of visual stimuli across areas of the rat visual cortex.

Disclosures: J.Y. Rhee: None. **C.E. Echavarria:** None. **D.D. Cox:** A. Employment/Salary (full or part-time):; MIT-IBM Watson AI Lab, IBM Research.

Poster

397. Vision: Extrastriate Cortex

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 397.16/HH10

Topic: D.07. Vision

Support: U01NS094330

Title: The functional organization of area MT neurons revealed by 2-photon microscopy in awake marmosets

Authors: *J. PATTADKAL, B. V. ZEMELMAN, N. PRIEBE
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Abstract: Area MT contains neurons that are exquisitely sensitive to visual motion and, based on extracellular recordings, is functionally organized for direction. Neurons within a cortical direction column share direction preferences, and preferences smoothly shift across cortex, much like the functional architecture for orientation selectivity in primary visual cortex of primates. The large-scale organization of area MT has not been accessible to optical imaging and electrode arrays, since in the macaque it is located near the bottom of the superior temporal sulcus. To examine the functional architecture of area MT and assay the selectivity of inhibitory neurons, we used the marmoset (*Callithrix jacchus*). These primates have lissencephalic brains in which we have access to activity of large neuronal populations. We used 2-photon microscopy to record from several hundred neurons at single-cell resolution over a 1 mm^2 region of area MT in awake marmosets. GCaMP expression was induced by injecting AAV constructs with promoters that provided specific expression in interneurons within area MT. The motion selectivity of the interneurons was assessed using a full-field patch of random dots moving in different directions. GCaMP signals from inhibitory neurons revealed similar degrees of motion selectivity as that

found from excitatory neurons (median DSI = 0.38, n = 301 cells). Nearby neurons tend to share direction preference, forming a map of direction preference with a period of approximately 300 microns. Finally, we found that the degree of orientation selectivity in MT neurons is weaker (median OSI=0.13) than direction selectivity. In sum, we have revealed the fine functional organization of area MT using 2-photon microscopy in awake marmosets and have demonstrated that MT inhibitory neurons are as direction selective as their excitatory neuron counterparts.

Disclosures: J. Pattadkal: None. **B.V. Zemelman:** None. **N. Priebe:** None.

Poster

397. Vision: Extrastriate Cortex

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 397.17/HH11

Topic: D.07. Vision

Support: Sir Henry Dale Fellowship jointly funded by the Wellcome Trust and The Royal Society (Grant Number 104285/B/14/Z)
Boehringer Ingelheim Fonds PhD Fellowship

Title: Intrinsic physiology and synaptic integration in genetically targeted thick-tufted layer 5 pyramidal neurons in mouse secondary visual cortex

Authors: *A. R. GALLONI, E. A. RANCZ
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Abstract: Thick-tufted layer 5 (ttL5) pyramidal neurons have broad dendritic arborizations that span all layers of the neocortex and receive disparate local and long-range inputs from both sensory and internal model-related brain regions. They output to many subcortical targets including the thalamus and brainstem, making them the principal output pathway for locally computed neocortical information, and therefore a good model system to study cortical computations. Here we describe the intrinsic and synaptic properties of a Cre-driver transgenic mouse line (Colgalt2), which selectively labels a sparse population of ttL5 neurons in medial secondary visual cortex (V2m).
We used whole-cell patch-clamp recordings in adult mouse brain slices to obtain a broad electrophysiological characterisation of intrinsic properties, including passive membrane properties, spike and firing properties as well as resonance frequency (4.9 ± 1.1 Hz, SD, n = 31). We find that Colgalt2-Cre neurons constitute a homogeneous population with intrinsic properties and morphology broadly consistent with previous descriptions of ttL5 neurons, with the exception that these neurons do not exhibit strong bursting responses associated with calcium electrogenesis in the apical dendrites, previously shown in ttL5 neurons in primary sensory cortical areas.

We next focused on the properties of two main input pathways, from primary visual cortex (V1) and retrosplenial cortex (RSC), by electrically evoking synaptic potentials. Paired-pulse ratios at 40Hz were similar (V1: 1.5 ± 0.54 SD, n = 14; RSC: 1.48 ± 0.32 SD, n = 14; p = 0.92). When stimulated together, V1 and RSC inputs were found to sum linearly (evoked sum 2.11 ± 0.70 mV, SD; arithmetic sum: 2.39 ± 0.61 mV, SD; n = 9, p = 0.38) and proved to be independent even at varying time-intervals of 5–75 ms. We are currently using spatially structured optogenetic stimulation to map these two different inputs onto the dendritic tree in order to establish the basis of their independence.
In conclusion, we present a genetically defined population of thick-tufted layer 5 pyramidal neurons showing linear summation of synaptic inputs and lacking some of the dendritic supralinearities generally associated with ttL5 neurons.

Disclosures: A.R. Galloni: None. **E.A. Rancz:** None.

Poster

397. Vision: Extrastriate Cortex

Location: SDCC Halls B-H

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Program #/Poster #: 397.18/HH12

Topic: D.07. Vision

Support: Sir Henry Dale Fellowship jointly funded by the Wellcome and The Royal Society (Grant Number 104285/B/14/Z)

Title: Whole brain input and output connectivity of layer 5 neuron subtypes in secondary visual cortex of the mouse

Authors: *Z. YE, E. A. RANCZ
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Abstract: The secondary visual cortex (V2) of mouse consists of multiple retinotopically organized areas and receives direct input from primary visual cortex (V1). Distinct areas of V2 receive differently tuned input from V1 and show preference for different visual features. Layer 5 neurons, with their dendritic arbour spanning most layers, are well placed to integrate disparate long-range and local cortical input streams. At the same time, they provide the majority of cortical output, broadcasting the end result of cortical computation. However, the input and output maps of V2 layer 5 neurons have not been systematically studied. By taking advantage of three layer 5 neuron specific transgenic mouse lines (Colgalt2-Cre, Tlx-Cre and Rbp4-Cre), we have mapped the inputs and outputs of different V2 layer 5 cell types.
We first identified the postero-medial area of V2 (PM) *in vivo* using intrinsic signal imaging to allow for functional targeting of subsequent viral injections. For output connectivity maps, we injected cre-dependent, GFP-encoding adeno-associated virus (AAV) in PM, which enabled us

to identify axonal projection targets. For input connectivity maps, we first injected 2 AAVs coding for the TVA receptor and rabies G protein in a Cre-dependent manner, followed by TVA-pseudotyped N2c rabies virus injection to label first order presynaptic partners. Brains were imaged on a block-face scanning 2-photon microscope, data were registered to the Allen Mouse Common Coordinate Framework and analysed using an automated pipeline.
The main projection targets of Colgalt2 neurons were in the midbrain (superior colliculus), thalamus (lateral posterior nucleus (LP), zona incerta), striatum and the pons. Tlx axons on the other hand remained mostly in cortex, with strong projections to lateral V2, retrosplenial cortex, striatum as well as contralateral medial V2. Rbp4 axons covered all areas typical for Colgalt2 and Tlx axons. Presynaptic input maps for all 3 cell types were qualitatively similar, with input from cortex (lateral V2, V1, retrosplenial cortex) and thalamus (dorso-lateral geniculate nucleus, LP, anteromedial nucleus of thalamus). We are currently focusing on detailed quantitative analysis of presynaptic input maps.

Disclosures: Z. Ye: None. **E.A. Rancz:** None.

Poster

398. Eye Movements: Central Mechanism in Animal Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 398.01/HH13

Topic: E.01. Eye Movements

Support: ZonMW TOP Grant 91215062

Title: Vestibular and optokinetic eye movement reflexes are both altered in nob mice

Authors: *B. H. WINKELMAN[1,2], M. KAMERMANS[1,4], C. I. DE ZEEUW[1,3]
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Abstract: In nob mice, a mutation in the X-chromosomal gene that encodes nyctalopin causes malfunction of the photoreceptor-to-bipolar cell synapse in the retina. This defect leads to congenital stationary night blindness (CSNB) and infantile nystagmus. Here we show that eye stabilization reflexes are severely affected in nob mice. The optokinetic reflex (OKR) responds only transiently to the on- and offset of horizontally moving sine-wave gratings (0.025-0.4 cycles/deg), and a clear gaze-holding defect is observed during presentation of stationary gratings. Remarkably, the vestibulo-ocular reflex (VOR), tested using vertical-axis sinusoidal oscillation (0.0625-4 Hz), deviates from the wildtype VOR in a velocity and frequency dependent manner. While the VOR is relatively normal at high peak velocities (up to 100 deg/s), the eye movement response at low peak velocities (down to 6.25 deg/s) shows a large shift in

phase angle that increases in lag with stimulus frequency and completely inverts the VOR at 0.5 Hz. This VOR behavior in nob mice can be modeled as a control system with two signal pathways: one linear and one nonlinear. The nonlinear pathway is of opposite sign as the linear component, saturates at about 10 deg/s and contains a band-pass filter (~0.6 Hz center frequency, ~2 Hz bandwith). Whereas the VOR is generally regarded to operate as a close-to-linear system, we show that marginal deviations from linearity observed in the wildtype VOR also conform to this model, albeit with a smaller gain of the nonlinear pathway (<0.4) compared to nob mice (>0.9). We hypothesize that the retinal motion detection defect in nob mice causes anomalous cerebellum-dependent VOR adaptation, resulting in an inverted VOR at low velocities.

Disclosures: B.H. Winkelman: None. **M. Kamermans:** None. **C.I. De Zeeuw:** None.

Poster

398. Eye Movements: Central Mechanism in Animal Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 398.02/HH14

Topic: E.04. Voluntary Movements

Support: Medical Research Council, MC_UP_1201/2
European Research Council, ERC Starting Grant STG 677029

Title: Genetics reveal the modular organization of movement vectors in the mouse superior colliculus

Authors: *L. MASULLO, L. MARIOTTI, N. ALEXANDRE, M. TRIPODI
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Abstract: We are surrounded by objects we are able to grasp and manipulate; in order to successfully interact with the environment, animals need to produce accurate movements towards specific positions in space. A crucial region of the brain that guides similar goal-oriented movements is the superior colliculus (SC), a multi-layered midbrain structure formed by a sensory and a motor domain. While several lines of research in different model organisms have confirmed that the SC contributes to the initiation of orienting movements, how functionally distinct neuronal groups within the SC are organized to support the production of such motor outputs is poorly understood.
One of the reasons why the intrinsic circuit organization of the SC remains elusive is the lack of genetic characterization of the neuronal populations of its motor domain. Here, we performed RNA sequencing to screen for genetic markers for neuronal subpopulations in the motor SC. We identified a genetically defined subpopulation of projecting glutamatergic neurons in the motor domain of the SC. Strikingly, this population of neurons displays a modular distribution within this domain, being organised in clusters along the mediolateral and anteroposterior axis.

We then asked whether these modules may act as functional units, each encoding a specific feature of head movement, the main ethologically relevant orienting behaviour in rodents. Optogenetic activation of this modular population in freely moving animals produced a stereotyped, robust head motion characterised by a pronounced quantal nature; furthermore, the amplitude of the elicited head movement varied based on the modular unit activated. Our results suggest that distinct clusters of genetically defined neurons produce head displacement along a characteristic vector.
In conclusion, we found that a population of premotor neurons in the SC is organised in a modular conformation and we suggest that such modularity may represent a physical implementation of a discontinuous motor map for orienting movements encoded in the mouse SC. Our study complements previous observations of periodicity in SC circuitry, as well as its afferent and efferent systems. Exploiting the genetic toolkit available in the mouse, our work begins to address the functional relevance of this modularity and paves the way for future experiments to investigate principles of sensorimotor integration in SC circuits.

Disclosures: L. Masullo: None. **L. Mariotti:** None. **N. Alexandre:** None. **M. Tripodi:** None.

Poster

398. Eye Movements: Central Mechanism in Animal Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 398.03/HH15

Topic: E.01. Eye Movements

Support: NIH Grant NS092623
NIH Grant EY027373

Title: Properties of the signals that drive directional learning in smooth pursuit eye movements

Authors: *D. J. HERZFELD[1], M. TRINGIDES[2], D. SUBRAMANIAN[3], S. G. LISBERGER[1]
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Abstract: To shed light on the neural signals that control directional learning in smooth pursuit eye movements, we conducted behavioral experiments that varied the instructive signals for learning and determined how learning generalizes to different contexts. Two monkeys tracked a smoothly moving target. During learning blocks of 100 trials, the target moved in an initial "pursuit" direction for 250ms and then added a component of motion in the orthogonal "learning" direction. We assayed learning in the final 25 trials of each block by measuring eye velocity in the learning direction just before the instructive change in target direction.
We varied the duration of target motion in the learning direction from 50 to 400ms, and found that learning scaled with duration when the target was continuously visible. If, however, the target was extinguished after the selected duration of the instructive stimulus and reappeared

later at the position it would have attained if it had continued to move for a full 400ms, then the magnitude of learning was considerably larger for each instruction duration and quickly saturated for durations longer than 100ms. We conclude that longer duration instructions cause image motion that engages competing learning mechanisms in the opposite direction, and that 100ms of motion is sufficient to instruct maximal learning.
We also varied the speed of target motion in the pursuit direction. Testing the expression of learning with multiple pursuit direction target speeds revealed that learning generalized linearly to speeds slower than the trained speed, but saturated for pursuit speeds faster than the trained speed. Given that learning must operate on signals that are related to initial pursuit, this pattern of generalization suggests that more inputs are recruited as pursuit target speed increases. We tested this hypothesis by running learning trials with a fast target speed, and then washing out the learning using a slower target speed. After learning had been extinguished, we switched to the fast target speed used during learning, revealing re-expression of the previously learned memory. We take this as evidence for inputs to the site of learning that respond during the fast target speed, but not the slow target speed.
Our findings on the effects of instruction duration are compatible with the proposed primacy of climbing fiber inputs in driving pursuit learning. We suggest that learning is a process based on multiple sites and/or mechanisms that, under some circumstances, compete. Our findings on generalization to target speed suggest that parallel fibers encoding target (or eye) speed are recruited in larger numbers as eye speed increases.

Disclosures: D.J. Herzfeld: None. **M. Tringides:** None. **D. Subramanian:** None. **S.G. Lisberger:** None.

Poster

398. Eye Movements: Central Mechanism in Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 398.04/HH16

Topic: E.01. Eye Movements

Support: NIH Grant RO1-EY027373
NIH Grant F30-EY027684

Title: Sensorimotor gain control as a mechanism of movement prevention during modulation of preparatory activity

Authors: *T. DARLINGTON, S. G. LISBERGER
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Abstract: In cells with firing seemingly tied to movement, an important question is how preparatory activity can evolve without actually producing movement. The smooth eye

movement region of the frontal eye fields (FEF_{SEM}) is a critical node in the neural circuit controlling smooth pursuit eye movement. Preparatory activity evolves in FEF_{SEM} during fixation. Under conditions where the animal can anticipate the ultimate target motion, FEFSEM preparatory activity signals expectation about the impending visual motion and eye movement. We recorded 164 single units in the FEF_{SEM} of two awake behaving rhesus monkeys while they smoothly tracked moving targets during two different blocks of trials: (1) a random 8-direction block to fit a linear model between the population response of our FEF_{SEM} cells during pursuit and eye velocity and (2) a repeated-direction block to provide predictable target motion and study preparatory activity. Recent work in the arm movement system suggests that population activity in M1 and PMd during movement preparation combines linearly in a way that cancels out, or remains confined to an "output-null" space. In contrast, we find that population activity in FEF_{SEM} evolves during fixation in a way that should cause movement. Using linear discriminant analysis, we also find that preparatory and pursuit-related activity progress along similar, "output-potent" dimensions. We understand the progression of preparatory activity along output-potent dimensions in terms of the previous finding that the output of FEF_{SEM} controls the strength, or "gain", of visual motion access to the oculomotor machinery. To test this, we presented brief 50 ms pulses of visual motion at random times during fixation. We find that eye speed responses are larger when the pulses are delivered later during fixation, at times when FEF_{SEM} preparatory activity has progressed further along output-potent dimensions. We conclude that the output from FEF_{SEM} operates downstream to enhance visual-motor gain in anticipation of behaviorally-relevant visual motion. These findings propose a novel mechanism by which preparatory activity in sensorimotor cortex can evolve without producing inappropriate movements: preparatory signals may act as a gain controller in anticipation of imminent command for movement.

Disclosures: T. Darlington: None. **S.G. Lisberger:** None.

Poster

398. Eye Movements: Central Mechanism in Animal Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 398.05/HH17

Topic: E.01. Eye Movements

Support: NIH Grant EY027373

Title: The relative contributions of area MT and the frontal eye fields to the latency of smooth pursuit

Authors: *J. MAYO, S. G. LISBERGER
Dept. of Neurobio., Duke Univ., Durham, NC

Abstract: Correlated features of neuronal activity constrain how the brain uses sensory information to guide behavior. While the majority of research on correlations so far has focused on the size of neuronal responses in terms of firing rate (e.g., spike count correlations), here we focus on correlations between the latency of responses between cortical neurons and their relation to the initiation of smooth pursuit eye movements. Previous work demonstrated that as much as 30-40 percent of the trial-by-trial variation in the timing of pursuit initiation could be accounted for by correlated variation in the timing of neuronal responses in each of (1) extrastriate visual area MT and (2) the smooth pursuit eye movement region of the frontal eye fields (FEFsem). Our goal here was to determine whether these cortical areas contribute independently or synergistically to pursuit latency. We recorded simultaneously in areas MT and FEFsem in rhesus monkeys using 24-channel Plexon V-probes. Monkeys were trained to fixate in the center of a video display and then pursue a patch of dots at various speeds and directions. Trials with saccades during stimulus motion onset or pursuit initiation were excluded, and we collected approximately 100 trials per stimulus condition. We used a quantitative procedure that effectively shifted and scaled each trial's eye speed trace and neuronal response to obtain precise latency and amplitude estimates (Lee et al., Neuron, 2016). Validating previous independent work in our laboratory, we found that trial-by-trial changes in the response latencies of MT and FEFsem neurons were positively correlated with the latency of pursuit initiation. Using simultaneously recorded MT-FEFsem neuron pairs, we assessed whether fluctuations in response latencies across trials covaried between areas. The response latencies of pairs of MT-FEFsem neurons were not correlated, despite the fact that (1) latencies were correlated for pairs of neurons within each area and (2) each area's latencies were significantly correlated with the latency of behavior. These results suggest that MT and FEFsem contribute independently to the timing of pursuit initiation.

Disclosures: J. Mayo: None. **S.G. Lisberger:** None.

Poster

398. Eye Movements: Central Mechanism in Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 398.06/II1

Topic: E.01. Eye Movements

Support: NIH Grant EY022854
NIH Grant EY024831

Title: Characteristics of saccades to moving targets

Authors: *K. J. MOHSENIAN[1,2], N. J. GANDHI[1,2]
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Abstract: The world is a dynamic environment filled with non-stationary sensory information that can be crucial to survival. Animals extract relevant information from their surroundings by aligning their gaze on both stationary and moving objects. Saccades, rapid eye movements, have been used to study sensory, motor, and cognitive processes in primates. The metrics and kinematics of saccades to stationary targets have been well characterized through countless studies. However, there have been relatively few in-depth studies that describe the kinematics of saccades to moving targets, called interceptive saccades, across a broad range of target speeds and directions. The goal of this study is to provide an understanding for how kinematics of saccades to stationary and moving targets vary as a function of target speed and trajectory. We recorded saccade kinematics from four rhesus monkeys who performed a delayed saccade task in which the delay duration, starting target location, moving target direction (inward and outward), and target speed (range: 15 to 45 deg/s) were varied randomly to elicit saccades with a broad spectrum of amplitudes and directions. Trials using stationary targets placed along the moving target paths were randomly interleaved with trials using moving targets. Eye position was recorded using magnetic search coils or an eye-tracker system. Preliminary analysis suggests that main sequence properties for monkeys may be more idiosyncratic than previously observed. Of the four subjects, two showed no difference in their main sequence properties between saccades to stationary and moving targets, as the peak velocity and duration of amplitude-matched saccades were similar. This effect was maintained across different moving target parameters (speed and direction). The other two subjects showed distinct differences: peak velocity was attenuated, and duration was longer for amplitude-matched saccades to moving targets. For all subjects, saccade latency for interceptive saccades was similar to saccades to stationary targets. Saccadic error, the error of the animal's gaze relative to the target location at saccade end, increased as a function of saccade eccentricity and target speed, and in general, was greater for trials with a moving target. Since all four subjects performed the same task, the range in kinematics for interceptive saccades most likely reflects subject-to-subject variability. These results therefore reveal a potential diversity in strategy used by different subjects to process a moving stimulus and direct the line of sight toward it.

Disclosures: K.J. Mohsenian: None. **N.J. Gandhi:** None.

Poster

398. Eye Movements: Central Mechanism in Animal Models

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 398.07/II2

Topic: E.01. Eye Movements

Support: NIH Grant EY014263

Title: A new tectal premotor input for the control of lens accommodation

Authors: *P. J. MAY[1], S. WARREN[1], I. BILLIG[2], J. J. QUINET[3], P. D. R. GAMLIN[3]
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Abstract: In order for the visual system to compensate for target distance and maintain stereopsis, three different motor actions are employed in the "near response": the activities of the medial and lateral recti muscles are modulated to produce vergence movements and so direct the eyes at the target, contraction of the ciliary muscle produces lens accommodation to focus the image on the retina, and sphincter pupillae muscle tone is increased to decrease pupil size and increase depth of field. We recently identified two populations of premotor neurons that control lens accommodation: one in the supraoculomotor area (SOA) and the other in the central mesencephalic reticular formation (cMRF). These were labeled using the retrograde trans-synaptic transport of the N2c strain of rabies virus from injections into the ciliary muscle of macaque monkeys. Here we describe a third population of midbrain neurons located in the midbrain tectum of the same animals. These neurons were labeled at survival times that allowed the virus to cross two synapses, the same time course as the SOA and cMRF groups, indicating that they are also premotor neurons supplying the preganglionic motoneurons in the Edinger-Westphal nucleus (EWpg). The labeled neurons were arranged bilaterally, in a rostrocaudally oriented column within the midbrain tectum. The rostralmost cells were located in the medial pretectal nucleus, while cells at more caudal levels were found just off the midline, medial to the intermediate gray layer (SGI) of the superior colliculus. This caudal region has been previously designated the tectal longitudinal column (TLC). The labeled neurons were relatively small, with a modest number of poorly branched dendrites. Labeled neurons were not found in the superior colliculus at these time points. Since this premotor population is a novel one, we also examined this projection with conventional tracers. Injections of wheat germ agglutinin conjugated horseradish peroxidase (WGA-HRP) into the SOA, which included EWpg, retrogradely labeled neurons in the same region, as well as within the SGI of the superior colliculus. Injection of BDA into the midline tectum, produced anterogradely labeled terminals that lay over EWpg motoneurons on both sides. The function of this tectal premotor population is unknown, but its presence may help explain changes in lens accommodation that have been reported in cats following electrical stimulation of the superior colliculus and pretectum.

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Poster

398. Eye Movements: Central Mechanism in Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 398.08/II3

Topic: E.01. Eye Movements

Support: NIH Grant EY014263

Title: Projection of the superior colliculus to the supraoculomotor area in macaque monkeys

Authors: *S. WARREN[1], P. MAY[1], A. K. HORN-BOCHTLER[2], S. UPADHYAYA[3], V. E. DAS[3]
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Abstract: In 1978, Edwards and Henkel reported that the rostral superior colliculus of the cat projects to the supraoculomotor area (SOA). They hypothesized that during saccades, this projection might allow the superior colliculus to directly control the activity of vertical gaze motoneurons that extend dendrites from the oculomotor nucleus (III) into the overlying SOA. However, the SOA contains a number of other cell populations that might be targeted, including: preganglionic motoneurons of the Edinger-Westphal nucleus (EWpg), peptidergic, centrally projecting Edinger-Westphal neurons (EWcp), motoneurons of the C- and S-groups that supply multiply innervated fibers (MIFs) within the extraocular muscles, and midbrain near response neurons that control vergence. We have investigated the projections of the superior colliculus onto the SOA in macaque monkeys in order to provide greater insight into the possible targets of this pathway. Anterograde tracers injected into the superior colliculus label terminals within SOA, whether they are placed in the rostral or caudal superior colliculus. The terminal field extends bilaterally within SOA, with an ipsilateral predominance, and includes EWpg and the region between the two sides of III, where S-group motoneurons are found. Retrograde tracer injections that included SOA produced labeled neurons in the intermediate gray layer (SGI) of the superior colliculus. When superior colliculus injections are combined with injections of the ipsilateral medial rectus and superior rectus muscles, no evidence of anterogradely labeled terminals contacting retrogradely labeled motoneurons within III was observed. Furthermore, only a few close associations were observed with retrogradely labeled medial and superior rectus MIF motoneurons found in the C- and S-groups, respectively. This suggests motoneurons are not a primary target of the SOA projection. In addition, data from trans-synaptic transport of the N2c strain of the rabies virus injected into the ciliary muscle suggests that the SC does not project directly to EWpg motoneurons. These negative findings suggest that the most likely target for this tectal projection may be the midbrain near response neurons. Indeed, the terminal field in the SOA is denser, caudally, where this premotor population is more numerous. Since these SOA cells do not modify their activity during conjugate saccades, the presence of such a projection would suggest that the superior colliculus may enhance the activity of near response neurons during disjunctive saccades or that the input is supplied by rostral superior colliculus neurons that encode near response signals, not saccades.

Disclosures: S. Warren: None. **P. May:** None. **A.K. Horn-Bochtler:** None. **S. Upadhyaya:** None. **V.E. Das:** None.

Poster

398. Eye Movements: Central Mechanism in Animal Models

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 398.09/II4

Topic: E.01. Eye Movements

Support: DFG Grant EXC307

Title: Saccadic directional precision across the horizontal and vertical meridians

Authors: *T. MALEVICH[1,2], Z. M. HAFED[1,2]
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Abstract: Saccades rapidly re-align gaze with objects of interest. Even though saccades are spatially precise, they do exhibit significant trial-to-trial variability in eye movement trajectories and landing positions. This may suggest that the oculomotor system is simply "sloppy", contradicting the idea that saccades precisely re-align gaze. Here we hypothesized that saccadic variability is related to variability in starting eye position due to fixational eye movements. Using scleral search coils, we measured eye movements precisely in 3 rhesus macaques performing horizontal or vertical 5 or 10 deg visually guided saccades. We observed significant saccadic curvature in all conditions even though the targets were placed along cardinal directions. We then binned saccades of a given size (e.g. 10 deg rightward saccades) according to initial fixational eye position at saccade onset. We found that saccade direction/curvature precisely corrected for deviations in fixational eye position that were present at the time of triggering the saccade. For example, rightward saccades with fixational vertical eye position at saccade onset in the lowest quartile of eye positions curved upwards, whereas rightward saccades with fixational vertical eye position in the highest quartile curved downwards. This suggests high precision of individual saccades, which we further demonstrated by presenting targets in the cardinal directions but having +/- 1.7, 3.5, or 5.3 min arc deviations in the orthogonal direction (e.g. a target at 10 deg rightward and 1.7 min arc upward from the fixation position). This resulted in "true" saccade directional deviations from the cardinal axes of only +/- 0.3, 0.7, and 1 deg for 5-deg saccades and +/- 0.15, 0.3, and 0.5 deg for 10-deg saccades. Saccade curvature in all cases was adjusted accordingly such that the angular deviations of the target were corrected for by saccade end. We then ran 5 human subjects on a similar task but asked them to report whether the target (disappearing at saccade onset) deviated upward (for horizontal saccades) or rightward (for vertical saccades) from the imagined meridian axis locations. Perceptional precision of the small angular deviations in target vector was significantly worse than for saccades, and it also exhibited strong biases; perceptual estimates were particularly poor for downward locations. Our results indicate that saccades are much more precise than assumed, and that fixational eye

movements render “purely” vertical and horizontal saccades rare events. This alleviates challenges of programming such “pure” saccades from neural tissue in which the visual fields are anatomically or functionally dissociated.

Disclosures: T. Malevich: None. **Z.M. Hafed:** None.

Poster

398. Eye Movements: Central Mechanism in Animal Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 398.10/II5

Topic: E.01. Eye Movements

Support: CIHR
NSERC

Title: Testing egocentric vs allocentric models in the frontal eye field (FEF) during a cue-conflict task in head-unrestrained monkeys

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Abstract: The brain could encode information about an external object in an egocentric or an allocentric fashion. By employing a classical memory-guided saccade task in head-unrestrained monkeys, we have already tested different egocentric models and have shown that the visual burst of the FEF neurons encodes for the target relative to eye (Te) and the motor burst best describes the gaze relative to eye (Ge) (Sajad et al. 2015; Sajad et al. 2016). Here, by using a cue-conflict paradigm (wherein the monkeys were trained to saccade toward a remembered target in relation to a shifting allocentric cue in different directions), we have recently examined the relative contributions of egocentric vs allocentric cues on goal-directed behavior in the saccadic system (Li et al. 2017) and found out that there was a 25% displacement of the gaze end-points toward the virtually displaced target, suggesting that gaze representation is weighted between the egocentric and allocentric cues. Here, we sought to examine the neural underpinnings of this weighing between the egocentric and allocentric frames in a cue-conflict task in head-unrestrained monkeys. Using single neuron electrophysiology in the FEF while the monkeys performed the cue-conflict task, we tested the best fitting of the visual and motor responses in different egocentric and allocentric reference frames, such as Te’ (virtually shifted target in eye-coordinates). It was revealed that the visual neurons (n=12) still encode Te and motor neurons (n=17) best encode the Ge, that is, so far the data are fitting and describing the canonical egocentric models better than the allocentric models. This suggests that the allocentric influence is processed at a different stage in the visual system. However, further analysis needs

to be performed with intermediate codes, especially the continuum between the Te and Te', and to see if it shifts partially like behavior.

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Poster

398. Eye Movements: Central Mechanism in Animal Models

Location: SDCC Halls B-H

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Topic: E.01. Eye Movements

Support: Brain/MINDS from MEXT and AMED
MEXT kAKENHI Grant Number 18K07359
the Center of Innovation Program from JST

Title: Microsaccades in blindsight monkeys

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Abstract: Patients with damage to primary visual cortex (V1) demonstrate residual performance on laboratory visual tasks despite denial of conscious seeing (blindsight). Macaque monkeys with a unilateral V1 lesion have been used as an animal model for blindsight. Previously, we have demonstrated that blindsight monkeys retain some cognitive and attentional capacities including visual spatial memory (Takaura et al 2011) and endogenous attention (Yoshida et. al. 2017). Here we examined whether a unilateral V1 lesion affects microsaccades, which are small, unconscious, fixational eye movements. Our first question was whether microsaccades during fixation are affected. After a surgical lesion in the left side of V1 in two monkeys, the number and/or amplitude of leftward microsaccades (towards the normal visual field) were larger than those of rightward microsaccades (towards the affected visual field). Analysis of horizontal eye positions at the onset of microsaccades (Tian et. al. 2018) revealed that the average horizontal eye position of blindsight monkeys was shifted towards the affected (right) visual field, suggesting that the increased number and/or amplitude of leftward microsaccades compensated for a lesion-induced rightward eye-position shift. We then asked whether microsaccades during peripheral and central cueing were affected. Before the V1 lesion, in a memory-guided saccade task (Takaura et al 2011), peripheral cue onsets transiently decreased the number of microsaccades. A rebound in frequency followed, and most rebound movements were directed away from the cues, consistent with our previous finding in monkeys and humans (Tian et. al.

2016). After the V1 lesion, the overall patterns of inhibition and rebound were not affected. However, the number of cue-directed microsaccades occurring <120 ms after cue onset ("express microsaccades", Tian et. al. 2018) increased for leftward microsaccades and was almost completely abolished for rightward microsaccades. In a Posner task with informative central cues (Yoshida et. al. 2016), the number of express microsaccades was also affected in a similar manner to the peripheral cues. These results suggest that: 1) V1 lesions affect the balance of leftward and rightward microsaccades, which is consistent with the push-pull equilibrium model of microsaccade dynamics (Hafed 2011); and 2) V1 is not necessary for transient inhibition of microsaccades after peripheral cues. These results help constrain models of microsaccade dynamics after peripheral and central cueing, and they highlight how V1 can contribute to such dynamics, in addition to visual-oculomotor areas such as FEF and the superior colliculus.

Disclosures: M. Yoshida: None. **Z.M. Hafed:** None.

Poster

398. Eye Movements: Central Mechanism in Animal Models

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Topic: E.01. Eye Movements

Support: NIH R01-EY014885

Title: Saccade target selection in superior collicular neurons during a categorical search task with naturalistic images

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Abstract: The target of visual search is not always defined with exacting specificity. Rather, orienting behaviors often direct attention using more broad constraints. Categorical search tasks, for example, have demonstrated search guidance via multiple features common to the target category (categorical target templates; Yang & Zelinsky 2002). Here we present the physiological responses from visual, visuo-movement, & movement (V, VM & M respectively) superior collicular (SC) neurons of non-human primates (NHPs) as they perform a visual categorical search task that utilizes images of naturalistic objects from various object categories. After an initial fixation period, NHPs were allowed to free-view an image array of 'real-world' objects (animals, kitchen utensils, cars, etc.). On target present (TP) trials, reward was contingent on fixating an image from one of two categorical image families (Teddy Bears or Butterflies) while target absence (TA) was indicated by fixating a specified location in the far periphery. We examined the visual response to stimulus onset as well as the first search saccade of each trial.

For the majority of cells sampled, when saccades were made into the response field (RF), responses were larger (for both targets and distractors) than when saccades were made elsewhere. This was pronounced in M as well as VM neurons with a negative visuo-movement (predominantly motor) index. It was observed that a number of V & VM neurons had a biphasic peak in visual activity. The first burst rarely displayed a significant difference in activity for saccades made to targets in the RF compared to distractors in the RF. However, often the differences in the second peak of activity carried significance. This is in keeping with results for search tasks using relatively simpler single feature search (Keller & McPeek 2002). Overall, a majority of cells had a stronger response for in RF saccades made to a target compared to that of in RF distractors. Furthermore, a subset of these target discriminating cells had significantly higher responses for saccades made to distractors in the response field on TP trials verses TA trials. We interpret this as reflecting a perceived categorical target template probability. This is supported by the finding that first saccade distractor fixations for TP trials had a higher likelihood of sharing feature similarity with the target category than the distractors fixated for TA trials. Taken together, these findings support a role for the SC in serving as a final gateway of a categorical target template selection process.

Disclosures: B.M. Cooper: None. **R.M. McPeek:** None.

Poster

398. Eye Movements: Central Mechanism in Animal Models

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Topic: E.01. Eye Movements

Support: DFG FOR1847

Title: Engaging spatial visual working memory expands superior colliculus neurons' response fields

Authors: *K. F. WILLEKE[1,2], A. BUONOCORE[1,2], X. TIAN[1,2], Z. M. HAFED[1,2]
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Abstract: Superior colliculus (SC) neurons are known to produce movement-related bursts before, during, and after the execution of high-speed, or saccadic, eye movements. Because these neurons show selectivity for saccade direction and amplitude, they are often described in terms of their response field (RF) location and size. However, saccade-related SC RF's have been typically mapped using visually-guided saccades. Here we show that the shape and size of SC RF's can be significantly altered by the demands that spatial visual working memory imposes on the oculomotor system. We trained two macaque monkeys to perform a memory-guided saccade

task. In this task, an eccentric flash was presented briefly (~50 ms). The monkeys maintained flash location in spatial visual working memory for ~300-1100 ms, after which the fixation spot disappeared, providing a "go" command to generate a saccade to the remembered target location. We then collected data from trials of random target eccentricities (0.1-16 deg) and directions (0-360 deg). The monkeys also performed a delayed-saccade task, in which the target flash was replaced with persistent stimulus presentation until trial end. This allowed for a direct comparison between saccades being triggered towards a memory-location versus saccades that were visually-guided. We recorded from 252 SC neurons that showed significant saccade-related activity in both tasks, and we characterized the size and location of their RF's. We found that SC RF's significantly expanded in size for memory-guided saccades when compared to visually-guided movements, such that saccades with identical amplitudes were associated with significant saccade-related discharge for memory-guided movements but not for visually-guided ones. Crucially, the RF expansion was not spatially uniform, but it exhibited a larger shift towards more central eccentricities. Thus, there was an "inward" RF expansion towards the fovea during memory-guided saccades. Despite this expansion, RF hotspot locations were not systematically altered. We conclude that SC RF's are highly malleable by task demands. These results offer a possible mechanism by which spatial visual working memory acts on the saccadic system, and thus could provide insights both into the nature of spatial visual working memory and also the SC's role in it. Our results also suggest that foveal magnification in SC topography might mediate inward RF expansions during working memory engagement, and therefore also mediate potential systematic errors that would be present for memory-guided saccades when compared to visually-guided ones.

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Poster

398. Eye Movements: Central Mechanism in Animal Models

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Topic: E.01. Eye Movements

Support: Hartwell Foundation Postdoctoral Fellowship (235938)

Title: Immunohistochemistry is required to visualize virally-mediated fluorescent protein transgene expression in the neuronal visuomotor circuitry of non-human primates

Authors: *M. O. BOHLEN[1], H. G. EL-NAHAL[2], M. A. SOMMER[2]
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Abstract: Optogenetics has been transformative for neural circuit analysis in rodents. To use optogenetics, primate researchers must resort to viral delivery of genes encoding opsin proteins that usually include a fluorophore conjugate. In ongoing work, we are characterizing a range of viral vectors to identify promising candidates for efficient transduction and patterns of intracellular labeling of targeted neuronal populations. This work will lead to new insights into the anatomical connectivity and physiology of the visuo-motor systems of macaque monkeys. To this end, we have targeted several gaze-related areas including the frontal eye field, V1, mediodorsal thalamus, superior colliculus, and brainstem motor nuclei. We have identified several AAV vectors which exhibit strong labeling of neurons either locally, anterogradely or retrogradely. Concurrently, we have tested the capacity for vectors to retrogradely label motoneurons following intramuscular injections in craniofacial musculature. To characterize a vector, we routinely use fluorescent microscopy and immunohistochemistry (IHC) to assess the efficacy of candidate viruses at delivering fluorescent protein genes. Different vectors contained different fluorescent proteins including GFP, eGFP, mCitrine, YFP, eYFP, RFP, tdTomato, and mCherry. In each monkey, adjacent sections were compared for the presence of fluorophores using (1) fluorescent microscopy and (2) immunohistochemical reaction using a primary antibody to the fluorophore and bright-field microscopy. To our surprise, there were dramatic differences in the degree of neuronal labeling observed using fluorescence compared to IHC visualization using bright-field microscopy across all monkeys. In some instances, fluorescent microscopy revealed no discernable labeling while IHC revealed dense neuronal labeling. In other instances, labeling could be seen under the fluorescent scope, but more neuronal details could be visualized and labeled neuronal structures could be observed using IHC. Our results suggest caution in assessing virally-mediated transduction solely using fluorescence microscopy, as this metric may substantially underestimate the true degree of neuronal labeling. Our results also suggest that IHC processing is necessary for detailed visualization of neuronal labeling in virally mediated experimental work, particularly anatomical and physiological optogenetic experiments.

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Poster

398. Eye Movements: Central Mechanism in Animal Models

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Topic: E.01. Eye Movements

Support: CIHR Grant FRN148365
Alzheimer's Society London and Middlesex scholarship
Natural Sciences and Engineering Research Council scholarship

Title: Muscarinic blockade modulates oscillatory activity in primate prefrontal cortex during rule-guided behaviour

Authors: *A. J. MAJOR, S. VIJAYRAGHAVAN, L. MA, S. EVERLING
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Abstract: Acetylcholine is heavily involved in executive processes such as attention and working memory and is important for optimal cognitive behaviour in primate prefrontal cortex (PFC; Croxson et al., *Nat Neurosci*, 2011). The disrupted cholinergic system of individuals with schizophrenia and Alzheimer's disease is thought to contribute to cognitive deficits such as impaired working memory. Although the effects of local cholinergic receptor modulation on neuronal discharge rate and tasked-related activity have been explored in nonhuman primate PFC (Major et al., *J Neurosci*, 2018), the effects of cholinergic compounds on oscillatory activity have been less thoroughly examined (Thiele et al., *Soc Neurosci Abstract #389.01*, 2016). Here, we have iontophoretically applied muscarinic receptor antagonist scopolamine to rhesus dorsolateral PFC during performance of pro- and antisaccades. In many recordings, we observed increased oscillatory power in the theta and alpha frequency bands. This was interesting considering scopolamine application unequivocally resulted in decreased neuronal discharge rate in PFC neurons (Major et al., *J Neurosci*, 2015). Others have reported cholinergic stimulation can enhance oscillatory power (Pafundo et al., *J Physiol*, 2013). These preliminary results suggest prefrontal muscarinic receptors are contributors to oscillatory activity during cognitive behaviour, and may contribute to the altered prefrontal oscillatory responses in conditions with altered muscarinic receptor expression, such as in Alzheimer's disease and schizophrenia (Kurimoto et al., *Neuroimage*, 2012; Senkowski and Gallinat, *Biol Psychiatry*, 2015).

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Poster

398. Eye Movements: Central Mechanism in Animal Models

Location: SDCC Halls B-H

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Topic: E.01. Eye Movements

Support: IISC Institute PhD fellowship

Title: Central and peripheral correlates of eye movement planning

Authors: S. RUNGTA[1], *A. N. MURTHY[2]
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Abstract: Accumulator models in which a signal rises to a fixed threshold have been used successfully to understand initiation of saccadic eye movements. In this study we examined

whether certain aspects of a central plan could be assessed by looking at the recruitment pattern of motor units from peripheral neck muscles using the accumulator framework. Towards this purpose, we recorded from frontal eye fields (FEF) and neck muscles in macaque monkeys while they were involved in a memory guided saccade task. We observed modulations in the neck muscles that paralleled the activity in FEF during the visual, delay and movement epochs. Not surprisingly, there were very few motor units which showed stimulus evoked response. Interestingly, the activity during the delay period in periphery could not be used to infer the direction of an upcoming movement. However, it was weakly correlated with the time it took to initiate saccades. For few sessions, we observed that the units that showed modulation during the delay period had smaller amplitude and higher spontaneous activity compared to the units which showed modulation only during the movement epoch. Similar accumulator models could explain the changes seen in FEF and neck muscles during planning. Our results suggest that the spatial information about the upcoming saccade gets gated out but temporal information leaks through possibly via smaller motor units into the periphery during planning. Also, accumulator models could be used to assess motor plans from periphery in more than one way.

Disclosures: S. Rungta: None. **A.N. Murthy:** None.

Poster

398. Eye Movements: Central Mechanism in Animal Models

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Topic: E.01. Eye Movements

Support: KAKENHI
Takeda Science Foundation

Title: Contextual changes in cortico-striatal transmission during time production in monkeys

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Abstract: Cortico-basal ganglia loop is involved in temporal information processing. Recent studies have revealed the scalable nature of timing-related neuronal activity, which exhibits expansion or shrinkage of entire temporal profile in proportion to the length of measured interval. However, the underlying mechanism for the generation of such neuronal activity remains largely unknown. To explore this, we recorded local field potentials (LFPs) at sites in the caudate nucleus where the timing-related neuronal activity was previously found. Three monkeys performed the oculomotor version of time production task, in which they generated a memory-guided saccade after the prescribed interval (400, 1000, or 2200 ms, instructed by color of the fixation point) following the visual cue (100 ms). We found that the magnitude of

visually-evoked potentials for the cue altered depending on the conditions, showing larger response for shorter interval (one-way ANOVA, $p < 10^{-3}$). When we assessed the spectral power, the low frequency components (< 25 Hz) clearly exhibited time-dependent modifications during the monitoring of elapsed time, but the power at higher frequency (30–40 Hz) did not. Furthermore, the power of low-frequency components before the cue presentation differed across interval conditions, showing greater spectral power for longer intervals ($p < 10^{-3}$). These results suggest that the network-level alteration in the state of the cortico-basal ganglia pathways before and during temporal monitoring might be important for the flexible adjustment of neuronal activity. Specifically, the power of low frequency components might be relevant to the gain of cortico-striatal transmission. To test this hypothesis, we applied single pulse electrical stimulation to the supplementary eye field before the cue onset and examined the short-latency response recorded from the caudate nucleus. We found that the magnitude of evoked response was indeed modulated depending on the interval conditions (one-way ANOVA, $p < 0.05$). Thus, the network state and the gain of cortico-striatal transmission appear to be adjusted before the monitoring of elapsed time, which might lead to the scalable nature of neuronal activity within the cortico-basal ganglia pathways for proper behavioral timing.

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Poster

398. Eye Movements: Central Mechanism in Animal Models

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Topic: E.04. Voluntary Movements

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Center for Nanoscale Microscopy & Molecular Physiology of the Brain (CNMPB)

Title: Contribution of the thalamic pulvinar to saccade and reach behavior in humans and monkeys

Authors: *M. WILKE[1], C. SCHMIDT-SAMOA[3], P. DECHENT[3], K. MILOSERDOV[2], S. MAHDAVI[2], J. LIMAN[4], M. HOLZGRAEFE[4], A. U. DOMINGUEZ-VARGAS[5], K. WILLIAMS[3], I. KAGAN[5], M. BAEHR[4]
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Abstract: Expansion of the pulvinar in primates and its anatomical connectivity to fronto-parietal cortices suggests its involvement in higher-order cognitive and visuomotor functions. While recent studies in non-human primates suggest a contribution of dorsal pulvinar portions to visuomotor functions, its causal involvement to behavior and cortical activity in humans remains poorly understood. Here we studied eight patients with pulvinar lesions (4 left, 2 right and 2 bilateral) with saccade and reach tasks involving different levels of spatial choices. Patient data in each task was compared to 9-20 healthy subjects. In the patients with a pulvinar lesion, saccade amplitudes to the contralesional visual field were significantly smaller than in controls and saccade latencies towards the contralesional hemifield were prolonged in free choice, but not in instructed, trials. Patients with a right, but not with left or bilateral pulvinar lesions exhibited an ipsilesional bias in the saccade free choice condition. Reach errors were most pronounced for the contralesional hand and hemifield, and when the target position was dissociated from the gaze position, thus resembling optic ataxia observed following parietal lesions. The main functional effect of the pulvinar lesions on remote brain regions consisted of a bilateral increase of fMRI BOLD activity in frontal cortices accompanied by a bilateral reduction of activity in the intraparietal sulcus during spatial cue presentation. Our previous findings in monkeys and new preliminary data show stronger impairments for contralesional hand and hemifield, and for dissociated reaches, after dorsal pulvinar inactivation. Taken together, these results suggest that visuospatial pulvinar function is partly lateralized in humans. The hand-specific deficits in both species imply that pulvinar function goes well beyond its subscribed role in visual cognition. Instead its role might be more appropriately conceived as an integrator of motor and sensory activity for the programming of voluntary eye and hand movements.

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Poster

398. Eye Movements: Central Mechanism in Animal Models

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Topic: E.01. Eye Movements

Support: NEI Intramural

Title: Role of superior colliculus neurons in value-based decisions

Authors: *A. GOPAL P A[1], O. HIKOSAKA[2]
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Abstract: There is a circuit in basal ganglia involving caudate tail and cdl SNr that can reliably distinguish the stable value of objects presented in the contralateral visual field (Hikosaka et al.2014). This network is responsible for the fast detection of valued objects among distractors and the reduction in reaction time (RT) when a saccade is directed to highly rewarded object compared to a low reward object. This behaviour may be achieved by sending the value sensitive information from the SNr to the SC neurons. In line with this hypothesis, a recent study from our lab showed that superficial neurons in SC fire strongly for objects consistently associated with greater juice reward compared to low-reward when presented in the contralateral visual field. For an animal to choose a valued object in it visual field, it is essential for the neurons in value network of BG and SC to be sensitive about the objects present in the ipsilateral visual field as well. To test this, we trained a monkey to perform a value based two alternate choice task in which the objects were presented, one in each hemifield. A set containing 8 fractal objects- 4 good (large juice reward) and 4 bad (small reward)- was used and presented in pairs of good-good, bad-bad, or good-bad combinations. After 10 days of training, multi-unit visual and visuomotor neurons from the SC was recorded while the objects were presented in the neuron's receptive field (RF) and in the diametrically opposite position (anti-RF). We found that SC neurons are sensitive not only to the value of objects present in the RF but also to the value of objects present in the anti-RF. Firing rates were higher when a good object in the RF was opposed by a bad object in anti-RF, compared to a good object in the RF opposed by good objects in the anti-RF. Since good objects were present in the RF in both conditions, the initial responses were identical but later diverged due to different valued objects in the anti-RF. When we compared conditions in which both RF and anti RF contained good or bad objects the neurons initially responded to the value of the object in the RF. Later the activity between the two conditions became indistinguishable due to the object of same value present in the anti-RF. Consistent with the neural response, the fastest RT was on good-bad trials while there was no difference between RTs on good-good vs. bad-bad trials that presented no objectively correct choice. We hypothesize that modulation of neuronal activity based on the object present in anti RF maybe mediated by mutual inhibition from the opposite hemisphere of the colliculus through the inter collicular connection.

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Poster

398. Eye Movements: Central Mechanism in Animal Models

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Topic: E.01. Eye Movements

Support: 100 talents program,Chinese Academy of Sciences

Title: Influence of reward on superior colliculus activity during spatial- and feature-based visual search

Authors: *H. LIAO, M. YANG, M. C. DORRIS
Inst. of Neuroscience, Chinese Acad. of Scie, Shanghai City, China

Abstract: Choosing a particular saccade from multiple visual targets engages both prior knowledge of our environment and immediate sensory information. To examine the mechanism by which prior information and sensory information may be combined in midbrain superior colliculus (SC), we had monkeys perform visual search tasks in which higher liquid reward was associated with a particular spatial location (Spatial Bias Task) or stimulus color (Feature Bias Task) while recording single SC neurons. Both tasks had the same basic structure in which four stimuli (1 target and 3 distractors) were simultaneously presented equidistant from central fixation and separated by 90 degrees. Monkeys received liquid reward for directing a saccade to the 'oddball' target that differed from distractors in brightness or color, respectively. One stimulus was always presented in the response field of the recorded SC neuron. *Spatial bias task* –Monkeys were required to saccade to the brighter target among isoluminant distractors. Monkeys performed 5 blocks of trials in which reward for the response field location ranged from ¼, ½, 1, 2, to 4 times the reward at other stimulus locations. There was an interaction between spatial reward and sensory information; choice was dominated by sensory information when discrimination was easy but predominantly directed towards highly rewarded locations when discrimination was difficult. Pre-stimulus baseline activity and stimulus-aligned sensory activity were concomitantly influenced by the spatial distribution of rewards. *Feature Bias Task* – Monkeys were required to look to the oddball color (either red or green) among isoluminant yellow distractors that were an equal mix of red and green. The same reward differences were used but, in this case, linked to color. Choices became progressively biased towards a stimulus color as its reward value increased. There was no effect on early baseline or initial visual SC activity. Only later, as the time of saccade initiation approached, did SC activity discriminate the colored target from distractor. Together our results suggest that the SC plays distinct roles when prior information is related to spatial or feature attributes. When prior spatial information is available, early baseline activity and immediate visual activity are strongly affected across the SC spatial map to influence target selection. However, the SC is largely "blind" when prior information is based on stimulus features. This suggests that prior feature information is largely processed upstream of the SC and, afterwards, the pre-selected target is simply relayed to the SC for execution.

Disclosures: H. Liao: None. **M. Yang:** None. **M.C. Dorris:** None.

Poster

398. Eye Movements: Central Mechanism in Animal Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 398.21/II16

Topic: E.01. Eye Movements

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Title: Tuned persistent activity in prefrontal cortex reliably follows every saccade

Authors: *I. CALANGIU, V. MANTE
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Abstract: Our understanding about prefrontal cortex (PFC) computations has been heavily shaped by the existence of pre-saccadic, memory-related activity, a form of persistent activity that is modulated by the direction of the *upcoming* saccade. Large-scale recordings (Utah arrays) from the dorso-lateral PFC (dlPFC) of awake primates engaged in a classic center-out saccade task however reveal a more prevalent form of persistent activity—overall, dlPFC responses were dominated by post-saccadic activity, which peaks after the onset of the saccade and is modulated by the direction of the *preceding* saccade. Critically, we analysed data from a version of the saccade-task that included a variable "hold-period" *after* the saccade, and before the delivery of the reward, during which the monkey had to keep fixation on the saccade target. The inclusion of this hold-period allowed us to study the properties of post-saccadic responses in dlPFC. Surprisingly, we found that post-saccadic responses overall are stronger, and occur in a much larger fraction of the recorded neural population than pre-saccadic responses. Like pre-saccadic responses, post-saccadic responses are tuned for the direction of the saccade. Neurons that show both pre-saccadic and post-saccadic responses fall into two categories: *stable* neurons and *flip* neurons, for which the pre- and post- saccadic responses have, respectively, similar or opposite preferred directions. This time-dependent selectivity appears to be a largely fixed property of each neuron, as it accompanies not just the instructed saccades required in the center-out task, but also spontaneous saccades occurring between successive trials.
We studied how this dynamic selectivity is reflected at the level of neural population responses by identifying a linear time-dependent mapping between single-trial population responses and saccade angle. We found that the mapping between responses and saccade angle is

approximately constant throughout a pre-saccadic (-0.4 to -0.1s before saccade onset) and a post-saccadic epoch (0.05-0.35s after) but differs between the two epochs. Moreover, the representation of saccade direction in the post-saccadic epoch is robust to the type of saccade type (instructed or spontaneous) and only represents the angle of the previous saccade, not that of the upcoming, next saccade.
In conclusion, our findings are at odds with previously proposed functions of post-saccadic activity (e.g. planning the next saccade or saccade-related visual transients) and portrait post-saccadic activity as a novel functional signal that is distinct from the better-known, persistent pre-saccadic activity.

Disclosures: I. Calangiu: None. **V. Mante:** None.

Poster

398. Eye Movements: Central Mechanism in Animal Models

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Topic: E.01. Eye Movements

Support: NIH T32 DC011499
R01 EY022854
R01 EY024831
F31 EY027688

Title: Ventral premotor control of head and eye movements

Authors: *I. SMALIANCHUK[1], N. J. GANDHI[2]
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Abstract: We redirect our visual axis to a stimulus of interest through a coordinated set of movements with our eyes and head. The specific properties of these gaze shifts are task dependent and rely on the initial head-in-space and eye-in-head positions. It is known that the premotor cortex plays a role in complex movements involving several muscle groups. In particular, a region of the ventral premotor cortex (PMv) projects polysynaptically to extraocular and neck muscles, through a pathway that bypasses the frontal eye field (FEF) and primary motor cortex (M1) (Billig and Strick, Program No. 371.05/LL12, SFN 2012). This relatively direct route suggests that PMv activity influences eye and/or head movements. Thus, we developed a paradigm in which a Rhesus monkey (*Macaca Mulatta*) was trained to produce an eye-only movement followed by a head-only movement, the same movements but in the reverse order, or a gaze shift without constraints on the effectors of action. We also incorporated in the design systematic control of initial eye-in-head and head-in-space positions as well as requirements to produce ipsiversive, contraversive, and centering movements, because all of

these factors have been associated with PMv function in previous physiological and/or anatomical studies. Neural activity in PMv and, for comparison, FEF was recording with single as well as laminar electrodes. Based on our current dataset, a majority of PMv cells exhibited enhanced activity ~100-200 ms before the movement onset and, notably, this activity returned to near baseline levels before movement onset; it's like the phasic burst typically observed in FEF was shifted ahead in time. Furthermore, many cells responded to ipsiversive movements, corroborating anatomical findings which show that PMv projects to neck muscles bilaterally. Additionally, a sub-population of cells responded to the first effector in the movement, regardless whether it was eye or head, potentially indicating the onset of a movement, but remaining agnostic of which effector is used to execute that movement. These observations are consistent with the notion that there is a neural code in PMV which coordinates the movements (in this case, head-unrestrained gaze shifts) across multiple effectors and muscle groups.

Disclosures: I. Smalianchuk: None. **N.J. Gandhi:** None.

Poster

398. Eye Movements: Central Mechanism in Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 398.23/II18

Topic: E.01. Eye Movements

Support: NIH EY022928
NSF-NCS 1734901

Title: A new computational model for decoding saccade-related activity in the frontal eye field

Authors: *M. A. SMITH[1,2], R. JOHNSTON[1,2], R. O. KONECKY[1,2]
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Abstract: The frontal eye field (FEF) is thought to play a major role in oculomotor function. For example, reversible inactivation of the FEF causes transient deficits in saccade production and electrical microstimulation at low currents elicits saccades with a characteristic direction and amplitude (Bruce et al., 1989). The FEF sends descending projections to subcortical structures that control saccades such as the superior colliculus (SC). Several computational models have been proposed to explain how brainstem circuitry decodes SC activity including a vector sum (VS) and vector average (VA). Although a similar scheme may be used to read-out FEF activity the precise nature of the computation is indeterminate. To address this issue, we microstimulated the FEF of two macaque monkeys using a 16-channel linear electrode array. The saccade evoked by simultaneously stimulating two contacts with equal current (90 or 100 υA) was compared to the saccades evoked by stimulating each constituent contact alone. We assessed the performance

of a novel polar average (PA) computation compared to the traditional VS and VA models. In the PA scheme, direction and amplitude are represented separately as the two constituent saccades are transformed into polar coordinates prior to averaging. Error, in terms of amplitude and direction, between actual and predicted saccades was significantly smaller for the PA model than the VS and VA. While the PA model was significantly better at predicting saccade direction overall, errors from the PA model were in the VS and VA direction when the difference in amplitude between the two constituent saccades was relatively large. These findings suggest that downstream regions may employ a PA computation to decode FEF activity. An underlying assumption of this model, which has key implications for current theories of oculomotor function, is that amplitude and direction are represented separately in the FEF.

Disclosures: M.A. Smith: None. **R. Johnston:** None. **R.O. Konecky:** None.

Poster

398. Eye Movements: Central Mechanism in Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 398.24/JJ1

Topic: E.01. Eye Movements

Support: CIHR Foundation Grant
CIHR Postdoctoral Fellowship

Title: Neural oscillations in the marmoset parietal cortex during a saccadic task

Authors: *L. MA[1], L. K. H. SCHAEFFER[1], K. JOHNSTON[2], S. EVERLING[3]
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Abstract: Over the past 50 years, macaque monkeys have been successfully used as the main nonhuman primate model for understanding the neural processes underlying saccadic eye movements. Although macaque monkeys are in many ways ideal due to their close evolutionary linkage to humans, many of the saccade-related brain regions lie deep in a sulcus in this species, which makes it difficult to access them for large-scale neural recordings and manipulations. The common marmoset (*Callithrix jacchus*) is a promising additional nonhuman primate model with a lissencephalic brain, which allows for easy targeting of frontoparietal brain regions. We have recently shown that marmosets, like humans and macaques, exhibit a gap effect—a decrease in saccadic reaction time when the fixation stimulus is removed prior to the onset of a peripheral visual saccade target (Johnston et al., 2018). Here, we took advantage of the marmoset smooth cortex and implanted 32-channel microelectrode arrays (Utah arrays) in the posterior parietal cortex of two marmosets and recorded both spiking activity and local field potentials during this task. We observed an enhancement in high gamma activities above 60Hz, during the gap period

compared to the same time period in step trials. Discharge rates in a subset of single units were also elevated during the gap period. During the fixation and gap periods, it was possible to predict whether the monkeys were about to make a short latency “express” (SRT>=110ms) or longer latency “regular” saccade (SRT>=120ms) based on high gamma band activities. On express-saccade trials, gamma activities were stronger during the gap than the fixation period, whereas in regular-saccade trials this pattern was reversed. Additionally, high gamma activities were stronger during the gap period in express-saccade trials than in regular-saccade trials. This effect was especially strong for trials with saccades contralateral to the recording sites. Our findings suggest that the common marmoset is a valuable additional nonhuman primate model for the study of the neurophysiology and pathology of saccades.

Disclosures: L. Ma: None. **L.K.H. Schaeffer:** None. **K. Johnston:** None. **S. Everling:** None.

Poster

398. Eye Movements: Central Mechanism in Animal Models

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 398.25/JJ2

Topic: E.01. Eye Movements

Support: CIHR

Title: Oculomotor effects evoked by microstimulation of posterior parietal cortex in the common marmoset

Authors: *K. D. JOHNSTON[1,2], M. GHAHREMANI[1], L. SCHAEFFER[3], S. EVERLING[4,3,2]
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Abstract: Frontoparietal networks have long been established as a critical neural substrate for cognitive processes and oculomotor control. The common marmoset (Callithrix jacchus) has recently emerged as a promising model for investigations of cortical circuitry, since the largely lissencephalic cortical surface of this small New World primate is readily amenable to laminar and high-density array recordings in frontal and parietal areas linked to oculomotor control such as the frontal eye fields (FEF) and the lateral intraparietal area (LIP), which are considerably more difficult to access in rhesus macaque due to their location within sulci.
Studies of cortical cytoarchitecture and comparative resting-state fMRI have established homology in the structure and connections of these areas between the rhesus macaque and common marmoset, but to date few studies have investigated in detail the functional properties of frontal and parietal areas in the marmoset model. Here, we sought to address one aspect of this gap by applying intracortical microstimulation in the marmoset posterior parietal cortex (PPC).

We implanted 32 channel planar electrode arrays (Utah arrays) in the PPC of two common marmosets. Array locations were determined using the stereotaxic coordinates of the area cytoarchitecturally defined as homologous to LIP, the location of resting-state fMRI functional connectivity between PPC and the midbrain superior colliculus, and for additional guidance the local cerebral vasculature of the cortical surface. Each array was positioned to cover as wide an area including and surrounding the putative LIP as possible. We applied 300ms trains of biphasic current pulses at variable amplitudes at sites across the arrays in awake animals while simultaneously monitoring oculomotor responses at 1000 Hz using a video-based eye tracker (EyeLink 1000). Similar to previous studies of PPC microstimulation in the macaque, we found that stimulation at some sites evoked contralateral saccades and eye blinks. In addition, we observed eye position effects such that the amplitudes of evoked saccades varied as a function of the initial eye position of the animals. These data provide evidence that PPC of the marmoset has a role in oculomotor function similar to that of the rhesus macaque. Together with structural studies, these data suggest that the common marmoset possesses a PPC homologus to that of rhesus macaques and humans, and further supports it's viability as a model species for investigating the detailed cortical circuitry underlying oculomotor control.

Disclosures: K.D. Johnston: None. **M. Ghahremani:** None. **L. Schaeffer:** None. **S. Everling:** None.

Poster

398. Eye Movements: Central Mechanism in Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 398.26/JJ3

Topic: E.01. Eye Movements

Support: AMED Brain/MINDS
KAKENHI 15K06709

Title: Comparison of saccadic behavior between common marmosets, rhesus macaques, and humans

Authors: *C.-Y. CHEN[1], D. MATROV[1], R. VEALE[1], M. YOSHIDA[2], K. MIURA[3], T. ISA[1]
[1]Dept. of Neurosci., Kyoto Univ., Kyoto-Shi, Japan; [2]Dept Syst. Neurosci, Natl. Inst. Physiol Sci., Okazaki, Japan; [3]Grad. Schl Med, Kyoto Univ., Kyoto-shi, Japan

Abstract: Rhesus macaques are commonly used to study the oculomotor circuits. However, precise circuit manipulation is difficult because the relevant cortical areas are deep in the sulcus. Recently, the common marmosets have been proposed to have great advantages in oculomotor research because their cortex is almost flat. Nonetheless, before we undertake large-scale use of marmosets, characterizing their oculomotor behavior under standard saccade tasks is essential.

Here, we directly compared saccadic behavior from marmosets, macaques, and humans during three standard saccade tasks. In free viewing task, we let subjects to passively view the video clips previously used by Yoshida et al. (2012). For visually guided saccade task, we first asked our subjects to fixate a fixation point (FP) at the screen center. After 200 ms, we simultaneously turned the FP off and illuminated 1 target randomly selected from 8 potential targets. The targets were spaced equally around the FP at a radius of 6° visual eccentricity. The subjects had to make a saccade to the target and maintain their gaze for 200 ms to receive a reward. In gap saccade task, the process was identical to the visually guided saccade task, except that an additional gap period (0, 48, 96, 144, 192, and 240 ms) was added between turning off the FP and illuminating the target. During this period, the subjects had to maintain their gaze close to where the FP had disappeared. For the free viewing task, we found saccade amplitude increased with increasing saccade peak velocity (known as the "main sequence") in all three species. However, we observed larger saccade amplitude, shorter intersaccadic interval, and shorter saccade duration in marmosets comparing to humans. Also, the marmosets showed faster saccade reaction time (SRT) (median: 134 ms) than human (median: 188 ms) in visually guided saccade task. Finally, in the gap saccade task, we found that the mean SRT was shorter in the trials with gap period > 0 ms than without gap period in marmosets, which is similar to those of humans. Taken together, we found both similarities and differences in the saccadic behavior of marmosets, macaques, and humans under standard oculomotor tasks. More studies are needed to fully characterize saccadic behavior to establish marmosets as a valuable animal model for oculomotor research.

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Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 399.01/JJ4

Topic: E.01. Eye Movements

Support: NIH Grant EY008313
Research to Prevent Blindness

Title: The paradox of leveraging eye movements: Ocular rotational axis varies with vergence

Authors: *J. L. DEMER, R. A. CLARK
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Abstract: Since the eye does not rotate about its center (Clark & Demer, *Ophthalmology*, 2018), determination of oculorotary torques requires consideration of both tensions & lever arms (LAs) of extraocular muscles (EOMs). We used magnetic resonance imaging (MRI) to determine

rotational axes & LAs of horizontal rectus EOMs.
In adult humans, 390μm resolution surface coil, T2 fast spin echo MRI was repeated in 2 mm thick axial planes in central gaze, abduction, and adduction during: unconverged monocular fixation; asymmetrical binocular convergence to a target at 20 cm; and fusional divergence to a symmetrically-placed accommodative near target at 20 cm with 8 PD base in prism, and divergence to a far target at 400 cm with 4 PD base in prism. Rotational axis was taken as the intersection of lines from cornea to globe-optic nerve junction in initial and final gazes. LA was then computed from axis to EOM scleral insertion for a 24 mm diameter eye.
Rotational axes were always highly eccentric ($P<0.002$). Mean (±SD, N=36 eyes) axis in unconverged abduction was 2.1±0.6mm medial & 0.6±1.1mm posterior to globe center, giving 12.6 mm LA for lateral rectus (LR) and 9.8 mm for medial rectus (MR). In unconverged adduction, axis was 2.3±0.01mm medial & 1.2±1.0 posterior, giving 14.6mm LA for LR & 10.7 mm for MR. Axis in 23±3° (N=10 eyes) asymmetric convergence was significantly more temporal than in adduction at 1.3±0.3 mm medial and 0.2±1.5mm anterior to globe center, giving 11.8mm LA for LR and 9.7mm for MR. Axis in 6.5±2.3° (N=20 eyes) near divergence was significantly less temporal than in abduction at 1.8±0.4 mm medial ($P<0.02$) and 0.1±2.4mm anterior to globe center, giving 12.1 mm LA for LR & 9.4 mm for MR. Axis in 3.6±1.3° (N=18 eyes) far divergence was also significantly less temporal than in abduction at 1.6±0.7mm medial ($P<0.002$) & 0.4±2.4mm anterior to globe center, giving a 12.4 mm LA for LR & 10.2mm LA for MR.
The LR lever arm is up to 36% longer than that of MR and varies more with eye movement type than for MR. The LR/MR lever arm ratio varies from 1.36 in adduction, 1.29 in abduction, to 1.22 for both convergence and divergence. Since torque, not simply tension, rotates the eye, eccentric rotational axis implies that neither motor neuron firing rate nor EOM tension should have constant relationships to eye movement. In convergence, for example, axis eccentricity predicts LR torque to decrease by 19%, and MR torque by 9%, without change in innervation of either EOM. Such co-relaxation and previously inexplicable relationship to motor neuron firing have been reported as a paradox during convergence in monkeys (Miller et al, *J. Neurophysiol.* 105:2863, 2011), but could be due to axis if also eccentric in monkey.

Disclosures: R.A. Clark: F. Consulting Fees (e.g., advisory boards); Nevakar, LLC.

Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 399.02/JJ5

Topic: E.01. Eye Movements

Title: Are vergence eye movements a myth? Observations from midline smooth pursuit

Authors: *S. J. HEINEN[1], A. CHANDRA[1], J. BADLER[1], S. N. WATAMANIUK[2]
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Abstract: Generally, during smooth pursuit the two eyes are thought to rotate through the same angles, resulting in conjugate eye movements. An exception is during vergence, when a target moves on the midline and the eyes rotate in opposite directions. The vergence system is thought to provide a single signal, but with opposite innervation to each eye, and is separate from the conjugate system. However, almost all natural pursuit movements include a depth component, raising the question of why a separate vergence system evolved. It would seem reasonable that instead of having a separate vergence system, the eyes are controlled independently as Helmholtz proposed almost 200 years ago. Here we tested the feasibility of a putative vergence system by having observers pursue a physical target that moved in depth with either both or one eye. Observers (n = 7 humans, 4 female) pursued a small letter "E" mounted on a pole that moved along a mechanical track periodically forward and backward on the midline (peak velocity = 50 cm/sec; cycle amplitude = 33.3 cm). Observers pursued during binocular viewing, or monocular viewing with each non-viewing eye occluded separately. Binocular eye position was always recorded, as occluders were infrared-passable filters that allowed recording from the occluded eye. Either an Eyelink 1000 eyetracker, or a Plusoptix power refractor (which simultaneously measured accommodation) recorded eye movements. We found that when both eyes were viewing, a robust vergence movement was generated. Similarly, in monocular conditions, the viewing eye always followed the target well. However, the two eyes showed very different behaviors from each other when one was covered. When one eye was occluded, it showed minimal evidence of vergence movements, and often no movement at all. When the other eye was occluded, in most observers it moved *conjugately* with the viewing eye. The results question the existence of a vergence system, and suggest instead that the eyes are independently controlled to override a brainstem conjugate eye movement system in situations where conjugate eye movements are not appropriate. Furthermore, the asymmetry with different covered eyes suggests control of one eye supersedes that of the other, evidence of an *oculomotor* dominance.

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Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

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Program #/Poster #: 399.03/JJ6

Topic: E.01. Eye Movements

Support: DFG La 952-6

DFG La 952-8

Title: Voluntary inhibition of saccadic adaptation depends on adaptation direction

Authors: *M. LAPPE, F. HEINS, A. MEERMEIER
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Abstract: Saccadic adaptation is often assumed to be driven by an unconscious and automatic evaluation of post-saccadic visual error. We investigated if saccadic adaptation is accessible to volitional control. We exposed eight human participants to a post-saccadic visual error in a standard double-step paradigm in which a target dot first jumped 12 deg to the right and then, during the elicited saccade, jumped again by between 2 and 4 deg either in or against the direction of the primary saccade. In one condition, participants were instructed to follow the target and look at its final position. In another condition, participants were instructed to observe the first target jump and then saccade to that position and remain at that position irrespective of any further jumps if the target. We found that gain change differed between the two conditions, showing an influence of volition on saccadic adaptation. The difference depended on the direction of adaptation. When told to remain at the first target position, gain change was close to zero for outward intra-saccadic secondary target jumps. For inward jumps gain change was smaller than when instructed to look at the final target but larger than zero so that some adaptation occurred also in this condition. Analysis of saccadic latencies also showed a difference between adaptation directions. Latency was not affected by the instruction during inward secondary jumps, but when the target was stepped outward during the primary saccade, latencies of the primary saccade were longer when subjects were instructed to remain at the position of the first target jump. The results show that volitional control can be exerted on saccadic adaptation. The differences between inward and outward adaptation suggest that volitional control affects the remapping of the target, thus having a larger impact on outward adaptation.

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Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

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Program #/Poster #: 399.04/JJ7

Topic: E.01. Eye Movements

Support: NSERC Discovery Grant to M.F. (RGPIN-2016-05296)

Title: Early excitatory oculomotor processing encodes task information

Authors: *D. H. KEHOE[1], J. LEWIS[2], M. FALLAH[2]
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Abstract: Influential accounts of oculomotor target selection processing posit that in critical oculomotor neural substrates like the superior colliculus (SC) and frontal eye field (FEF), early excitatory activity encodes sensory information about a potential saccade target, while later inhibitory activity encodes top-down information (Fecteau & Munoz, 2008). Recently, Kehoe and Fallah (2017) developed a non-invasive technique to measure the time course of excitatory and inhibitory activity encoding a peripheral distractor in which human saccade curvature is modeled as a continuous function of *saccade-distractor onset asynchrony* (SDOA): the time between the transient onset of a task irrelevant distractor and the initiation of a saccade to a target. The distractor processing time course observed using the non-invasive SDOA technique was closely corroborated by the reported time course of visuomotor neural activity in SC during target selection processing (McPeek & Keller, 2002). Here, we used the SDOA technique to investigate how varying the degrees of visual similarity between a distractor and the target affect the time course of excitatory and inhibitory distractor-related processing while human observers (N = 35) performed a discrimination saccade task for pairs of complicated, novel objects. Consistent with differences in target-distractor discrimination time between similar and dissimilar distractors in SC (Shen & Paré, 2012) and FEF (Sato et al., 2003) neurons, we observed that the latency of the distractor-related inhibitory response was 40-60 ms later for the high similarity distractor than for the intermediate/low similarity distractors. Interestingly, we also observed that the latency of the initial rapid excitatory response was ~60 ms longer for the high similarity distractor than for the intermediate/low similarity distractors, which suggests that the early excitatory response observed during oculomotor target selection processing does encode top-down information under certain task-related conditions. Such observations have important implications for the primacy of top-down "attentional control settings" over sensory salience observed during attentional capture (Folk et al., 1992, 1994; Yantis & Egeth, 1999) and also suggest that differences in search efficiency for similar and dissimilar distractors (e.g., Duncan & Humphreys, 1989; Treisman & Gelade, 1980; Wolfe et al., 1989, 1994) may be equally well attributed to activation of relevant features as supposed to just the inhibition of irrelevant features.

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Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

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Program #/Poster #: 399.05/JJ8

Topic: E.01. Eye Movements

Support: NIH Grant 5T32EY025201-03

Title: Saccadic localization of moving objects is influenced by illusory perception

Authors: *Z. MA, S. J. HEINEN
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Abstract: Internal drift dramatically shifts the perceived location of a translating Gabor patch. Lisi and Cavanagh (2015) showed that unlike perception, saccades target a Gabor's physical location but not its perceived illusory one. In their experiment, a Gabor patch translated diagonally in the periphery, either with an orthogonal internal drift (illusory condition) or not (control condition). In the illusory condition, a dissociation between perceptual and saccadic localization happened: while the subjectively perceived translation path appeared vertical, saccade endpoints were arranged diagonally with the veridical motion. However, in a separate perceptual experiment, they showed that the perceived illusory path was shifted toward the Gabor's starting position. But in their saccade experiment, the Gabor underwent multiple direction reversals, potentially providing different "starting positions" at the reversal locations within a single trial. This might have obscured the influence of the Gabor's starting position and hence obscured an illusory bias in saccade endpoints. Here, we replicate their experiment, but using Gabors that followed a single, non-reversing motion path. The Gabor traversed a diagonal trajectory, and started with one of two possible horizontal displacements (9 or 11 degree eccentric) and moved upwards or downwards toward the other horizontal displacement. This resulted in two different horizontally displaced illusory trajectories, despite the fact that the veridical motion trajectories were exactly the same. We found that in the illusory condition, although the path of the saccade endpoints was not oriented strictly vertical as is the illusion, it was tilted away from the veridical diagonal path, ($t(4)=3.83$, $p<0.05$). More importantly, the starting position of the Gabors influenced saccade endpoint. We found that horizontal saccade amplitude was smaller when the Gabor started from a position that was closer to the fixation point ($t(4)=7.55$, $p<0.05$), suggesting that similar to the illusory perception, saccades were biased by the Gabor's starting position. Our results suggest that saccade endpoints are biased by perceived illusory position offsets, supporting that the saccadic system receives input from neural pathways subserving perception.

Disclosures: Z. Ma: None. **S.J. Heinen:** None.

Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 399.06/KK1

Topic: E.01. Eye Movements

Title: Is saccade vigor modulated by subjective effort cost?

Authors: *C. KORBISCH[1], A. A. AHMED[2]
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Abstract: Saccade vigor has been shown to reflect how individuals evaluate different financial decision outcomes and correlate with their subjective temporal discounting rate, as well as the intrinsic value of the saccade target [1,2]. Movement decision outcomes can be considered in similar ways as financial decisions. In both paradigms, individuals wish to maximize the utility of the decision outcome [3]. We propose that the same modulation of saccade vigor will hold when individuals evaluate movement decisions that differ in their metabolic costs. Just as individuals subjectively evaluate financial decision outcomes, we propose that metabolic costs are subjectively evaluated and discounted either exponentially or hyperbolically. To probe this, we examined how individuals' saccade vigor changed as they were given metabolically costly decisions. We measured the vigor of their
saccades as they chose between a reference incline and duration (either 4%/6min or 5%/5.5min) and an alternative movement of varying inclines and durations ranging from 0 to 10% and 0.5 to 10 minutes respectively. Participants' decisions were made by saccading to their preferred movement presented on either side of the screen. We then fit each participant's decisions using an iso-cost curve and compared the fit to assess objective (based on metabolic cost) and subjective cost models. A variational Bayesian method was performed to select the most likely cost model based. Our preliminary data (N=3) suggest that participants' decisions were best explained by a subjective model of effort in which the total metabolic cost is hyperbolically discounted by time. Using this subjective cost model, we then assessed how saccade vigor differed as a function of the cost difference of the two options presented. On a whole, we found that participants were more likely to saccade at faster peak velocities as the perceived cost difference increased and found a weaker relationship when considering the objective metabolic cost difference. Total deliberation time and reaction time followed the same trends. Our findings suggest that individuals modulate their saccade vigor and make movement decisions based on an individual perception of the metabolic cost of the movement, rather than simply the objective cost.

REFERENCES

1. Reppart et al. (2015) Modulation of Saccade Vigor during Value-Based Decision Making. Journal of Neuroscience 35: 15369-15378
2. Xu-Wilson et al. (2009). The intrinsic value of visual information affects saccade velocities. Exp Brain Res 196: 475-481
3. Shadmehr et. al. (2016) A Representation of Effort in Decision-Making and Motor Control. Current Biology (26) 14:1929-1934

Disclosures: C. Korbisch: None. **A.A. Ahmed:** None.

Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 399.07/KK2

Topic: E.01. Eye Movements

Support: NIH 1R01NS078311
ONR N00014-15-1-2312
NSF 1723967

Title: Vigor of movements in a normative framework: An extension of the marginal value theorem

Authors: *R. B. GEARY[1], T. YOON[2], R. SHADMEHR[1]
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Abstract: Reward and effort modulate vigor of movements. Current theories account for this observation by suggesting that movements are governed by a utility, combining measures of reward, effort, and time. However, behavior of an animal depends on not only the contingencies of the current option, but the animal's history. To account for this behavior, we adopted a normative approach where the objective was to maximize average net capture rate, defined as the sum of all rewards and efforts accumulated, divided by total time. We found a novel solution to this finite horizon optimal control problem by extending marginal value theorem and tested the theory in two experiments. Our theory predicted the average net capture rate, reflecting a history-dependent utility, acts as a local cue that dictates vigor.

Theoretical results: Consider a scenario where the animal performs an action to acquire a reward, then repeats a similar action to acquire a reward at another location. During the time to travel between locations t_m, effort is expended. The animal spends time t_h to harvest the reward via $f(\alpha,t_h)$. The animal chooses its vigor t_m and harvest period t_h to maximize not the utility of the current action, but the average utility J across all actions. By extending marginal value theorem, we found J was maximized when the rate of effort expenditure during movement equaled the negative average utility. The basic prediction was that vigor should depend on the average net capture rate.

Experimental results: To manipulate J, we controlled harvest duration t_h. Subjects performed a saccade task, viewing images of faces. We considered the time period of looking at the image as the harvest period. If the harvest function $f(\alpha,t_h)$ is concave-downward, then $f(\alpha,2t_h) < 2f(\alpha,t_h)$. This implies as t_h decreases, J increases, resulting in faster saccades. To test this prediction, in Exp. 1 we recruited n = 16 healthy volunteers. On each trial, an image was presented at horizontal positions $\pm 20^o$ from the center, with a $0\text{-}5^o$ random vertical offset, for a variable duration. We observed that as harvest duration increased, saccade vigor decreased, and as harvest

duration decreased, saccade vigor increased. In Exp. 2 (n=19 subjects), we manipulated the history of harvest durations (short, long, or medium), followed by a control harvest (medium duration). We found the period of long harvest durations produced slower movements in the control environment, whereas the short harvest durations produced more vigorous movements. Together, these results suggest movement vigor may be a reflection of a process that attempts to maximize a global utility, the sum of rewards and efforts accumulated, divided by total time.

Disclosures: T. Yoon: None. **R. Shadmehr:** None.

Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

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Program #/Poster #: 399.08/KK3

Topic: E.01. Eye Movements

Support: NIH 1R01NS078311
ONR N00014-15-1-2312
NSF 1723967

Title: Movement vigor and decision-making in a patchy reward environment

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Abstract: Stimulus value modulates the vigor with which animals move toward a stimulus. Current theories account for this observation by suggesting a utility that combines measures of reward, effort, and time. However, behavior depends not only on the contingencies of the animal's current option, but also the animal's history. Here, we adopt a normative approach where our objective is to maximize average capture rate, defined as the sum of all rewards acquired and efforts expended, divided by total time.
Theoretical results: In a patchy reward environment, we considered the problem of maximizing average capture rate by controlling time spent harvesting reward, and time spend travelling between patches of reward. By including a realistic metabolic cost of travel, we found a novel solution to this problem through an extension of marginal value theorem (MVT). The theory predicting two aspects of behavior: how much time the subject will spend at the reward site, and how fast the subject will move between reward sites.
Experimental results: To test the theory, we measured human behavior in environments where each reward patch was a small image on a screen, and movements were executed via saccadic motion of the eyes. We manipulated reward magnitude via image content, and effort magnitude via image location. In Exp. 1 (n=17 subjects), on each trial subjects were presented with two

visual stimuli simultaneously and had 2 seconds to freely gaze. As theory had predicted, time spent gazing at a stimulus increased with its reward value, and decreased with required effort. Importantly, for a given eccentricity, saccade vigor not only increased with stimulus value at the destination, but also with stimulus value at the previous location. In Exp. 2 (n=21 subjects), we presented a patchy environment where on each trial the subjects were free to gaze at the current stimulus, and then move to another location, where a new stimulus would be displayed upon arrival. We found that time spent at the patch depended on both the past experience (increased with the effort expended to get there), and the expected future (increased with the effort expected to move to the next patch). In Exp. 3 (n=19 subjects), we modulated background rate (long term history and predicted future) by having sessions of differing distributions of reward and effort and found that long-term history of capture rate affected gazing duration and movement vigor. In summary, we found that both the time spent harvesting reward, and vigor of movements between reward sites, depended on history of reward and effort, in such a way predicted by our extended MVT.

Disclosures: T. Yoon: None. **R. Shadmehr:** None. **A.A. Ahmed:** None.

Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 399.09/KK4

Topic: E.01. Eye Movements

Title: Dynamic overshoot in saccadic movement of pupil inside iris during pro- and anti-saccade tasks

Authors: *S. YAMAGISHI, M. YONEYA, S. FURUKAWA
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Abstract: Typical saccades have a dynamic overshoot. One previous study reported that the dynamic overshoot was larger for pupil movement than for iris movement (Nyström et al., 2013). The present study explores mechanisms that might determine the properties of the dynamic overshoot of pupil/iris movements. Specifically, we compared the properties during pro- and anti-saccade tasks. The execution of an anti-saccade is assumed to require top-down inhibition of a reflexive pro-saccade and is known to involve activity of the frontal cortex and basal ganglia (Munoz & Everling, 2004). Eye movements were recorded with high-speed camera at a sampling rate of 500 Hz. On the basis of the recorded images, we extracted trajectories of pupil and iris centers during pro- and anti-saccades. At the beginning of a trial, the participant (total n=13) was asked to fixate on a filled square (side 1°) at the center of the display, ignoring the two empty peripheral boxes on the left and right of the filled square (1.5 - 2 s). Then, after the filled square changed to an empty one (200 ms), one of the peripheral squares changed to a filled square

(Saccade part: 1.5 - 2 s). In the pro-saccade task, the participants were asked to make a saccade in the direction of the filled square. In the anti-saccade task, the direction of the saccade was opposite. After the saccade part, all visual stimuli disappeared (1 - 1.5 s). As in the previous report, we confirmed that the post-saccadic overshoot was larger for the pupil movement than for the iris movement. Interestingly, the overshoot of the pupil movement tended to be greater for pro-saccade than for anti-saccade, while the iris movement exhibited generally comparable overshoot between the conditions. This discrepancy between the pupil and iris movements indicates that the overshoot cannot be attributable solely to the characteristics of neural commands to the eyeball movement, whose effects on the pupil and iris should not be sensitive to the saccade task. We propose an explanation that considers the elasticity inside the iris: it is possible that the elasticity inside the iris is higher during pro-saccade, while the iris is relatively rigid so that it reflects more accurately the movement of the whole eyeball.

Disclosures: S. Yamagishi: None. **M. Yoneya:** None. **S. Furukawa:** None.

Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 399.10/KK5

Topic: E.01. Eye Movements

Support: DBT-IISc Grant

Title: Optimal feedback control of saccade trajectories can explain saccade variability

Authors: *V. VASUDEVAN, A. MURTHY, R. PADHI
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Abstract: Movements are thought to make use of a predictive forward model in conjunction with sensory feedback to allow for motor control and motor learning. This work aims at gaining further insights into the principles of motor control by investigating the nature of movement variability. Although earlier systems level descriptions of motor control were largely deterministic in nature, more recently developed optimal stochastic feedback control models provide a better framework to study motor control in the presence of noise (Todorov and Jordan, 2002). However, with a few exceptions ((van Beers, 2007),(West et al., 2009)), the predictions of these models have been confined to predicting the average kinematic profiles and the main sequence in case of saccades (Harris and Wolpert, 2006)(Chen-Harris et al., 2008). This work studies the inter-trial variability of repeated saccades made by 20 human subjects. Eye position was recorded at 240 Hz using a desktop mounted IR based camera (ISCAN, Boston USA) while subjects made 12° horizontally. Variability was calculated for each subject over normalised saccade time. Generally, the variability profile was observed to be an increasing function with

saturation or decrease towards the end of the saccade in a few cases. To examine the contribution of feedback control, two versions of optimal control model which optimized error and control effort were tested. In one version, a feedforward optimal control (FOC) model that minimized end-point variance without feedback (Harris and Wolpert, 2006) was tested. The second version tested a model (OFC model) that minimized the error in trajectory and velocity of saccades in the presence of feedback (Chen-Harris et al., 2008). Both models were able to predict the mean saccade trajectories, with root mean square prediction errors equal to 1.9 ± 0.39^{o} ($5.2 \pm 0.93\%$) for the FOC model and 1.53 ± 0.93^{o} ($4.19 \pm 0.73\%$) for the OFC model. However, the FOC model completely failed to explain the trajectory variances. The prediction errors were 1.94 ± 1.004 degree^2 ($99.7 \pm 0.03\%$), while the OFC model predicted trajectory variances with errors of 0.42 ± 0.198 degree^2 ($25.2 \pm 14\%$). Overall, the OFC model performed significantly better at predicting the trajectory variance than the FOC model (Wilcoxon signed-rank test: $p < .001$). These results suggest that realistic noisy saccadic system may use an internal feedback mechanism. Our analysis also suggests that understanding variability in movements is a powerful tool to investigate the architecture of movement control.

Disclosures: V. Vasudevan: None. **A. Murthy:** None. **R. Padhi:** None.

Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

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Program #/Poster #: 399.11/KK6

Topic: E.01. Eye Movements

Support: NIH 1R01NS078311
ONR N00014-15-1-2312
NSF 1723967

Title: Reward-prediction-error modulates learning from sensory-prediction-error

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Abstract: Decades of psychophysical evidence suggests that there are at least two error signals that drive behavioral learning: reward-prediction-error (RPE), the difference between the predicted and actual reward, and sensory-prediction-error (SPE), the difference between the expected and realized sensory consequences of an action. During learning, we likely experience both types of errors, raising the question of whether these two systems interact with each other. Here, we hypothesized that during learning of a motor task, coincidence of an RPE with an SPE may modulate how the brain learns from that sensory error. We recorded eye movements as subjects were presented images with high (faces of people) and low (noise) value. Subjects

experienced two types of errors: sensory- and reward-prediction-errors. We controlled SPEs by manipulating the position of the stimulus on the fovea: on some trials, the position of the target was jumped while the eye was in motion, resulting in a foveal error at the end of the saccade. In response to this SPE, subjects performed a corrective saccade to the final target location, and then learned from this error and altered their next saccade. We controlled RPEs by manipulating the content of the image: the image that was presented as the initial target was occasionally switched to a new image after the eye began moving, resulting in an RPE. We considered two kinds of images: faces (high value), and noise (low value). In this way, we could produce positive RPEs in some trials (noise image switched to face), and negative RPEs in other trials (face image switched to noise). We quantified how the RPEs affected learning from the SPEs. We found that both the primary and the corrective saccades had shorter reaction times and greater peak velocities when they were made towards a high valued stimulus (face). RPE affected the corrective saccade: a positive RPE increased the velocity of the corrective saccade and reduced its reaction time, while a negative RPE had the opposite effects. Regardless of this prediction error, during gain down saccade adaptation, subjects learned more from their sensory errors when the final foveated target was more valuable (face). In contrast, during gain up adaptation, learning from SPE was modulated by the value of the initial stimulus. Together these findings demonstrate that vigor of movement is not only affected by the value of the stimulus but also the reward-prediction-error that coincided with experience of that stimulus. Furthermore, learning from a sensory-prediction-error is modulated by the reward-related events that took place at the time of experience of that sensory-prediction-error.

Disclosures: E. Sedaghat Nejad: None. **D. Herzfeld:** None. **R. Shadmehr:** None.

Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 399.12/KK7

Topic: E.01. Eye Movements

Support: K99EY027846

Title: Modeling the triggering of saccades, microsaccades and saccadic intrusionsModeling the triggering of saccades, microsaccades and saccadic intrusions

Authors: *J. OTERO-MILLAN[1], L. M. OPTICAN[2], S. L. MACKNIK[3], S. MARTINEZ-CONDE[4]
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Abstract: When we explore a static visual scene, our eyes move in a sequence of fast eye movements called saccades, which are separated by fixation periods of relative eye stability. Based on uncertain sensory and cognitive inputs, the oculomotor system must decide, at every moment, whether to initiate a saccade or remain in the fixation state. Even when we attempt to maintain our gaze on a small spot, small saccades, called microsaccades, intrude on fixation once or twice per second. Because microsaccades occur at the boundary of the decision to maintain fixation versus starting a saccade, they offer a unique opportunity to study the mechanisms that control saccadic triggering. Abnormal saccadic intrusions can occur during attempted fixation in patients with neurodegenerative disorders. We have implemented a model of the triggering mechanism of saccades, based on known anatomy and physiology, that successfully simulates the generation of saccades of any size—including microsaccades in healthy observers, and the saccadic intrusions that interrupt attempted fixation in parkinsonian patients. The model suggests that noisy neuronal activity in the Superior Colliculus controls the state of a mutually inhibitory network in the brain stem formed by burst neurons and omnipause neurons. When the neuronal activity is centered at the rostral pole the system remains at a state of fixation. When activity is perturbed away from this center, a saccade is triggered. This perturbation can be produced either by the intent to move one's gaze or by random fluctuations in activity.

Disclosures: J. Otero-Millan: None. **L.M. Optican:** None. **S.L. Macknik:** None. **S. Martinez-Conde:** None.

Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

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Topic: E.01. Eye Movements

Support: NIH 1R01NS078311
ONR N00014-15-1-2312
NSF 1723967

Title: Signatures of the fast and slow learning processes in the motor commands that move the eyes during a saccade

Authors: *S. P. OROZCO[1], D. J. HERZFELD[2], R. SHADMEHR[3]
[1]Biomed. Engin., Johns Hopkins Univ., Baltimore, MD; [2]Dept. of Neurobio., Duke Univ., Durham, NC; [3]Dept Biomed. Eng, Johns Hopkins Univ. Dept. of Biomed. Engin., Baltimore, MD

Abstract: Behavior during motor adaptation suggests that learning may be supported by two parallel processes, one that learns significantly from error but forgets quickly (fast process), and

one that learns little from error but forgets slowly (slow process). A limitation has been the inability to directly measure a behavioral proxy for each of the two hypothetical states. Here we report on motor commands that move the eyes during a saccade and show that during adaptation, separate components of the commands carry the signatures of the putative fast and slow processes. We hypothesized that changes to the later part of the saccade would behave like the fast process, whereas changes in the early part of the movement would proceed like the slow process. To test this idea, we designed a saccade adaptation paradigm in which the primary saccade direction was vertical but the target jump was horizontal. This design is akin to force field adaptation where the change in motor commands is perpendicular to the baseline commands, allowing for clear identification of the commands that result from error-dependent learning. Subjects (n=25) made 15^{o} vertical saccades and experienced 5^{o} horizontal perturbations of the target. We divided each saccade into early and late halves based on peak speed and found that the motor commands that were generated in the first half of the movement learned little from error, whereas the motor commands that came in the second half of the movement were more strongly affected by the same error. With passage of time, the motor commands that came before peak velocity showed strong retention, whereas the motor commands that came after peak-velocity showed rapid forgetting. These results suggest that during vertical saccades, the motor commands that arrived late in the movement show carried signature of the fast learning system. To test for this directly, we collected data from n = 21 subjects in a single trial learning (STL) experiment in which every trial had an equal chance of a 5^{o} leftward perturbation, no perturbation, or a 5^{o} rightward perturbation. We found that following a single error, the motor commands on the next movement change, correcting the error only in the second half of the movement, as the eye is decelerating. Together, signatures of the hypothetical fast and slow learning processes appear to be measurable in the motor commands that move the eyes.

Disclosures: S.P. Orozco: None. **D.J. Herzfeld:** None. **R. Shadmehr:** None.

Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 399.14/KK9

Topic: E.01. Eye Movements

Support: NSERC

Title: A 10-minute bout of moderate to very-heavy intensity aerobic exercise improves executive function in older adults at risk for cognitive decline

Authors: *A. PETRELLA, M. HEATH
The Univ. of Western Ontario, London, ON, Canada

Abstract: A 10-minute bout of moderate to heavy intensity aerobic exercise provides a boost to executive-related oculomotor function in healthy young adults. Notably, research suggests that older adults (> 55 years of age) at-risk or with early signs of cognitive decline may accrue the largest benefit from both single-bout and chronic exercise participation. Thus, and because the prevalence of older adults with executive deficits is an increasing population challenge it is important to determine whether engaging in brief, yet specific intensities of aerobic exercise ameliorate the deficits associated with cognitive decline. In the present study, participants with and without a self-reported cognitive complaint (sCC) completed a VO_2max test. Subsequently, participants completed three separate 10-minute treadmill-based aerobic exercise sessions at participant-specific moderate (i.e., 80% below lactate threshold), heavy (i.e., 15% of the difference between lactate threshold and VO_2max) and very-heavy (i.e., 50% of the difference between lactate threshold and VO_2max) intensities. Pre- and post-exercise executive function was examined using the antisaccade task (i.e., goal directed eye-movement mirror-symmetrical to a visual stimulus). Antisaccades provide a reliable tool for evaluating executive function in persons experiencing cognitive decline due to the temporal precision of the measurement technique combined with the task's hands- and language-free nature. Furthermore, work has shown that directionally correct antisaccades are supported via the same frontoparietal networks that show modulation following single and chronic exercise protocols. Results demonstrated that older adults with and without sCC experienced reliable decreases in post-exercise antisaccade reaction times without a concomitant change in the frequency of directional errors - a result that was independent of exercise intensity. Accordingly, our results demonstrate that older adults with and without a sCC show a benefit to executive function following moderate to very-heavy intensities exercise sessions as brief as 10 minutes.

Disclosures: A. Petrella: None. **M. Heath:** None.

Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 399.15/KK10

Topic: E.01. Eye Movements

Title: The effect of normal ageing on minimally delayed oculomotor response (MDOR) task performance

Authors: *P. C. KNOX, N. PASUNURU
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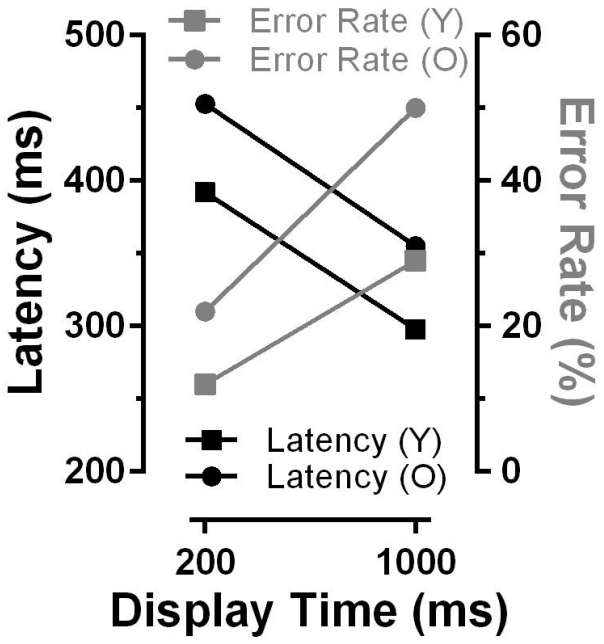
Abstract: The MDOR task (participants inhibit saccades to target onsets and instead saccade to target offsets), provides an oculomotor method of measuring behavioural inhibitory control (BIC; Wolohan & Knox, 2014, Exp Brain Res 232:3949). As the extent to which older patients

with neurodegenerative disease show BIC deficits is of considerable interest, we investigated the effect of healthy ageing on MDOR task performance. We compared 13 older, healthy participants (Group O: mean age:60y; range:50-70y) and 51 younger participants (Group Y: mean age:22y; range 19-27y) using a synchronous MDOR task. After a randomised fixation period (1-1.5s), a central fixation target was extinguished and a saccade target immediately appeared 5° to either left/right with a display time (DT) of either 200ms or 1000ms (DT and direction randomised). Participants were instructed to maintain fixation centrally and saccade to the target position on target offset. Eye movements were recorded using an infrared eye tracker; latency and amplitude of target directed primary saccades was measured. Saccades occurring <80ms post target *offset* were classed as errors. The pattern of response in Group O was identical to that observed previously; latency to offsets was much longer than for reflexive prosaccades. Latency also increased and error rate decreased for DT 200ms tasks (intersubject mean±SD latency: 453±88ms; error rate: 22±13%) compared to DT 1000ms tasks (latency: 355±111ms; error rate: 50±18%). Latency (Fig:Black) was longer and error rate (Fig: Grey) higher in Group O. When tested with a repeated measures ANOVA (DT: 200 vs 1000 within; O vs Y between factor), both factors returned a statistically significant result for latency (DT: $F_{1,62}=51$; $p<0.001$; Group: $F_{1,62}=15$; $p<0.001$) with no interaction. Error rates were affected similarly (DT: $F_{1,62}=140$; $p<0.001$; Group: $F_{1,62}=16$; $p<0.001$; DT x Group: $F_{1,62}=8$; $p=0.006$). These results confirm a significant effect of normal ageing on MDOR task performance, implying that normal ageing affects both oculomotor inhibition specifically and BIC more generally.



Disclosures: P.C. Knox: None. **N. Pasunuru:** None.

Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 399.16/KK11

Topic: E.01. Eye Movements

Title: Voluntary saccade training in healthy adults

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Abstract: During neurologically healthy aging, function of ballistic eye movements known as saccades gradually decreases. Saccades are key to maintaining the image of salient features of the environment on the high visual acuity fovea of the retina. Older adults display increased saccade latency and duration. Additionally, older adults perform greater numbers of compensatory saccades, which devote more cognitive resources to reaching a target(1). Studies have shown that visual function can be improved through various techniques, but none have shown improvement in voluntary saccade function using saccade training. We report on a training method that follows the principle of specificity to improve centrally guided voluntary saccade function. Five adults, between the ages of 40 and 65, were trained to perform voluntary saccades to points at angles 10°, 20°, 30°, 40°, 45°, and 50° from the center of a computer screen at the eight cardinal and intercardinal directions. In each of the eight sessions of the four-week intervention period, participants trained at three amplitudes. Participants performed 40 trials for each amplitude, in randomized directions, such that five trials were performed for each direction at that amplitude. All amplitudes were trained equally over the course of the intervention. Saccades were recorded with targets at 10°, 20°, and 30° pre- and post-intervention. During this testing, participants' saccades were recorded using the SR Eyelink-II system (SR Research Ltd., Ottawa, Ontario, Canada) and analyzed for amplitude, latency, and duration of the first saccade, as well as the number of saccades to reach the target. An ANOVA of mixed effects showed a decrease in saccade latency for the right eye, p-value = 0.03 (SAS Institute Inc, Cary, NC, USA). Increases in normalized saccade amplitude and decreases in saccade count needed to reach the target yielded p-value = 0.1 and p-value = 0.1 respectively. A statistical power calculation estimates the required sample size of n = 10 participants to achieve significance for these three measures of saccade performance. Decreased voluntary saccade latency may be caused by a decrease in required perceptual processing time. The results of the intervention on these outcomes are considered for the use of saccade training in healthy aging adults and neurological diseases that affect these aspects of saccades, such as Parkinson's disease.
1. Fernandez-Ruiz J, Peltsch A, Alahyane N, Brien DC, Coe BC, Garcia A, Munoz DP. Age related prefrontal compensatory mechanisms for inhibitory control in the antisaccade task.

NeuroImage. 2017;165:92-101. doi: 10.1016/j.neuroimage.2017.10.001. PubMed PMID: 28988829.

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Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 399.17/KK12

Topic: E.01. Eye Movements

Support: NRF No.2016R1D1A1B01006813

Title: A study on characteristic of spontaneous eye blink and sustained attention between young and old adults while watching visual stimuli

Authors: *Y. SHIN[1,3], W. KIM[3], I. SHIN[3], D. CHOI[3], S. CHAE[3], K. KIM[2], D. JUNG[4]
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Abstract: Many researches confirmed that eyeblink rate reflects visual attention, and many attempts have been made to understand the audience preferences through the changes in spontaneous eyeblink rate according to concentration levels on different visual stimuli. However, it is hard to find studies that considered the characteristics of spontaneous eyeblink rate and sustained attention while watching visual stimulus, therefore a study on the characteristics of spontaneous eyeblink rate and sustained attention while watching various types of visual stimulus between young adults and elders were compared in this research.
A total of 95 healthy subjects were participated in the experiment. The young adults group was consisted of 45 adults aged from 20 to 30(M = 24.0, SD = 3.1). The elderly group was consisted of 50 adults aged from 59 to 90(M=70.9, SD=6.6). There were five different types of visual stimuli: fixation (5min), documentary (5min), advertisement video clip (5min), TV program (60min), modified vigilance task (10min), and the order of task lists was randomized. First of all, the results from vigilance task were analyzed to verify the characteristics of sustained attention and spontaneous eyeblink rate. For analysis, (1) mean RT (mRT; the mean response times for all trials) and (2) Correct Rate (CR; the number of correct answers/all responses) were used to compared with the spontaneous eyeblink rate and the correlations between each element were evaluated.
The average response time (mRT) in young adults (0.50.1) was approximately 5 seconds faster than the average response time in elderly (1.00.4), and the rate of correct answer(CR) was

significantly higher($p<0.01$) in young adults (97.0%0.7) than it was in elderly (92.8%.4). Moreover, the rate of eye blink in young adults (0.470.12) was significantly lower ($p<0.01$) than the rate of eyeblink in old adults (0.930.19). Also, there was a significant positive correlation between eyeblink rate and the average response time ($r2=.456, p<0.01$), and a negative correlation between eyeblink rate and the rate of correct answer($r2=-.469, p<0.01$).
This research verified the characteristics of spontaneous eyeblink rate and sustained attention in young and old adults. Also, it was confirmed that the EBR and sustained attention ability are correlated. These results are expected to be used as an intuitive index for visual attention to explain the audience preferences in elderly.
This research was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education(No.2016R1D1A1B01006813)

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Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 399.18/LL1

Topic: E.01. Eye Movements

Title: The interaction of eye and hand movements in visual reaching task in hereditary spinocerebellar degeneration

Authors: *S. INOMATA-TERADA[1], S.-I. TOKUSHIGE[1], S.-I. MATSUDA[2], M. HAMADA[3], S. TSUJI[3], Y. UGAWA[4], Y. TERAO[1]
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Abstract: Introduction: Eye and hand movements are known to be closely linked in daily actions (eye-hand coordination), and the cerebellum plays important role not only in each movement but in their coordination. Negative reciprocal interactions between the eye and hand movements are reported when patients with cerebellar dysfunction perform a target tracking task (van Donkelaar et al.,1994). We devised a system in which we can record simultaneously the trajectory of hand and eye when subjects performed a visually guided reaching task, in a manner similar to the finger-nose test performed to detect the cerebellar dysfunction in clinical practice. The aim of this study was to investigate how cerebellar dysfunction affects this coordination.
Methods: Subjects were 8 SCA patients (SCA6 or SCA31) with pure cerebellar symptoms and 9 age-matched normal controls (NC). In a visually guided reaching task, a fixation spot was presented in the center of the touch panel, which the subjects fixated and touched with the index finger. At a random interval, this fixation spot moved to a peripheral position, to which the

subjects were to instructed to move their finger from the center position by sliding on the monitor. A video-based eye tracker recorded eye movements, while a touch panel recorded the trajectory of finger movements during the task. **Results:** In most trials, the eyes preceded the finger movements to the target. In SCA, the amplitude of both eye and finger movements was more dispersed than in NC, with the magnitude of dispersion is more pronounced for the eyes. The latency of finger movements in SCA was longer than that in NC. The interval between last saccade and start of the finger movement was also longer in SCA than in NC, resulting in further delay of finger movement. **Conclusion:** The accuracy of eye movements did not directly translate into that of hand movements. The time interval between the onsets of saccades and finger movements was significantly greater in SCA patients than in NC. This was not only because the eye took longer to reach the target location, due to the multiple saccades or curved trajectory of the saccade, but also because the start of hand movement after gaze fixation was delayed.

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Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 399.19/LL2

Topic: E.01. Eye Movements

Support: NSERC Discovery Grant
CFI John R. Evans Leaders Fund

Title: Eye and hand movement characteristics during rapid go/no-go decisions in Parkinson's disease

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Abstract: Parkinson's disease (PD) is a neurodegenerative movement disorder that classically causes motor symptoms such as muscle rigidity, tremors, and changes in gait. However, the majority of PD patients also experience non-motor deficits in sensation, perception, and cognition as well as characteristic eye movement impairments. Eye movements are critically important for a large range of natural tasks, including interactions with dynamic objects, such as hitting or catching a flying ball.
Here we investigate the role of eye movements in sensorimotor decision making and manual interception in early stage PD patients (n=12) with mild to moderate symptoms, tested ON and

OFF medication, and age-matched healthy controls (n=8). We developed EyeStrike, a go/no-go manual interception task, in which observers had to predict whether a ball-like target, shown on a computer screen, would pass through (hit) or go by (miss) a designated strike box. Observers were instructed to judge hits or misses by either intercepting the ball with their index finger in the strike box (hits), or by withholding the hand movement (misses). Only the initial launch (300, 500, or 700 ms) of the ball was shown, requiring observers to extrapolate the ball trajectory to decide whether and where to intercept. Observers tracked the ball with a combination of smooth pursuit and saccadic eye movements and received feedback at the end of each trial. Eye and hand movements were recorded with a video-based eye and magnetic hand tracker.
As expected, patients showed impairments in eye movement initiation, hand movement execution, and an overall reduction in movement speed. However, patients compensated for this deficit by initiating hand movements earlier, and exhibited overall similar hitting accuracy and decision-making ability as controls, regardless of medication status. These results indicate that early stage PD patients show preservation of function in rapid go/no-go decision making and are able to compensate for motor slowing by adapting their hand movement strategy.

Disclosures: J. Fooken: None. **G.K. Mann:** None. **C.B. Jones:** None. **M.J. McKeown:** None. **M. Spering:** None.

Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 399.20/LL3

Topic: E.01. Eye Movements

Support: Force Health Protection Program of the Office of Naval Research (SAA 402925-1)

Title: Increased dependence on saccades for ocular tracking with low-dose alcohol

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Abstract: The decline in smooth pursuit performance following moderate-dose alcohol has been shown in previous studies. Fransson and colleagues (Clin Neurophysiol, 121(12):2134-2142, 2010) showed that a steady-state gain decrease of 2% and 7% at 0.06% and 0.1% blood alcohol concentration (BAC), respectively. The current study examines the detailed features of both the pursuit and saccadic components of the tracking response over a wider range of BACs extending down to zero. Thirteen healthy subjects (8 females, mean age $\pm$ SD = 25.2 $\pm$ 2.1 years) participated. Subjects completed a 3-day at-home pre-study schedule that included 8.5 hours in

bed at night with the timing verified by actigraphy, call-ins, and self-reported sleep logs. They then participated in a 2-day laboratory study where they consumed a single low-dose of ethanol (40% ABV Vodka with juice) adjusted to target either 0.02 or 0.06% initial BACs, and completed three pre-dose and 6-9 post-dose oculomotor test runs of a 5-minute Rashbass-like ocular tracking task with highly randomized target trajectories (Krukowski & Stone, Neuron, 45(2):315-323, 2005). From this oculomotor test, a set of largely independent measures of oculomotor (Liston & Stone, JOV, 14(14):12, 2014) and pupillary (Tyson, Flynn-Evans, & Stone, JOV, 17(10):660, 2017) performance were computed. For each of these metrics, for each subject, we computed the within-subject % deviation of each test run from their baseline (the average across their three pre-dose runs). We then combined the data across subjects and used linear regression to compute the slope and x-intercept (extrapolated threshold) of the mean % deviation from baseline as a function of BAC. We found significant linear decreases in steady-state pursuit gain (-3.8%/0.01%BAC, $p < 0.001$) and proportion smooth (-1.0%/0.01%BAC, $p < 0.01$) with estimated thresholds of +0.013 and -0.007%BAC, respectively. We also found a significant decrease in the precision of visual motion processing for direction (+9.2%/0.01%BAC with a threshold of +0.008%, $p < 0.01$), but not for speed ($p = 0.48$). Conversely, we found a significant increase in catch-up saccade amplitude (+9.4%/0.01%BAC, $p < 0.001$) and rate (+2.38%/0.01%BAC, $p < 0.05$) during steady-state tracking, with thresholds of +0.009 and -0.019%, respectively. Our study, using high-uncertainty target motion, reveals that the smooth pursuit system is more sensitive to ethanol than previously thought. Ethanol consumption generates a dose-dependent increase in reliance on the saccadic system to maintain steady-state tracking as pursuit becomes substantially less effective, starting at BAC levels around 0.01%.

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Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 399.21/LL4

Topic: E.01. Eye Movements

Support: NSERC Canada (RGPIN-2014-04361)

Title: Changes in motor cost affect gaze-limb coordination

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Abstract: The brain usually tries to minimize the cost to move (or motor cost). When walking, for example, we normally select a step length and width that minimizes the motor cost of foot placement and the overall walking pattern. However, when the terrain and decision of where to step becomes more complex, moving optimally is not always an option. In these situations, we rely on vision to help select a step location. Here, we determined how motor cost affects the coordination of gaze behavior and foot placement. In one experiment, participants (N=5) walked while stepping on the center of four targets in sequence. To create six different levels of motor cost, we manipulated the step vector on a trial-to-trial basis by shifting the second or third stepping target from the established preferred step width based on the participant's leg length. Participants maintained the same level of foot-placement accuracy across all step motor costs. Participants also made a saccade away from the target they were about to step on sooner when encountering the lowest two motor cost targets compared to the highest two cost targets (one-way ANOVA: p=0.001). This suggests that participants adapted to the greater stepping motor cost by increasing the visual online control of the limb to maintain foot-placement accuracy. In a second experiment, participants (N=4) performed a similar walking task but had a choice of two targets in certain positions of the sequence. One target was always at the participant's preferred step width location (or lowest motor cost), whereas the other target was in one of the five remaining higher motor cost locations, as described above. When participants were free to choose where to step, they directed gaze to the low cost target and always stepped on it as well. In fact, they rarely fixated the higher cost target. However, when we forced participants to step onto the higher cost target, they had more variable gaze behavior. Specifically, we found an increase in the probability to direct gaze to both low and high cost targets, rather than just the latter (main effect of instruction: p=0.018). There are at least two possible explanations for this finding. First, the visuomotor system is primed to prioritize targets that are associated with low cost. Second, the visuomotor system explores both target choices to help calculate the change between the low and high cost target to generate the appropriate motor plan for the step. Taken together, our results suggest that motor cost affects gaze behavior, and that people can readily adjust their gaze patterns to ensure movement accuracy in complex environments.

Disclosures: J. Dominguez-Zamora: None. **D.S. Marigold:** None.

Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 399.22/LL5

Topic: E.01. Eye Movements

Support: CAS Grant QYZDB-SSW-SMC032
NSF Grant 31722025

Title: Eye-hand coordination during motor learning in humans

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Abstract: When we reach for an object, our eyes often guide toward the object as well. The behavioral pattern of both eye and hand movements directing to an object is well known as eye-hand coordination. We investigate interactions between eye and reaching movements in two types of behavioral tasks: 1) motor control, where subjects were asked to reach a target randomly located in one of 13 positions in a half circle (from 0^o to 180^o, by every 15^o). 2) motor learning, where subjects were given visual feedback information of the reaching endpoints after a zooming transformation defined by a linear function. When introducing a zoomed visual feedback on a single position, subjects adapted their behavior performances toward to the zoomed point. This learned effect was generalized to all other positions after learning. When applying the function on two (or more) positions, subjects were able to gradually learn the function on the whole variable space. A Gaussian Process Regression model based on Bayesian Average reproduces the statistics of a complex set of behavioral data.

Disclosures: Y. Yang: None. **D. Sun:** None. **T. Zhu:** None.

Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 399.23/LL6

Topic: E.01. Eye Movements

Support: CIHR

Title: To reach or not to reach: Coordination of eye, head and hand movements during visually guided reach

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Abstract: Much emphasis has been placed on trying to understand the motor control principles behind the coordination of complex multi-movement systems in humans. Previous research has provided insight into eye-head and eye-hand coordination within a 2D plane, but there is little understanding of the more natural condition of eye-head-hand coordination during a 3D reach in humans. This study investigates the relative contribution of the head to gaze movement, in a head free paradigm. In this experiment, participants (n = 6) were instructed to perform a saccade

with and without an arm movement towards a visual target at any of the 25 predefined locations on a 40° horizontal x 20° vertical (visual angle) computer screen. Right eye movements were measured using *EyeLink II* eye tracker and two Optotrak cameras were utilized to monitor hand and head position in space. Preliminary results revealed that average head movements were greater when a saccade was accompanied by an arm movement towards a visual target (2.901 ± 0.103º) compared to the control gaze condition (0.722 ± 0.084º). Additionally, the overall peak velocity of head movements that were accompanied by arm movements were faster (6.065 ± 0.231º/sec) compared to the gaze control condition (2.014 ± 0.187º/sec). Contrary to previous research on rhesus monkeys and humans, analysis on eye movements revealed no significant interaction between the average peak eye velocity and the type of condition from 16.862 ± 0.339º/sec to 17.151 ± 0.4115º in gaze control and reach condition, respectively. However, the overall eye gaze error is significantly less when a saccade is accompanied by an arm movement from an average of 3.290 ± 0.088º in the gaze control to 2.766 ± 0.114º in the reach condition. These results show that reaching influences not only gaze behavior, but also eye-head coordination patterns, possibly to optimize 3D vision for grasping.

Disclosures: J. Crawford: None.

Poster

399. Eye Movements: Saccade Behavior and Psychophysics in Humans

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 399.24/LL7

Topic: E.01. Eye Movements

Title: Eye movements and their relation to motor performance during a simultaneous whole-body imitation task

Authors: *P. I. BURGOS[1,2], M. BOCAZ[2], K. MUÑOZ[2], J. MARIMAN[2]
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Abstract: Previous studies state that when imitating a motor task the gaze focuses on distal points of the segment to be imitated. However, these studies use static images of isolated body segments, partial segments of the body such (as the hand or face), which may limit their projections.
The aim of our study was to identify the patterns of eye movements that are related to a better motor performance during the simultaneous imitation of movements of whole-body in the frontal plane (video).
19 participants performed the task of simultaneous imitation, with recordings of eye movements and kinematics of 4 joints (shoulders and hips) by inertial sensors.
The results showed that the participants with better performance in the imitation sequence, had

fewer fixations, longer duration of fixations, shorter duration of saccades, and a greater preference for distal areas.
The partial conclusion of our analysis allows us to relate a pattern of fixations with a better performance during a simultaneous imitation of the whole body. These results may have implications in disciplines that require teaching this type of gestures such as physical therapy, sports or video games.

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Poster

400. Voluntary Movements: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

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Program #/Poster #: 400.01/LL8

Topic: E.04. Voluntary Movements

Support: DFG Grant He 6368/1-1
ERC Grant EU-ERC-283567
NWO Grant NWO-VICI 453-11-001

Title: Dynamic coding of sensory and motor information during hand movements directed to tactile targets

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Abstract: The location of tactile information is initially coded with respect to the skin surface. To move towards the touch, skin location must be integrated with postural information to compute the external location to which the effector must be directed. Posterior parietal cortex (PPC) has been linked to external spatial coding, as well as to the transformation between different spatial codes in touch. However, it is currently unclear which PPC regions contribute to tactile-spatial coding, which spatial codes they employ, and how spatial transformations proceeds between them.
We performed multi-voxel pattern decoding of fMRI BOLD brain signal changes in a delayed, goal-directed pointing paradigm with tactile targets. Human participants pointed, with the right hand, to the precise location of tactile stimuli delivered to their feet, while the legs were either uncrossed or crossed over the shins. With crossed limbs, skin-based and external coordinates are in opposite sides relative to the body midline (e.g., right foot in left space). In each trial, a stimulus was presented, followed by a delay (sensory phase, 1-4 TR, with TR = 1.88 s). Hereafter, a cue instructed either a pro-pointing towards the precise stimulus location, or an anti-pointing towards the same location on the other foot, followed by a second delay (motor

planning phase, 1-4 TR). Anti-pointing, in combination with limb crossing, dissociates the movement goal from both skin-based and external sensory stimulus information. A final cue instructed movement execution.
During the sensory phase, skin-based coding was evident in primary somatosensory and an anterior region of PPC. External coding was evident in a distinct, more posterior PPC region. During the movement planning phase, both skin location and external stimulus location were no longer decodable from PPC activity. A network including primary motor, premotor and PPC now represented information about goal location of the movement. Many of these regions are known to be active when pointing and reaching to visual targets. Thus, goal-directed movement planning appears to recruit similar regions independent of the sensory modality of the movement target. Critically, the network active during movement planning included the regions in which skin-based or external coding had been decodable during the sensory phase. This result suggests that these anterior and posterior PPC regions dynamically adapted their spatial coding from different kinds of sensory-based codes to a common movement-based code during the course of a trial.

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Poster

400. Voluntary Movements: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

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Program #/Poster #: 400.02/LL9

Topic: E.04. Voluntary Movements

Support: AMED-CREST
Grant-in-Aid for JSPS Research Fellows

Title: Cerebellar outputs contribute to the activity of the primary motor cortex during arm-reaching movement in macaque monkeys

Authors: *N. SANO[1,2,3], Y. NAKAYAMA[1], E. HOSHI[1], S. CHIKEN[4,5], A. NAMBU[4,5], Y. NISHIMURA[1]
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Abstract: The cerebellum (Cb) plays an important role in coordination and execution of limb movements, as evidenced by the fact that Cb dysfunctions cause delayed movement initiation, dysmetria, and intention tremor. Cb outputs project to the contralateral motor cortices, including the primary motor cortex (M1), via the ventral lateral thalamic nucleus. Thus, Cb may contribute

to control of motor outputs by this cerebello-thalamo-motor cortical pathway. However, it is unclear how Cb outputs contribute to activity of the M1 during movements. In order to address this question, we identified M1 neurons that received Cb outputs (Cb-M1 neurons) and investigated their activity during arm-reaching movements in macaque monkeys. We recorded neuronal activity in the forelimb region of the M1 and examined Cb inputs by responses to the electrical stimulation (0.1-1.0 mA, 0.3 ms duration, single pulse) of the deep Cb nuclei through chronically implanted electrodes. Then, we recorded their activity during voluntary arm-reaching movements. The following results were obtained: 1) Cb-stimulation induced initial excitatory or inhibitory responses in 144 neurons among 559 neurons recorded in the M1. Initial excitatory responses were usually followed by inhibitory responses. Based on the initial response, M1 neurons can be classified into Cb-M1 neurons with excitatory response, Cb-M1 neurons with inhibitory response, and non-Cb-M1 neurons. 2) The majority of the Cb-M1 neurons with excitatory response to Cb-stimulation increased their activity during reaching movements. 3) The Cb-M1 neurons with inhibitory response decreased their activity more frequently than the Cb-M1 neurons with excitatory response. 4) Mean firing rate of the Cb-M1 neurons was higher than that of the non-Cb-M1 neurons during all period of the task. 5) Direction selectivity (feature of neuron that shows preferred direction in arm movement) of Cb-M1 neurons found to be similar to that of non-Cb-M1 neurons. 6) Cb-M1 neurons with excitatory response, those with inhibitory response, and non-Cb-M1 neurons showed different activity patterns during arm-reaching movement. These findings suggest that Cb outputs contribute to the activity of M1 neurons during arm-reaching movements.

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Poster

400. Voluntary Movements: Reaching Control: Action and Sensation

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Program #/Poster #: 400.03/LL10

Topic: E.04. Voluntary Movements

Support: MEXT KAKENHI (25240019)
MEXT KAKENHI (16H01610)
MEXT KAKENHI (18H03502)

Title: Explicit sense of agency emerges from dynamic network of neural oscillations

Authors: Y. SAKURAGI, *H. MIZUHARA
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Abstract: Consciousness is a core function of human beings, and attracting many researchers in neuroscience. Sense of agency (SOA) is one of topics to investigate the underlying mechanism of consciousness. It is a sense that an action or its result is caused by a one's own intention. "Comparator model" could imply an importance of neuronal networks for an implicit SOA (feeling of agency). However how neuronal networks contribute to an explicit SOA (judgment of agency) is still an open question. We investigated neuronal networks for judgment of agency based on EEG synchronization.
Nineteen people participated in a scalp EEG experiment. The experiment procedure was approved by the institutional ethics committee, and all participants provided an informed consent before the experiment. During the experiment, the participants performed a reaching task of the right hand within a virtual reality environment wearing a head mount display. We manipulated SOA by a delayed visual feedback of the participant's right arm movements. The experiment was consisted of two conditions, including SOA judgment and delay judgment. The SOA judgment condition included the movement retrieved by the participant's own past actions as distractors. Participants were required to judge whether the arm movement was their own present action for the SOA judgment condition, and whether there was a temporal delay in the visual feedback for the delay judgment condition.
The behavioral results showed that the number of own action responses and no-delay responses were modulated with a function of temporal delay. The own action responses reached chance level with a large delay, while the no-delay responses were almost perfectly reported. We thus succeeded to manipulate the explicit SOA with our task. The EEG results showed that the low beta amplitude of the frontal midline electrodes increased in association with SOA judgment. Phase synchronization decreased between the lateral-central and occipito-parietal electrodes, and increased between the lateral-central and frontal electrodes. These results indicate that SOA emerges from the local network in the frontal region in combination with desynchronizing the global network between the sensorimotor and occipito-parietal regions.

Disclosures: Y. Sakuragi: None. **H. Mizuhara:** None.

Poster

400. Voluntary Movements: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 400.04/LL11

Topic: E.04. Voluntary Movements

Title: A comprehensive analysis of human wrist proprioception through robot-based assessment

Authors: *F. MARINI, P. MORASSO
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Abstract: “A PubMed search on the subject of *proprioception* and *rehabilitation* will generate close to 2,300 related publications. If one adds the term *wrist*, however, the number of articles is dramatically reduced to 20”. This is how the scientist Elisabet Hagert introduced her brilliant review about proprioception of the wrist in 2010[1]. If one carries out the same search today, 7 years later, he will find 7640 scientific articles on proprioception in general, with 86 articles specific for the wrist joint. Although such numbers suggest an increasing interest in the field, nonetheless it remains an area of research not comprehensively and extensively investigated. An understanding of proprioception is essential to adequately rehabilitate patients with sensorimotor deficits resulting from neurological damages. Yet, at present, there is no established objective method for the assessment of proprioception, and despite clinical rating scales are available and currently in use, they allow to obtain only qualitative information with low resolution. Recent advancements in haptic interfaces, provide the starting point for an innovative robot-aided approach for the assessment of proprioceptive function. Such technologies may allow the implementation of tests for proprioceptive acuity assessment and for the collection of large normative data sets through a reliable procedure that yields objective data at a high resolution. Here we intend to present the results of a robot-aided assessment of joint position sense acuity for the three degrees of freedom of the wrist/hand complex in a cohort of adults and typically developing children. The task employed a joint position matching paradigm in which individuals must replicate a previously assumed reference joint position in the absence of vision. This test allowed us to characterize proprioceptive abilities of each degree of freedom (DoF) of the wrist (Flexion/extension, radial/ulnar deviation, pronation/supination) in term of acuity, measured as the error made while actively replicating a reference joint angle. Furthermore, we investigated the age-related changes of proprioceptive acuity during development and determine when children begin to reach adult levels of proprioceptive acuity. Finally we studied the contribution of external forces on our perception of body and joint configuration. The proprioceptive acuity was tested under five different external forces, in order to understand to what extent they affect proprioceptive acuity. [1]E. Hagert, “Proprioception of the wrist joint: a review of current concepts and possible implications on the rehabilitation of the wrist.,” J. Hand Ther., vol. 23, no. 1, p. 2-16;

Disclosures: F. Marini: None. **P. Morasso:** None.

Poster

400. Voluntary Movements: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 400.05/LL12

Topic: E.04. Voluntary Movements

Support: NIH Grant 5R01NS050256

Title: Information encoded by motor cortical neurons changes discretely during reaching

Authors: *S. B. SUWAY[1], A. B. SCHWARTZ[2]
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Abstract: Activity patterns of motor cortical (M1) neurons during reaching are often complex. Direction is encoded by M1 neurons during reaching, but this parameter by itself does not account for the complexity observed in firing rates. A recent study from our lab (Suway et al, 2017) found that simple components helped explain these complex patterns. We showed that directional tuning of M1 neurons is associated with discrete time epochs during a single reach. Tuning remains stable within an epoch, and changes abruptly between epochs. Although changes in encoding were found to be discrete upon analysis, the nature of the drivers responsible for these changes was unclear. Here, we present new findings that suggest separate neural operations may be taking place in each epoch. Two macaques were implanted with electrode arrays in M1 and were trained to make reaches in a virtual-reality (VR) environment. The animals could not see their hands directly, but instead observed a cursor in VR representing the hand's position. In the standard task, the direction of cursor movement matched the direction of hand movement. In a second condition, the direction of cursor movement was rotated relative to the direction of hand movement. Most neurons exhibited the same epochs in both conditions, but the tuning within an epoch was often better described in terms of the cursor direction rather than the hand direction. Crucially, this relationship changed discretely and abruptly between epochs for single neurons: a neuron with "hand direction" tuning in one epoch would often also display "cursor direction" tuning in another epoch. This effect was consistent over a large range of cursor rotation angles. There was no obvious temporal order across the population; some neurons displayed a "hand direction" epoch prior to a "cursor direction" epoch, and other neurons displayed the opposite ordering. While reach direction is a known driver of M1 activity, visual information is thought to be less prominent in this brain area (Schwartz et al, 2004). Here, we found visual information (the cursor direction) was strongly represented by many neurons throughout a trial, and reasoned this information would be evident in a low-dimensional view of population activity. Using a canonical correlation analysis, we found two orthogonal vectors in neural space that robustly represent the x- and y- dimensions of cursor movement, tracking its orientation accurately before and after the cursor direction was dissociated from the hand direction. Our findings lend key new insight into the operational principles of M1 and bolster the idea that tuning changes in single M1 neurons are related to discrete representations of information.

Disclosures: S.B. Suway: None. **A.B. Schwartz:** None.

Poster

400. Voluntary Movements: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 400.06/LL13

Topic: E.04. Voluntary Movements

Support: NRF-2016R1D1A1B03936326
NRF-2017M3C7A1047227

Title: The relationship between the sensory information and the awareness of action in reaching movement

Authors: ***J.-K. RYU**[1], S. KIM[2]
[1]Inst. for Cognitive Sci., Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Neurosci. Res. Inst., Gachon Univ., Incheon, Korea, Republic of

Abstract: The feeling of being in control and of being the author of one's movements is an important aspect of bodily self-consciousness. During the voluntary motor performances the actor should experience physical phenomenon and sensory consequences about body movements. The consciousness of action can be constituted through the integration of information from the central motor command and the peripheral sensory signals. Those experiences are the most important sources of the sense of agency and the belief of being the author of one's movements.

To explore the relation between sensory information and the consciousness of action in perceptual motor performance, ten healthy adults were recruited and were instructed to perform a visually guided reaching tasks. The task was to trace sagittal lines on a perceptual-motor experimental device. The hand was hidden by a mirror in which participants saw the lines projected on a computer monitor. In unperturbed trials the cursor seen in the mirror exactly corresponded to the participant's hand. In perturbed trials a bias was introduced so that the cursor was deviated in one direction by experimental conditions. Subjects consistently compensated their hand in the opposite direction for producing a target trajectories. After each trial they were asked in which direction they thought their hand had moved. Data was processed and analyzed with customized computer software.

In perturbed trials participants largely could not estimate the actual hand direction. Multiple linear regression analysis revealed that the verbal responses were determined by visual feedback during early and late phase of the reaching tasks. In a second session using the same experimental paradigm a motor response was asked for subjects to retrace their hand trajectory during each trial by drawing a line without visual information. Participants' responses generally indicated an incorrect conscious monitoring of motor performance as well as verbal response. In addition the motor responses during late phase of the perceptual motor tasks largely reflected the kinesthetic feedback information.

The conclusions are as following:

First, Sensory information which arises during performing motor tasks are important ingredients of consciousness of motor action.

Second, in early phase of reaching movement consciousness of action mostly rely on visual information.

Third, Consciousness of action include information which can be presented by body movements whereas not be presented by verbal report.

Disclosures: J. Ryu: None. **S. Kim:** None.

Poster

400. Voluntary Movements: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 400.07/LL14

Topic: E.04. Voluntary Movements

Support: NSERC

Title: Spinal stretch reflexes show sophisticated tuning that supports hand control

Authors: *J. WEILER[1], P. L. GRIBBLE[3], A. PRUSZYNSKI[2]
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[3]Brain and Mind Institute, Dept. Psychology, Western University, Canada, London, ON, Canada

Abstract: The nervous system generates movement that adheres to the so-called 'minimum intervention principle' but it is unclear which neural circuits implement the control laws that produce such behaviour. One idea is that these control laws are exclusively derived within a transcortical feedback pathway centered on primary motor cortex. Here we show that even the fastest spinal feedback pathway can produce solutions consistent with the minimum intervention principle. In our first experiment, participants placed their hand on a spatial target and we mechanically flexed their elbow - stretching the triceps muscle - and simultaneously flexed, extended or did not change their wrist angle. These perturbations displaced the participants' hand off the target, but critically, the perturbation that yielded the largest hand displacement relative to the target did so with the least amount of elbow flexion. We found that the triceps' spinal stretch reflex was tuned to the hand's displacement, and thus the amount of elbow extension needed to return the hand to the target, and not the amount of elbow flexion. In our second experiment we ruled out the possibility that our initial results simply reflected hardwired connections from wrist afferents to triceps motorneurons. We applied the same perturbations as before, but critically, participants responded using two different arm orientations that diametrically altered how local wrist rotation translated to movement of the hand. For example, for one orientation, flexing the wrist moved the hand away from the target, whereas in other orientation, flexing the wrist moved the hand towards the target. Strikingly, the triceps' spinal stretch reflex was again tuned to the hand's displacement from the target rather than the elbow's rotation. In fact, changing the arm's orientation diametrically altered the pattern of the triceps' spinal stretch reflex and did so in a way that was appropriate for returning the hand to its initial location. In our third experiment, we tested whether our initial results are also observed during reaching actions. We occasionally applied the same perturbations as before, but did so when participants began reaching to a goal-target that required pure elbow extension. We found that the triceps' spinal stretch reflex was tuned to the hand's distance from the goal-target, and not the amount the elbow was flexed.

These collective findings reveal that the fastest spinal feedback pathway is capable producing corrective responses that adhere to the minimum intervention principle, forcing a re-evaluation of the how the nervous system derives the sophisticated control laws that support natural motor behaviour.

Disclosures: J. Weiler: None. **P.L. Gribble:** None. **A. Pruszynski:** None.

Poster

400. Voluntary Movements: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 400.08/MM1

Topic: E.04. Voluntary Movements

Support: DFG Hi 1371/1 - 2

Title: Out of sight, not out of reach: Activity in the superior colliculus associated with reaching for tactile targets

Authors: *N. G. PRABHU[1,2], M. HIMMELBACH[1]
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Abstract: The superior colliculus (SC) has been implicated in orienting gaze and arms towards an object of interest. Electrophysiological studies in non-human primates so far have reported the involvement of the SC only in visually guided movements. Two previous studies from our lab reiterated this finding in the human SC using fMRI. With this study we sought to decipher if the SC is exclusively involved in visually guided reaching movements or might also be involved when targets are specified by proprioceptive and tactile information, i.e. somatic in nature.
The study was conducted using 3T BOLD fMRI on 6 subjects (5 females, 1 male, age range 22-44) with each subject performing the experiment 4 times, one session per day. Each experimental session had 4 conditions - visually guided reaching (VM), somatically guided reaching (SM), visual sensory (VS) and somatic sensory (SS) - with 2 conditions alternating in each of 4 runs. The left hand of the participants was fixed on a handrest with pneumatic stimulators under the fingertips and optic fibers presenting visual target stimuli above each finger. The right hand rested on a button box fixed on the participant's chest. In complete darkness, the participants either executed reaching movements with the right forearm to pneumatically stimulated left hand fingertips (SM) or to target lights presented through optic fibres (VM). In sensory control conditions, participants pressed a button if visual (VS) or somatic (SS) double stimuli occurred. Fixation at a central, dim fixation light was maintained throughout each run.
Our results showed activation in the deep layers of the SC for somatically guided reaching movements in addition to visually guided reaching movements. This goes beyond the

conventionally established role of the SC and opens up new avenues for exploration in the integration of sensory information towards reaching movements in subcortical systems.

Disclosures: N.G. Prabhu: None. **M. Himmelbach:** None.

Poster

400. Voluntary Movements: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 400.09/MM2

Topic: E.04. Voluntary Movements

Support: NWO Rubicon grant (446-17-003)

Title: Grip force in preparation for collisions

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Abstract: From research on grip force during movements it is known that people modulate grip force in phase with the characteristics of an object held to make sure the object does neither fall nor slip (e.g. Flanagan & Wing, 1993; Bleyenheuft *et al.*, 2009). Grip force planning and anticipation for interactions have not been studied extensively yet. Both Bleyenheuft et al. (2009) and White at al. (2011) found that the peak of the grip force is after the collision, suggesting that the grip force motor command anticipates the impact of the collision. In this study, we explore both temporal and force characteristics of anticipatory grip force in collisions with the static hand.
Participants (N=19) were asked to hold both handles of a bimanual endpoint KINARM (BKIN) in a precision grip with custom made integrated grip force sensors. In a collision task, participants were asked to hold the hands at the same positions while an object collides with one of their hands. We analyzed grip force patterns during preparation and collision.
Objects were presented in series of 6 collisions (right and left hand alternated) with the same mass and stiffness. We used 3 masses (2/4/8 kg) and 2 stiffnesses (1000/6000 N/m) resulting in 6 objects, all with the same visual appearance. Each object was presented in 30 series. In each series a catch trial was introduced to get direct insights in the motor planning for the interaction.
The results show that for the first collisions of each series the grip force at collision was consistent (~15N). Starting from the second collision (which was with the opposite hand) the grip force at collision diverged based on the characteristics of the objects. For collisions 3-6 grip force at collision was consistent and adjusted to the mass and stiffness of the objects.
We compared the onset of grip force increase, i.e. time at which grip force exceeds 5 STD of the baseline grip force, for each collision. The anticipation was longer for the first collision

(~150ms) compared to the following collisions (~120ms), but did not depend on the characteristics of the objects. The difference in grip force at collision between objects can be explained by the findings that both the grip force rate at collision and the baseline grip force in between collisions were related to the mass and stiffness of the object. Evidence in the results of the catch trials showed that the grip force maximum is not at collision, but ~80ms later, with a slight modulation related to stiffness.
To conclude, the timing of the anticipatory grip force is very robust and there seems to be a standardized temporal component in predictive motor planning. Furthermore, grip force is adjusted very fast to the characteristics of the target object.

Disclosures: I.A. Kuling: None. **P. Lefevre:** None.

Poster

400. Voluntary Movements: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 400.10/MM3

Topic: E.04. Voluntary Movements

Support: Biomedical Data Driven Discovery

Title: Divergent encoding of arm kinematics in the cuneate nucleus and primary somatosensory cortex

Authors: *C. VERSTEEG[1], E. R. GASPER[2], R. H. CHOWDHURY[3], T. TOMLINSON[5], L. E. MILLER[4]
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Abstract: Single neurons in the cuneate nucleus (CN), the initial site of somatosensory afferent convergence, have never been recorded during active behavior. To understand how the sensorimotor system processes feedback signals, we must first understand how sensory information is transformed as it moves centrally. We present novel data that show that the encoding of arm kinematics in the cuneate nucleus is quite different from its representation in the somatosensory cortex (S1). In S1, limb state is represented similarly, whether the result of a voluntary movement or an unexpected perturbation applied to the hand. In contrast, these kinematically-similar active and passive movements result in different responses in CN. The source of differences in CN, and subsequent processing that eliminates these differences, are unknown.
We implanted Utah multi-electrode arrays (MEAs) chronically in CN of two monkeys. One also had an MEA in area 2 of S1. These animals were trained to control a cursor by making reaching

movements while grasping a robotic manipulandum. We also used the robot to apply forces to the hand when it was at rest, that generated endpoint kinematics similar to those of reaching. We recorded from single neurons in CN and area 2 and fit Generalized Linear Models (GLMs) to neural firing as a function of hand movement. Neurons from area 2 had PDs that were approximately evenly distributed in all directions in both active and passive conditions, while neurons in CN had a significant directional bias in the active condition but not in the passive condition. This bias differed in the two monkeys. One had active CN PDs predominantly pointing away from the body, the other pointing towards the body. To confirm these biases were not an artifact simply of the highly stereotypic center-out task, we also fit GLMs to data from a random-target task in which the monkeys made rapid sequential movements to a series of target in random locations. CN PDs computed under these conditions were similarly biased, affirming that the encoding of arm kinematics in CN is dependent on movement context: whether movements are made voluntarily, or the result of a perturbation to the limb. In addition to these velocity PDs, a significant number of CN neurons were tuned to the static position of the monkey's hand in the workspace. By using vibration to activate muscle spindles, we also found CN neurons that responded to vibration of more than one muscle, an observation that conflicts with previously reported estimates of sensor convergence in CN. This knowledge has important implications for understanding how muscle length information is processed by spinal, brainstem, and transcortical loops for the control of movement.

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Poster

400. Voluntary Movements: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 400.11/MM4

Topic: E.04. Voluntary Movements

Support: NIH T32 AG052375
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NIH T32 GM081741

Title: Dynamic measures provide unique insights into motor dysfunction after stroke

Authors: A. B. THOMAS, E. V. OLESH, A. ADCOCK, *V. GRITSENKO
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Abstract: The most common long-term consequence of stroke is motor impairment, yet movement complexity makes the extent of impairment difficult to quantify. Standard clinical

assessments include kinematic measures such as speed of movement, or measures such as strength of individual muscle groups. However, the relationship of these measures with the underlying cause of movement deficit is non-linear and often subjective. We propose the use of dynamic torque-related measures into the assessment of motor disability that are more linearly related to the muscle activity underlying movement. We recruited 8 post-stroke individuals (MCA, n=4; lacunar, n=2; brainstem, n=2), 6 age-matched controls, and 9 young controls. During the experiment, participants reached to virtual targets in center-out task, as described in Olesh et al. (2017). Kinematics were recorded using 3-dimentional motion capture with Impulse by PhaseSpace, while electromyography (EMG) of 12 arm muscles was recorded with Motion Labs system. Kinematics were then used to calculate active muscle torques (τ_M), or joint moments that result from muscle activity, using inverse dynamic simulations with a 5-DOF model of the arm in MATLAB. By running simulations with and without gravity, τ_M can be divided into two components: support of limb segments against the force of gravity (τ_{MG}), and a residual dynamic torque related to phasic EMG (τ_{MD}) (Olesh et al. 2017). Principal Component Analysis was then applied to normalized EMG and torque components to compare features between groups and between affected and less affected limbs. To quantify the changes in EMG captured by τ_{MG} and $\tau_{MD,}$we projected the affected arm EMG onto the torque principle components from the less-affected arm. Despite differences in individual morbidities, EMG patterns in stroke group were consistent across repetitions of each center-out and return movement and across individuals. We observed larger EMG bursts in proximal muscles in the stroke group compared to age-matched controls (n=6), suggesting a smaller contribution of distal muscles to reaching movements in post-stroke individuals. Torque components captured a significant amount of variance in EMG in all groups, with the least amount captured in some post-stroke individuals. The proportion of variance captured by each component also varied between post-stroke individuals, potentially providing a unique profile of impairment. We suggest that dynamic measures capture aspects of stroke deficits that are more linearly related to the underlying deficit in neural motor control.

Disclosures: A.B. Thomas: None. **E.V. Olesh:** None. **A. Adcock:** None. **V. Gritsenko:** None.

Poster

400. Voluntary Movements: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 400.12/MM5

Topic: E.04. Voluntary Movements

Support: NIH T32 Training Grant 5T32HD055180-08

Title: Neurobehavioral effects of dissonant sensory feedback

Authors: *J. T. JOHNSON[1], F. L. HAMMOND[2], L. A. WHEATON[1]
[1]Applied Physiol., [2]Mechanical Engin., Georgia Inst. of Technol., Atlanta, GA

Abstract: Motor planning and control are influenced by the availability and reliability of sensory feedback. Following an upper-extremity amputation, the patient loses not only motor and structural functionality, but also somatosensory feedback from both the hand and the hand's interaction with the world. Decreases in sensory availability have been shown to modulate shifts from feedback control to predictive feedforward control, and to increase recruitment of parietofrontal areas. Substituting a prosthetic limb's end effector for the hand can enable basic object interactions, but does not compensate for the dissonance between motor output and somatosensory feedback. This dissonance is exacerbated by the use of proximal muscles to control the prosthesis. Further, while prosthesis-to-object interactions may resemble grasp, whether neural areas specialized for grasp are recruited during prosthesis use is not known. Similarly, whether a prosthesis is treated as a tool during motor planning and control is not known. We hypothesized using a pair of hand-held tongs during reduced visual availability would result in an increase in recruitment of parietofrontal areas relative to a full-vision group. We also hypothesized increased parietofrontal activity during prosthesis use for both full vision and occluded vision. Electroencephalography data were gathered while persons (n=26) with sound limbs used either a prosthetic simulator (n=18) or a pair of hand-held tongs (n=8) to perform a repetitive reach and grasp task involving discs of three sizes. Results confirm our hypotheses, showing increased parietofrontal activity in the tongs group with occluded vision, and in both full vision and occluded vision in the prosthesis group. Increased predictive control in both prosthesis groups may be due to the dissonance of somatosensory feedback from the prosthesis and the change from distal to proximal muscles to control the end effector.

Disclosures: J.T. Johnson: None. **F.L. Hammond:** None. **L.A. Wheaton:** None.

Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 401.01/MM6

Topic: E.04. Voluntary Movements

Support: Arizona State University and Mayo Clinic Partnership for Collaborative Research Seed Grant Program

Title: Control of arm movements during activities of daily living

Authors: *N. DOUNSKAIA[1], D. SANSGIRI[1], D. MARSHALL[1], J. SARBOLANDI[1], M. VIDT[2]
[1]Arizona State Univ., Phoenix, AZ; [2]Pennsylvania State Univ., State College, PA

Abstract: The ultimate goal of human movement control research is to understand how natural movements performed in daily activities are controlled. Natural movements require coordination of multiple degrees of freedom (DOF) of the arm. We examined patterns of arm joint control during daily functional tasks, which are performed through rotation of the shoulder, elbow, and wrist with the use of seven DOF: shoulder flexion/extension, abduction/adduction, and internal/external rotation; elbow flexion/extension and pronation/supination; wrist flexion/extension and radial/ulnar deviation. Analyzed movements imitated the following 7 activities of daily living: moving an empty soda can from a table and placing it on a shelf set to 3 different heights, bringing the can to the mouth as for drinking, combing hair, turning a book page, and bringing the right hand to the left side of the chest. Kinematic and kinetic analyses were conducted. The studied kinematic characteristics were displacements of the 7 DOF and contribution of each DOF to hand velocity. These characteristics demonstrated that the shoulder and elbow transported the hand in space and the wrist provided the required hand orientation. The kinetic analysis involved computation of 3-dimensional vectors of muscle torque (MT), interaction torque (IT), gravity torque (GT), and net torque (NT) at each joint. Using a relationship NT = MT + GT + IT, we assessed the role of active control and the passive factors in rotation of each joint by computing MT contribution (MTC) to NT using the ratio of the signed MT projection on NT to NT magnitude. Despite the variety of joint movements required across the different tasks, 3 patterns of shoulder and elbow coordination prevailed in each movement: 1) active rotation of the shoulder and predominantly passive rotation of the elbow; 2) active rotation of the elbow and predominantly passive rotation of the shoulder; and 3) passive rotation of both joints. At the wrist, MT mainly compensates for passive torque and provides adjustment of wrist motion according to requirements of each task. We conclude that the 3 shoulder-elbow coordination patterns during which at least one joint moves largely passively represent a joint control strategy underlying performance of well-learned arm movements. We discuss that the advantage of this control strategy is that it requires minimal neural effort for joint coordination, and thus increases neural resources that can be used for cognitive tasks.

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Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 401.02/MM7

Topic: E.04. Voluntary Movements

Title: Co-contraction improves upper limb endpoint tracking in the presence of perturbations

Authors: *C. M. SALIBA[1], M. J. RAINBOW[1], W. S. SELBIE[3], K. J. DELUZIO[1], S. H. SCOTT[2]
[1]Mechanical and Materials Engin., [2]Dept Biomed. and Mol. Sci., Queen's Univ., Kingston, ON, Canada; [3]C-Motion Inc, Kingston, ON, Canada

Abstract: The objective of this study was to determine the influence of co-contraction on the response to a physical perturbation during an upper limb tracking task. We examined the effect of pre-perturbation muscle activity on the motor response of muscles stretched and shortened by the perturbation.
Subjects interacted with a KINARM Exoskeleton robot (BKIN Technologies, Kingston, ON, Canada) to track a target between 80 and 100 degrees of elbow flexion at a constant angular velocity. Subjects were given visual feedback of their fingertip position and the target. A single trial consisted of an initial movement, tracking the target from one end of the trajectory to the other, and a return, tracking the target back to the initial position. A perturbation torque (5 Nm, 50 ms pulse) was applied near the midpoint of both the initial and return movements. Three perturbation torques were possible: no perturbation, or perturbations that flexed or extended the elbow. Subjects were instructed not to anticipate the perturbation and to return to the moving target as quickly and accurately as possible once perturbed. Surface electromyography (EMG) signals were recorded for muscles spanning the elbow. Trials were performed with and without intentional antagonistic co-contraction of the arm muscles.
Co-contraction produced a faster corrective response to the perturbation and improved task performance by decreasing the return time to the target and the maximum error between the elbow angle and the target angle. This was true for perturbations in the same direction and in the opposite direction of the movement. Decreases in elbow motion with co-contraction were evident less than 50 ms post-perturbation. This decrease can be primarily attributed to mechanical properties of the joint due to feedback delays in the motor response, indicating increased joint stiffness when co-contracting. When tracking with and without co-contraction, muscles stretched by the perturbation displayed a burst of EMG activity starting 60 ms post perturbation. When co-contracting, muscles shortened by the perturbation displayed an inhibition in EMG activity 60 ms post-perturbation.
Co-contraction exploits the reciprocal nature of the motor system to use two muscle groups for control. By increasing pre-perturbation muscle activity, co-contraction allows an inhibitory response in the shortened muscles to contribute to the overall response in addition to the excitatory response in the stretched muscles. This inhibitory contribution is not possible without pre-activation.

Disclosures: C.M. Saliba: None. **M.J. Rainbow:** None. **W.S. Selbie:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C-Motion Inc. **K.J. Deluzio:** None. **S.H. Scott:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BKIN Technologies.

Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 401.03/MM8

Topic: E.04. Voluntary Movements

Support: NSERC
CFI
FRSQ

Title: An investigation of reach decision preferences during ongoing action control

Authors: J. MICHALSKI, A. M. GREEN, *P. E. CISEK
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Abstract: Studies of decision-making, in both psychology and neuroscience, have primarily focused on situations in which subjects must first make a choice and then report it with an action. Such “decide-then-act” paradigms have led to theories in which the brain weighs the costs and benefits of different options, selects one, and then prepares and executes the associated action. However, in our daily lives, we often make decisions while we are already acting (e.g., when playing almost any sport), and the choice is between an action that is being executed and a new potential action that has become available. Given that the human brain evolved primarily to guide real-time behavior, our theories of decision-making must be able to address decisions made in real-time, during “decide-while-acting” paradigms. Here, we examine how subjects make decisions during ongoing action control as compared to static situations. In the “continuous tracking” task, human subjects use a hand-held cursor to track a target that moves in the horizontal plane. At certain moments, a new target choice is presented somewhere on the screen. The subjects can either ignore it and continue tracking the current target, or they can choose to switch to the new target, which then starts to move and becomes the tracked target. The placement and timing of the new target is designed to present subjects with decision scenarios that are analogous to situations already well-studied in “decide-then-act” paradigms. We found a strong preference for closer and larger targets, as expected from prior studies, as well as a preference for targets aligned with the current axis of hand movement. However, in contrast to previous observations in static paradigms where strong preferences were found for movement directions incurring lower biomechanical costs, during continuous tracking we found no such directional preference. To further investigate this difference in results, we examined a “discontinuous tracking” version of the task, in which the tracked target jumped discontinuously so that the choice was always between two point-to-point movements. In contrast to the continuous tracking task, we observed almost no effect of angular alignment with the current axis of movement, but larger directional preferences consistent with the influence of

biomechanical cost. Finally, we examined a version of the task in which subjects were presented with identical choice scenarios but outside the tracking task context. Here, we again observed an effect of biomechanical costs. Taken together, these data show that subject preferences are differentially affected by a variety of factors depending on the context in which choices are presented.

Disclosures: J. Michalski: None. **A.M. Green:** None. **P.E. Cisek:** None.

Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 401.04/MM9

Topic: E.04. Voluntary Movements

Title: Effect of repeated explicit instructions on visuomotor adaptation and intermanual transfer

Authors: *S. WERNER, C. RICKERS, H. K. STRUEDER
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Abstract: Intermanual transfer of visuomotor adaptation is related to explicit learning (Poh et al., J Neurophysiol, 2016). Yet, it is unknown whether intermanual transfer can be increased by repeated explicit instructions. To clarify this issue, we conducted an extensive experimental programme modulating the amount of awareness of visuomotor adaptation in four groups (GNI, SNI, SOI, SSI). All participants performed centre-out-and-back pointing movements with their right arm. During baseline they received veridical visual feedback about their movement. In a subsequent adaptation block, feedback was rotated by 60°. This rotation was either introduced suddenly (SNI, SOI, SSI) or gradually in steps of 3° (GNI). The groups either received no explicit instructions (GNI, SNI), instructions once before the onset of adaptation (SOI) or several instructions before and during adaptation (SSI) about the nature of the rotation. After adaptation an awareness test was conducted. The experiment concluded with an intermanual transfer block, in which movements were performed with the left arm under rotated feedback, and a washout block again under veridical feedback. We determined adaptation, awareness, unawareness, transfer and washout indices from the subjects pointing directions. Analysis of adaptation indices revealed less adaptation during gradual than during sudden adaptation and less adaptation without than with instructions. Repeated instructions did not increase adaptation compared to one-time instruction. These differences were pronounced during beginning of adaptation; however, at the end of adaptation all four groups reached a similar adaptation level. Besides, we found significant group differences only for awareness index with less awareness in GNI than SOI and SSI. Unawareness, transfer and washout indices did not differ between groups. However, we found a significant correlation between awareness and intermanual transfer indices.

The findings are discussed with reference to implicit and explicit processes in visuomotor adaptation.

Disclosures: S. Werner: None. **C. Rickers:** None. **H.K. Strueder:** None.

Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 401.05/MM10

Topic: E.04. Voluntary Movements

Title: Effect of practice and expertise on producing slow, smooth movements

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Abstract: Point-to-point arm movements have been shown to have smooth, bell-shaped velocity profiles. However, when the duration of the movements is sufficiently slow (i.e. with a duration longer than approximately 500 ms), individuals are unable to produce smooth movements. Rather, they produce jittery movements where multiple peaks can be observed in the velocity profile. This naturally leads to the question of why are we unable to produce these movements? One possible explanation is that the motor system is simply unable to produce slow, smooth movements. An alternative explanation is that we can't make these movements because of a lack of practice. In day to day life, we rarely perform very slow movements, rather we want to achieve our movement goals in a relatively short amount of time. We want to bring the cup of coffee to our mouth quickly (without spilling it)! The implication of this is that slow, smooth movements may not be a part of our motor repertoire. To test whether this is the case, we examined whether training can add these movements to our motor repertoire, and whether experts in making slow movements already have these movements in their motor repertoires. We trained a group of naïve subjects to make slow, smooth movements using the single-player mirror game, where subjects track rhythmic stimuli which move at different frequencies and amplitudes using their arm. We found that following 10 days of training, 15 minutes per session, subjects improved in their ability to perform slow, smooth movements. Additionally, we tested experts in producing slow movements - long term Tai Chi practitioners. In this group of subjects, who all have extensive training in performing slow movements, we found that most of the participants were able to produce slow, smooth movements at frequencies at which participants from the general population were unable. In conclusion, the results from these two studies show that the previously observed lower limit in our ability to produce slow, smooth movements was likely due to a lack of practice in these movements and is not the true lower limit. An

understanding of the other causes which contribute to the lower limit in our ability to produce slow movements requires further investigation.

Disclosures: J. Friedman: None. **L. Noy:** None.

Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 401.06/MM11

Topic: E.04. Voluntary Movements

Support: Campus Research Board of the Unviversity of Illinois at Urbana-Champaign
Center for Health Aging and Development of the University of Illinois at Urbana-Champaign
Jump Applied Research for Community Health Research Grant

Title: Dimensionality reduction in the control of quasi-static force production tasks in humans

Authors: N. C. SPEIDEL[1], P. CHEMBRAMMEL[4], R. N. MCNISH[2], S. N. MCKEEMAN[5], P. B. CAMACHO[3], *C. LOPEZ-ORTIZ[5]
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Abstract: Human muscle activation patterns have proven difficult to characterize due to the large number of degrees of freedom present in the system. The underlying characteristic behind the reduction in dimensionality is the ability to group together individual degrees of freedom (typically muscles) together to create new variables that act as the input to the system. In these experiments, two common grouping methods are explored: principal component analysis (PCA) and non-negative matrix factorization (NMF) subjected to a generalized Akaike information criterion (AIC) to serve as a quality of fit estimator. These are used to group the muscle activity of individuals during quasi-static force production tasks to synthesize reduced-order models that account for 90% of the muscle activity contributing to the task. Regression techniques are then utilized to obtain mathematical models that describe the system's behavior. Ultimately, we show that the system's response is a multi-valued function and linear combinations of predetermined functions perform poorly in terms of goodness of the t at capturing the data. However, a Fourier series parameterization through time yields promising results in terms of validity of studying reduced order models and how they may be used to further study robotic systems or form movement characterization and rehabilitation infrastructure.

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Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 401.07/MM12

Topic: E.04. Voluntary Movements

Support: NIH grant R01NS058659-01A1

Title: Control of forelimb joints during accurate stepping

Authors: *H. N. ZUBAIR[1,2], E. E. STOUT[1], I. N. BELOOZEROVA[1], N. DOUNSKAIA[2]
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Abstract: Natural locomotion often requires accurate stepping when ground conditions change. How movements of limb joints are coordinated and how this coordination is adjusted to expected and unexpected ground perturbations remains unknown. In this study, coordination among forelimb joints was investigated during the swing phase of locomotion in the cat. Unperturbed and perturbed accurate stepping along a horizontal ladder was examined. In the unperturbed condition, all crosspieces of the ladder were spaced equally, 25 cm apart. In the perturbed conditions, one of the crosspieces was displaced 5 cm toward to or away from the cat, which required the cat to adjust step. The crosspiece was displaced at three different time points along the cat's progression: before the cat stepped on the ladder (known perturbation), two steps ahead (long notice), and one step ahead (short notice). Joint kinematics and kinetics were analyzed during the unperturbed and perturbed steps with a focus on the roles of the active muscle torque, passive gravitational torque, and interaction torque in rotation of each joint. We found that the paw was transported in space through translation of the body and shoulder and elbow rotations while the wrist provided paw orientation required to step on crosspieces. The underlying joint control pattern was consistent in all conditions. The movement was initiated with an active elbow flexion and mainly passive shoulder flexion. This was followed by a period of predominantly passive rotations of both joints. Shoulder muscle torque crucially contributed to generation of the last movement portion. This torque caused active deceleration of the shoulder flexion, while the elbow continued to move mainly passively, mainly due to interaction torque. The wrist was rotated predominantly passively, with the muscle torque contributing only at the very beginning and end of the motion to stabilize the joint against high interaction torque. This pattern of control was preserved in all perturbed conditions. Changes in the step length were accomplished predominantly by a change in shoulder movement during the last quarter of the swing motion. The influence of the timing of the crosspiece displacement was minor. We

conclude that joint coordination during locomotion is produced mainly passively, by exploiting gravity and the limb's inter-segmental dynamics, which may simplify neural control of locomotion. The use of shoulder musculature at the movement end enables flexible responses to environmental disturbances.

Disclosures: H.N. Zubair: None. **E.E. Stout:** None. **I.N. Beloozerova:** None. **N. Dounskaia:** None.

Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 401.08/MM13

Topic: E.04. Voluntary Movements

Support: University of South Carolina Magellan Scholar Grant

Title: Scaling of muscle activation during 3D reach actions

Authors: *C. R. SMITH[1], A. HETHERINGTON[1], S. SILFIES[2], J. STEWART[1]
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Abstract: Scaling of kinematic measures of reach control to targets that vary in distance is well-described. Scaling of muscle activity has also been shown, but such work has primarily been in single-joint or 2D workspaces. Many functional tasks of everyday living occur in 3D environments. Therefore, the purpose of this study was to investigate the scaling of shoulder and elbow muscle activity for 3D reaches to targets of increasing distance.
Twelve young, right-hand dominant participants (5F/7M, 23±4yrs) reached across midline with the right arm to 3D virtual targets placed at 7, 14, and 21cm. Participants were instructed to reach "as fast as possible" to targets presented in a pseudo-random order in 6 blocks of 18 trials each. EMG (2000Hz) and kinematic data (100Hz) were collected simultaneously. Hand position and joint angles were collected using electromagnetic sensors. Wireless surface electrodes were placed on the biceps, lateral triceps, anterior deltoid and posterior deltoid. All data were collected and time-synced using the MotionMonitor.
As expected, movement distance, peak velocity, peak acceleration, shoulder flexion, and elbow extension showed significant scaling with target distance ($p<0.001$). Anterior deltoid activity scaled significantly with target distance ($p<0.001$); posterior deltoid, biceps and triceps activity also scaled to target distance ($p<0.05$) although this scaling was less distinct. Anterior deltoid activation was positively correlated with movement distance across participants (mean $r=0.714$); the correlation between movement distance and posterior deltoid (r range: -0.723 to 0.166), biceps (r range: -0.025 to 0.694), and triceps (r range: -0.209 to 0.887) activation varied between individuals, suggesting different movement strategies were used to meet the demands of the task.

As the primary mover for this reach task, anterior deltoid activation scaled with target distance and had a strong correlation with movement distance. The role of the posterior deltoid, biceps, and triceps during this task was variable which may be due to differences in individual approaches to task performance. While the task required a 3D reach, some individuals moved with a relatively straight hand path, leading to activation patterns that mirror previous 2D findings; other individuals moved with a more curved hand path, resulting in muscle activation patterns that differed from those previously observed. An understanding of the movement patterns and scaling of muscle activation for reaches in 3D space can assist in the development of models that can be used to compare scaling used by individuals that have known motor deficits, such as after stroke.

Disclosures: C.R. Smith: None. **A. Hetherington:** None. **S. Silfies:** None. **J. Stewart:** None.

Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

Location: SDCC Halls B-H

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Program #/Poster #: 401.09/MM14

Topic: E.04. Voluntary Movements

Support: Portage Health Foundation

Title: Aging and the neurocognitive mechanisms underlying corrective movements for obstacle collision avoidance

Authors: *I. FLINT[1], K. TREWARTHA[2]
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Abstract: The objective of this research is to identify the neurocognitive mechanisms that contribute to our ability to make rapid evasive actions in response to sensory feedback during an ongoing movement, and to establish how these mechanisms are impacted by aging. Rapid motor corrections allow us to make evasive actions to avoid knocking over objects in a cluttered workspace, and navigate around other people in a crowded room. In the current study, we used a robotic device (Kinarm, BKin Technologies) to apply unpredictable visual "cursor shifts" while participants reached for visual targets and tried to avoid visible haptic obstacles. The obstacles were positioned to the right and left of a straight hand path from the start position to the target, and upon contact with the participant's cursor a repulsive force was applied by the robot to simulate a collision with a real obstacle. On each trial the cursor briefly disappeared behind a rectangular occluder positioned in front of the start position, and emerged either unperturbed, or shifted by a small, medium, or large distance to the left or right of a straight line to the target. The medium-shift trials placed the cursor in a collision course with one of the obstacles, and

required a movement correction to avoid the obstacles. For the no-shift and small-shift trials, and the large-shift trials a movement correction was not necessary to guide the cursor between or around the outside of the obstacles, respectively. We tested the prediction that older adults (65-85 years old) would perform less optimal corrections more frequently than younger adults (18-35 years old). We also recorded EEG data while participants performed the obstacle avoidance task to examine age differences in the neurocognitive mechanisms involved in responding to the visual perturbations. Finally, we probed individual differences in the efficiency with which older adults made rapid corrective actions by administering a battery of perceptuomotor and cognitive tasks. The battery included simple and choice reaction time tests, a finger tapping test, a letter-digit substitution task, the flanker task, a task-switching test, the Wisconsin card sorting test, and an N-back task. This battery allowed us to identify the factors that best predict impairments in performance on the obstacle avoidance task in later adulthood. The results of this study add to a growing literature examining the impact of aging, and age-related cognitive decline, on adaptive motor behavior. The data also contribute to our understanding of the nature of cognitive contributions to rapid feedback control.

Disclosures: I. Flint: None. **K. Trewartha:** None.

Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

Location: SDCC Halls B-H

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Program #/Poster #: 401.10/NN1

Topic: E.04. Voluntary Movements

Support: T32EB009406

Title: The effect of the flexion synergy on arm and hand kinematics in hemiparetic cerebral palsy: Preliminary results

Authors: *N. M. HILL, J. P. A. DEWALD
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Abstract: Introduction: Unilateral brain injury before, during, or soon after birth resulting in hemiparetic cerebral palsy (HCP) causes movement impairments on one side of the body. One clinically observed impairment is the upper extremity flexion synergy, an involuntary coupling between shoulder abduction and elbow, wrist and finger flexion. With the use of haptic robotics and motion capture technology, these involuntary coupling patterns can be explored in the pediatric population. The purpose of this study is to test the feasibility of an integrative measurement technique to record reaching and hand opening/closing abilities in individuals with HCP. Retention of high resolution ipsilateral corticospinal projections in earlier injuries may reduce the presence of involuntary joint coupling observed in individuals with later injuries. We

hypothesize that individuals with earlier injuries (PRE) will be able to reach farther and open their hands more at higher shoulder abduction loads compared to those with later injuries (PERI and POST). **Methods:** Participants completed a set of reaching and hand tasks in an admittance controlled haptic robot which allows arm movement in three dimensions. With the arm rigidly coupled to the robot, participants were instructed to reach forward towards a virtual target set near full arm extension while lifting a percentage of their maximum shoulder abduction torque generation ability. Reaching ability was quantified using the reach distance deficit defined as the percent change between the overall max distance achieved and the max distance for each load level. A motion capture system using specialized markers with embedded optical patterns was used to measure hand movement during a grasping task. Hand opening ability was quantified using a pentagon area calculated from fingertip positions. **Results:** A total of eight participants (ages 11y-19y) completed the reaching tasks of this study with two individuals in each injury timing group and two typically developing (TD) controls. Preliminary results show a decrease in reaching ability at higher load levels with trend towards greater deficits in the latest injury timing group compared to earlier groups. Similar trends are expected with the hand pentagon area. **Conclusions:** Integration of haptic robotics and motion capture technologies enable quantification of the flexion synergy in pediatric populations. Knowledge gained from this study will enable the development of targeted therapies to address the underlying causes of motor impairments in different subpopulations of HCP.

Disclosures: N.M. Hill: None. **J.P.A. Dewald:** None.

Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

Location: SDCC Halls B-H

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Program #/Poster #: 401.11/NN2

Topic: E.04. Voluntary Movements

Support: NIH-R01-HD087089
NSF-NRI-1637854

Title: Interaction with a complex object: Exploiting stability to render perturbations predictable

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Abstract: Interactions with objects are ubiquitous in our daily life and present specific challenges to our motor skill. When guiding acup of coffee to one's mouth, the hand applies forces on the cup and the coffee inside, and the sloshing coffee simultaneously exerts forces on the hand. To avoid spilling, subtle control is required to predict and compensate for those

complex interaction forces. To date, motor neuroscience has focused on simple movements but extrapolation of insights to tasks with complex dynamics is questionable. For such complex interactions, slow neural transmission and intrinsic noise make feedback control insufficient while prediction based on internal models of dynamic objects seems implausible. Previous work on rhythmic interactions showed that humans exploit dynamic stabilityor increased predictability of object dynamics to facilitate control. This exploratory study examined discrete movements and hypothesized that actors render the interaction stable to preempt and compensate for perturbations. Since discrete movements are not at equilibrium, traditional stability analyses are inapplicable. Instead, contraction analysis was used to identify contraction regions that stabilize trajectories, specifically in the presence of perturbations.Using a virtual set-up, a simple cart-and-pendulum model mimicked the task of carrying a cup of coffee: the pendulum bob represented the liquid moving inside a cup defined by the bob's semicircular path. Participants moved a robotic manipulandum to control the virtual cup with the ball "rolling" inside; the goal was to move the cup to a target as fast as possible without losing the ball. A small perturbation assisting or resisting the motion was presented at a fixed location along the path. Hypotheses: 1) For both perturbations, subjects learn to harness contraction regions to stabilize the interaction. 2) Contraction regions are utilized differently for assistive and resistive perturbations. To compute the contraction regions, a metric was obtained by solving a partial differential equation. Experimental results show that subjects stabilized their trajectories and attenuated the perturbations by moving through contraction regions of the free, unforced system. This stabilized performance and made the dynamics more predictable. Moreover, subjects chose the contraction regions with relatively slow guaranteed convergence rates, which is a trade-off between stability and flexibility. These results demonstrate that humans are sensitive to subtle stability properties of the task and exploit them to enable safe interaction with dynamically complex objects.

Disclosures: D. Sternad: None. **S. Bazzi:** None. **N. Hogan:** None.

Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

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Program #/Poster #: 401.12/NN3

Topic: E.04. Voluntary Movements

Support: NIH-R01-HD087089
NSF-NRI-1637824

Title: Input shaping to control dynamically complex objects

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Abstract: Physical interaction with objects, including tool use, is ubiquitous in activities of everyday life and humans have mastered this skill to a remarkable degree. The ability to manipulate objects with complex dynamics is particularly intriguing due to the subtleties required to perform such tasks. To date the primary focus of neuroscience studies has been on grasping or transporting rigid objects, but the insights gained thereby are unlikely to be sufficient to understand manipulation of dynamically complex objects. Motivated by our ability to transport a cup of coffee with minimal sloshing and without spilling, this study examined discrete movements of an object with nonlinear dynamics and internal degrees of freedom. The task of transporting a cup of coffee was implemented as a simplified 2D cart-and-pendulum model simulated in a haptic virtual environment; the cart represented the cup and the pendulum's bob emulated the liquid moving inside the cup. This study explored the strategies that humans use when the goal is to move the cup-and-ball to a target without any terminal oscillations. In the control theoretical literature, a strategy called input shaping was developed for such a task: if a desired motion profile is convolved with impulses at appropriate times, determined by the natural frequency of the pendular load, all terminal oscillations are eliminated. In the experimental study participants controlled the virtual "cup and ball" system by moving a robotic manipulandum. Subjects were instructed to move the cup into the target box without losing the ball along the way. They were allowed to move at their own pace, as long as the cup's forward velocity above a minimum, which was instructed verbally. Results showed that subjects used two different control strategies to complete the task, distinguished by trial completion time. Subjects who took a relatively longer time to complete the task used a minimum jerk profile. However, the jerk minimized was of the ball motion and not the cup motion. Other subjects took a shorter time to complete the task; their movement kinematics revealed a pattern with two pulses, consistent with input shaping. These results show that humans can apply finely-timed pulses dictated by the object's dynamics. We submit that, while free movements may reveal some aspects of neural control, to provide insight about the control of physically interactive tasks, new experimental paradigms are needed.

Disclosures: H. Guang: None. **S. Bazzi:** None. **D. Sternad:** None. **N. Hogan:** None.

Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

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Program #/Poster #: 401.13/NN4

Topic: E.04. Voluntary Movements

Support: European Space Agency (ESA)
Prodex (BELSPO, Belgian Federal Government)

Title: Modification of the control law in reaching movements induced by online target change

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Abstract: In order to control movements efficiently, the nervous system takes into account sensory inputs (kinaesthetic and visual signals) and provide appropriate reactions to mechanical perturbations applied to the limb. These feedback responses have delay of ~50 ms and ~100 ms respectively, depending on whether the perturbation engages the somatosensory or the visual pathway. However, whether this feedback controller is sensitive to an online modification of the structure of the goal, for example a change of the size of the target, has not yet been documented. The interest of considering such a change is that it cannot be handled only by modifying the state of the system, as for a target jump, but requires recomputation of the control policy. More specifically, we studied whether change in the structure of the goal occurring during movement could elicit rapid changes in the control law. We analysed this issue by modifying the size of the target, which switched from a square (2.5x2.5 cm) to a rectangle (40x2.5 cm) or vice versa during movement. The change in the size of the target occurred either before or after the mechanical perturbation. In addition to this target size modification, we applied mechanical perturbations to the hand, orthogonally to the main reach direction, to enhance change in the control policy associated with the target size. We analysed kinematics of the hand movement as well as surface electromyography (EMG) of shoulder flexor and extensor muscles involved in the movement to determine when the feedback responses to mechanical perturbations reflected changes in the goal structure. Position of the hand at the end of the reaching showed significant dependency ($p<0.05$) on the occurrence of a target change, such that earlier change from a square to a rectangle were followed by a larger hand deviation, and the opposite organisation of reach end-point was observed when the target changed from a rectangle to a square. When the target changed before the mechanical perturbation, the end-point position was closer to the one of the perturbed reaching to the modified target. In contrast, when the target changed after the mechanical perturbation, the end-point position was closer to the one of the perturbed reaching to the unmodified target. Surface EMG averaged in a window of [100 150] ms after the change in target displayed a significant modulation consistent with changes in behaviour (linear mixed ANOVA, $p<0.01$). Our results highlight a rapid and online modification of the control law following the modification of the size of the goal target.

Disclosures: A. De Comite: None. **F. Crevecoeur:** None. **P. Lefevre:** None.

Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

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FRSQ

Title: Fronto-parietal neural activity during multi-attribute decision-making with conflicting value information

Authors: *A. NAKAHASHI, T. LUSIGNAN, P. E. CISEK
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Abstract: Many studies have shown that delay-period activity in sensorimotor regions not only represents the action plans to be executed, but also reflects the reward values of the potential actions. This has led to the proposal that value-based decisions between actions emerge as a biased competition between potential actions taking place in sensorimotor regions. Here, we investigate how neural activity in dorsal premotor cortex (PMd) and posterior parietal cortex (PPC) unfolds when multiple attributes jointly indicate the reward value of a potential reach target. In our task, monkeys are presented with one or two reach targets whose reward value is determined by two attributes: a bottom-up (BU) feature based on luminance, and a top-down (TD) feature based on the orientation of a line drawn in the target. Each feature has three levels of desirability scores and the total of the two scores determines the number of rewards received upon a successful reach to that target, after a GO signal. When the decision is based entirely on the BU feature (EasyBU trials; line orientation is the same in both targets) or on the TD feature (EasyTD trials; luminance is the same for both targets), monkeys reliably select the more valuable target. In these trials, choice specific activity appears almost simultaneously in both PMd and PPC, within 100ms of target onset, at which time the activity associated with the unselected target begins to decrease sharply. When the targets are identical in terms of both BU and TD features and the monkey can choose randomly, choice predictive information appears earlier in PMd (within 100ms) than PPC (within 150ms). Most interesting are trials in which the targets differ in both attributes, but the total reward value is the same (Conflict trials). In these trials it doesn't matter which target the monkey chooses, but he must choose one, and we examine how neural activity unfolds when he chooses the target with a higher valued BU feature (ConfBU) versus when he chooses the target with a higher valued TD feature (ConfTD trials). In ConfTD trials, the activity looks similar to EasyTD trials - both PMd and PPC reflect the choice within 100ms, at which time the activity associated with the unselected target starts to decrease. In contrast, in ConfBU trials both regions show prolonged activity for both targets, with the unselected target activity decreasing at around 150ms in PMd and slightly later in PPC. These results are consistent with the proposal that reach choices are determined by a biased competition in sensorimotor regions, and suggest that in cases of indecision the conflict resolution appears to be primarily driven by activity in PMd.

Disclosures: A. Nakahashi: None. **T. Lusignan:** None. **P.E. Cisek:** None.

Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 401.15/NN6

Topic: E.04. Voluntary Movements

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Title: Humans do not minimize muscle effort to control constrained motion

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Abstract: Tool use is a hallmark of human behavior, yet how humans control physical interaction is still poorly understood. For unconstrained motion, it is often proposed that musculo-skeletal coordination minimizes muscle effort. This study examined if this notion holds true for motor tasks that involve physical interaction, specifically when humans interact with a circular kinematic constraint. In the experiment, human subjects were instructed to move a robot handle around a virtual, circular constraint at a constant tangential velocity in both clockwise and counter-clockwise directions. To reduce the effect of forces that might arise from incomplete compensation for neuro-musculo-skeletal dynamics, the target tangential speed was set to an extremely slow pace (13.3 seconds per revolution). This ensured that subjects moved around the constraint under quasi-static conditions. First, under quasi-static conditions, subjects should exhibit the same behavior, irrespective of the direction of rotation (H1). Second, in this task, minimizing muscular effort requires muscles to be deactivated at positions where they cannot generate force tangent to the constraint and hence cannot contribute to mechanical work (H2). Third, if deactivated at these positions, muscles cannot offset radial components of force that may result from activation of other arm muscles. We hypothesized that subjects would exert repeatable patterns of workless, radial forces on the constraint (H3). While subjects exerted repeatable patterns of workless forces, supporting H3, those patterns differed with direction of rotation, contradicting H1. Most importantly, subjects did not deactivate muscles when they could not contribute to mechanical work, contradicting H2. These findings demonstrate that minimizing muscle effort is not a prominent factor in human performance of constrained motion.

Though no doubt important in some contexts, minimization of muscular effort or metabolic energy consumption may be subordinate to other concerns, such as predictability, in the context of tool-using behavior.

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Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

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Support: NIH Grant R01 AR-050520
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Title: Small errors in movement paths can induce dramatic changes in musculotendon velocities

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Abstract: Deciding how to move the hand along a given path to produce activities for daily living (ADLs) involves multiple factors such as limb kinetics, choice of muscle activation patterns, online error corrections, robustness to perturbations, accounting for length/velocity muscle mechanics and time-sensitive reflex modulation. In Hagen & Valero-Cuevas (2017) we show how the joint rotations associated with different paths induce different musculotendon (MT) velocities. While *different* paths will naturally produce *different* MT velocities, we found that *similar* paths can exhibit *different* MT velocity profiles. This matters at the level of individual muscles because differences in MT velocities will require unique muscle activation strategies to compensate for force-length/force-velocity properties and modulation of their spinal reflex mechanisms. Importantly, these differences in MT velocities within and across paths establish the neuromechanical landscape that determines the robustness of muscle coordination patterns and reflex modulation strategies during ADLs.
Here we study how initial variability in the direction of a hand path affects the subsequent time histories of MT velocities. As in our prior work, we used an 18 muscle, 2 joint planar arm model to produce 100 random reaching paths for 6 different pairs of points on a tabletop (3 pairs shared the starting position, 3 pairs shared the ending location). Each valid, smooth reaching path was generated (and its MT velocities found) using a pseudo-random clamped cubic spline, parameterized to follow a bell-shaped tangential velocity curve, simulating reaching movements of 35 cm in length with initial errors compatible with those seen in reaching studies in humans. We find that uniform initial error (i.e., variability) across paths induces non-uniform distributions of MT velocities. That is, although the induced MT velocities follow a similar profile for each reaching movement, their distributions/deviations change across them. This implies that some reaching paths may be more or less forgiving to initial errors in muscle coordination or reflex modulation. This has important consequences to the study of healthy movement, as well as the rehabilitation of movement for ADLs in neurological conditions and stroke.

Disclosures: D.A. Hagen: None. **C.M. Laine:** None. **S. Chakravarthi Raja:** None. **F.J. Valero-Cuevas:** 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 R01 AR-050520, NIH R01 AR-052345, DoD MR150091.

Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

Location: SDCC Halls B-H

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Topic: E.04. Voluntary Movements

Support: German Research Foundation (DFG, grant SFB-889)

Title: Spatial averaging and inference of decision time in go-before-you-know tasks is not restricted to rapid movements

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Abstract: Movement trajectories in go-before-you-know action selection paradigms provide a continuous marker for ongoing decision processes. When tight time constraints require subjects to initiate reaching movements before knowing which one of multiple potential targets to choose ('go-before-you-know'), reach trajectories are initially often aimed at the spatial midpoint of the potential targets ('spatial averaging'). The time course at which the trajectory is corrected towards the ultimately chosen target serves as marker for the decision time. These effects are thought to require rapid movements, usually achieved with tapping a touchscreen under tight time constraints. It is unclear if spatial averaging depends on movements with such reduced need for motor control or if slower and more controlled movements can reveal underlying decision processes equivalently.
To test this, we asked subjects (N = 22) to reach towards two potential targets with different reward value using a haptic manipulandum and cursor feedback in 3D space. Different to a previous, touchscreen-based rapid-reach version (325ms reaction time limit and 425ms movement time limit, 40cm distance to target) of this task [1], movement here covered 18cm distance within a 325ms reaction time limit and 500, 700 or 900ms movement time limit. Since subjects did not receive haptic feedback on target acquisition and had to hit a spherical target zone (15mm radius) in 3D, movements required more visuomotor control than tapping on a screen. Actual average movement times were 318, 442, and 639ms, respectively.
Subjects showed spatial averaging with the 900ms time limit, but often guessed and performed direct reaches to one of the two targets with the 500 and 700ms limits. The movement time limit had a larger impact on optimal choice rates (on average 53%, 68% and 94% for 500, 700, and 900ms, respectively) than on successful target acquisition rates (68%, 73%, and 78%).
Our findings suggest that spatial averaging is not restricted to rapid movements, but that there is a minimum movement time needed to elicit these behavioral effects, set by the level of control the movements require. Thus, movements with higher level of control still allow for using go-before-you-know tasks as long as the movement time windows are adequately large. This allows studying decision processes in a wider range of applications, such as effort- based choice tasks , where effort can be operationalized via movement-resistive forces on the manipulandum.
[1] Chapman, S, Gallivan, JP, Wong, JD, Wispinski, NJ, & Enns, JT (2015). Journal of Experimental Psychology: General, 144, 4.

Disclosures: P. Ulbrich: None. **A. Gail:** None.

Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

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Program #/Poster #: 401.18/NN9

Topic: E.04. Voluntary Movements

Support: NSERC
OGS
Campus France

Title: Examining online adjustments to visual and somatosensory target perturbations

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Abstract: Movements to somatosensory targets could be planned using either a gaze-dependent or gaze-independent reference frame. The use of a gaze-dependent frame requires the remapping of somatosensory targets onto an extrinsic coordinate system. Because these additional remapping processes require time and could lead to transformation-induced errors, it is unclear if similar transformation processes are employed for limb trajectory amendments during movement execution. The purpose of the present study was to examine if different online control mechanisms exists for movements to somatosensory targets. For movements to somatosensory targets, it was hypothesized that reaches, made without visual feedback of the limb, would be controlled using gaze-independent reference frame. Because fewer sensorimotor transformations should be required, movements to somatosensory targets would exhibit earlier online trajectory corrections and greater correction amplitudes compared to movements to visual targets. Participants performed reaches to both somatosensory and visual targets in complete darkness. The target either remained stable (control) or was perturbed: 300 ms prior to the movement (before), or 100 ms, or 200 ms after movement onset (that is requiring online corrections). As hypothesized, participants exhibited shorter latencies and online corrections of greater amplitude when aiming to somatosensory targets compared to visual targets. These results provide evidence for the existence of a sensorimotor transformation network capable of performing rapid corrections in gaze-independent coordinates.

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Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

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Program #/Poster #: 401.19/NN10

Topic: E.04. Voluntary Movements

Support: NIGMS T32 GM081741
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COBRE P20GM109098

Title: Estimating functional muscle parameters using high-density electromyography with a flexible array

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Abstract: Recent advancements in musculoskeletal modeling have bolstered the feasibility of myoelectric control of closed-loop myoelectric prostheses and functional electric stimulation. However, the transformation of electromyographic (EMG) signals into a device control signal is dependent upon accurate estimation of motor neuron recruitment. Several studies have shown the use of high-density surface EMG for extracting parameters of muscle activity and motoneuron recruitment, such as conduction velocity. We used a flexible, high-density surface EMG grid over extrinsic hand muscles to extract motor unit action potentials from healthy, human participants performing single and multijoint movements. The flexible grid of 96-electrodes 3mm in diameter, spaced 10 mm apart was manufactured by Ripple Inc. (UT). We recorded myoelectric potentials from single-ended electrodes at 30kHz with high-pass filter at 10Hz. Multiple sites provided correlated recordings from finger extensor muscles. A conductive electrolyte gel was applied under the recording sites and was shown to decrease electrode impedance (2-4kΩ) and facilitated the recording of motor unit action potentials. This resulted in selective EMG patterns for single finger extensor muscles with high signal-to-noise ratio, including small thumb muscles. We also isolated individual motor unit action potentials from specific muscles spanning the wrist and hand and calculated muscle fiber conduction velocity to approximate motor neuron recruitment levels. These high-quality signals can provide a more dexterous control signal to drive myoelectric prosthetics.

Disclosures: R.L. Hardesty: None. **V. Gritsenko:** None. **S. Yakovenko:** None.

Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

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Topic: E.04. Voluntary Movements

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IRPHA Grant

Title: Neural correlates of kinematic planning and execution

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Abstract: Studies of movement kinematics have largely focused on representations of target location and displacement concerning eye/hand position. Much less is known regarding the representation of other aspects of kinematics such as the velocity of the movement. To study this aspect of kinematic planning, we designed a delayed reaching task where subjects made hand movements with either slow or fast velocities during which we recorded EEG signals from the parietal area, motor cortex and premotor cortices in 10 human subjects. We hypothesized that such a task should reveal a velocity planning signal in the instruction period and hold time delay which would facilitate planning. However, no distinct velocity related signal was observed in the time domain in all the above mentioned cortical areas after normalizing for differences in reaction time. Furthermore, we analyzed the LRP signal in the frequency domain following movement onset. Even though alpha (8-12 Hz) and beta (12-30 Hz) band powers decreases during reaction time and movement duration, we observed that neither frequency power distinguished between the velocity conditions. However, interestingly, gamma band activity (30-70 Hz), which has been shown to correlate with target selection (Aoki et al., 1999), showed a modulation with velocity during movement execution. i.e., higher gamma band power (1.31 dB +/- 0.43) for higher velocity and lower (0.31 dB +/- 0.14) for slow velocity trials. Further, this modulation began relatively earlier for parietal cortex compared to premotor and motor cortices with respect to movement onset. Taken together, our study indicates that velocity signals are more robustly expressed in gamma oscillations and suggest that these computations may be generated during the execution of movements.

Disclosures: V.N. Thakur: None. **A.N. Murthy:** None.

Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

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Topic: E.04. Voluntary Movements

Support: NIH R01 NS053813
T32 EB009406

Title: Motor planning associated with voluntary and involuntary movements occurs equally fast

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Abstract: The preparation of a desired motor action can influence both voluntary and involuntary movements, but it is unknown to what extent planning processes are shared between these mechanisms of movement initiation. Voluntary movements are relatively slow, but motor planning allows us to move more quickly in simple reaction time tasks. External stimuli can trigger faster initiation when a motor plan has been prepared. For example, a startling acoustic stimulus (SAS) can trigger initiation of a prepared movement faster than is possible with voluntary initiation. Differences in movement latencies are thought to result from the initiation mechanisms. SAS-triggering is mediated by subcortical pathways and causes a faster rise to threshold for motor output than voluntary initiation which is triggered cortically by the decision to move. However, voluntary initiation can be forced to occur faster than in typical reaction time tasks when the decision to move is made independently of planning processes to meet task requirements. It is unknown if shorter latencies result solely from differences in initiation mechanisms or if differences in the time required for motor planning also contribute. The objective of this work was to determine if a motor action is planned and accessible at the same latency for voluntary and involuntary movements. Ten subjects (26±4 years old, 6 females) were instructed in a ballistic reaching task. The dominant, right arm was supported on a table with a low-friction interface. Subjects were trained in a forced initiation paradigm to time voluntary reaches with a predictable cue given by a sequence of auditory tones (similar to Haith et al., *J Neurosci* 2016). One of four possible targets was presented 0-380 ms before the cue to reach. SAS was delivered 150 ms before the cue in 16% of reaches to investigate a subset of target presentation times, 20-200 ms. We quantified the probability of reaching to the correct target versus the time between target presentation and movement onset. We quantified motor planning time when the probability was 0.625. We chose 0.625 as it is halfway between chance and 1. Motor planning time for SAS-triggered movements did not significantly differ from voluntary initiation ($p>0.05$). Average motor planning time was 146 ms for voluntary (95% CI: [138, 156] ms) and 148 ms for SAS-triggered (95% CI: [112, 156] ms) movements. These results suggest a

motor action is planned and accessible for voluntary and involuntary movements at the same short latency. The novel use of SAS in a forced initiation paradigm suggests planning processes may be shared between the voluntary and involuntary initiation mechanisms used to trigger ballistic reaches.

Disclosures: R.L. Heckman: None. **D. Ludvig:** None. **E.J. Perreault:** None.

Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

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Topic: E.04. Voluntary Movements

Support: Carl von Ossietzky University of Oldenburg

Title: BOLD representations of arm movement rate and speed in fMRI

Authors: *S. SHIRINBAYAN, A. DREYER, J. RIEGER
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Abstract: Among the kinematic variables of arm movements, movement speed (displacement per time unit) and movement rate (number of movement initiations per time unit) have been well-studied using electrophysiological and neuroimaging techniques. However, in most of the previous studies rate and speed of movement were highly correlated. This correlation hinders an unequivocal interpretation of the corresponding observed brain activations. In the current study we employed a two-by-two factorial design to investigate the pattern of the fMRI activation associated with rate and speed when they vary independently. We analyzed fMRI data acquired from 21 healthy subjects (10 females, age range: 20 – 32, mean age: 26) who were instructed to perform a target tracking task using a steering wheel. To examine whether rate and speed are the characteristic of successive or individual movements we performed block-based and event-related second-level GLM analyses. When data were analyzed with a block-based approach, no significant effects (FWE corrected at $p < 0.05$) of speed variation was observed, but we found differential rate-related BOLD-activation in contralateral primary sensorimotor cortex (SMC), bilateral putamen, contralateral thalamus and ipsilateral cerebellum. On the other hand, when we analyzed the data with an event-related GLM, taking individual movements into account, we found no significant rate-related activations, but contrasting levels of movement speed revealed activation in the contralateral primary SMC, contralateral thalamus, and ipsilateral cerebellum. The differential results for the block-based and the event-related analyses suggest that movement speed variations have stronger representation in fast BOLD-modulations following individual movements whereas rate variations have stronger representation in mean BOLD-activations. The latter effect could be due to build up effects caused by overlapping BOLD-responses. These

different representations of speed and rate in the BOLD-responses need to be considered when disentangling their effects.

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Poster

401. Voluntary Movements: Reaching Control: Movement Selection and Strategy

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Program #/Poster #: 401.23/NN14

Topic: E.04. Voluntary Movements

Support: CRC Research Program
Benno Nigg Chair in Biomechanics

Title: There is an energetic cost to movement jerk during human reaching

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Abstract: When people perform reaching movements, their hands typically follow smooth trajectories that may be predicted by minimizing the time-derivative of acceleration, or jerk. Minimization of jerk underlies a number of computational models for reaching, but its functional role remains unexplained. Reaching has been thought to entail trivial metabolic energy cost but non-trivial demands for speed and accuracy. In particular, accuracy is negatively affected by non-smooth neural control signals ("signal-dependent noise," Harris and Wolpert, 1998). The functional benefit of minimizing jerk might therefore be to improve accuracy. However, recent evidence (Huang et al., 2012) shows that reaching does exact a small but meaningful metabolic cost, in contrast to previous assumptions. But it remains unknown whether there are metabolic consequences to jerky movements. Here we show that jerky arm movements are indeed energetically costly, in an amount that increases with the time-derivative of acceleration. This suggests that energetic cost may help explain the minimization of jerk and its role in stereotypical reaching trajectories.

We tested the cost of movement jerk while healthy adults (N=12) performed reaching movements with their arms supported by a planar manipulandum (Kinarm, BKin Technologies, Kingston Canada). They performed cyclic movements between two points, at a series of four predetermined amplitudes and frequencies. Movement frequencies varied between 0.75 and 1.5 Hz, while amplitude was scaled with frequency to the -3/2 power, a relationship intended to increase jerk while keeping the average positive mechanical power approximately constant. We recorded reaching kinematics as well as oxygen consumption (Cosmed Inc,) to estimate the energetic cost of movement. Each trial lasted 6 min to allow time for steady state respirometry.

Despite mechanical power being held approximately constant, the metabolic power expended for reaching increased with greater jerk, from 25.4 to 52.2 W (P = 0.012). The cost increased more than twofold when movement frequency doubled from 0.75 to 1.5 Hz. These results support the hypothesis that jerky reaching movements have a metabolic energetic cost and may thus be one reason people tend to minimize it.

Disclosures: J.D. Wong: None. **T. Cluff:** None. **A.D. Kuo:** None.

Poster

402. Voluntary Movements: Plasticity

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Program #/Poster #: 402.01/NN15

Topic: E.04. Voluntary Movements

Support: Startup to AJN

Title: The effects of biological sex and ovarian hormones on exercise-induced neuroplasticity

Authors: *J. EL-SAYES[1], C. V. TURCO[2], L. E. SKELLY[2], C. NICOLINI[2], M. FAHNESTOCK[3], M. GIBALA[2], A. J. NELSON[2]
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Abstract: Acute aerobic induces short-term increases in excitability and reductions in inhibition of the motor cortex, as assessed via transcranial magnetic stimulation (TMS). To date, it is unknown whether biological sex and ovarian hormones impact exercise-induced neuroplasticity. Biological sex may influence the propensity for plasticity, as females show greater neuroplasticity induction via repetitive TMS compared to males. Further, ovarian hormones may impact the opportunity for exercise-induced neuroplasticity. Repetitive TMS yields greater neuroplasticity during ovulation, when estradiol levels are higher, compared to menstruation. The present study investigated the effects of biological sex and ovarian hormones on the magnitude and direction of neuroplasticity induced by acute aerobic exercise. Fourteen females and fourteen males (ages 18-30 years) participated in two sessions in which dependent measures were acquired before and following a single bout of aerobic exercise. The two groups were matched for cardiorespiratory fitness (VO_2max) as a function of fat-free mass (F: 58.9 ± 7.0; M: 55.7 ±7.4 ml/kgFFM/min). Females were tested in the follicular (~day 7) and luteal (~day 21) phases of the menstrual cycle. Males were also tested on two sessions separated by ~14 days. The exercise intervention consisted of 5 minutes of warm up, 20 minutes of moderate intensity cycling at 65-70% maximal heart rate, and 5 minutes cool down. Ratings of perceived exertion and heart rate were recorded throughout. TMS was used to obtain motor evoked potential (MEP) recruitment curves, short-interval intracortical inhibition (SICI), and intracortical facilitation

(ICF) from the right first dorsal interosseous muscle. MEP recruitment curves were assessed as a function of resting motor threshold (90-200%RMT). SICI and ICF were assessed using 80% and 90% active motor threshold as the conditioning stimulus intensities with interstimulus intervals of 2ms and 10ms, respectively. In addition, blood measures of estradiol, progesterone, testosterone, and brain-derived neurotrophic factor were assessed on both testing sessions. Preliminary results indicate that females show greater increases in MEP recruitment curve following exercise compared to males, and this occurs regardless of menstrual cycle phase. ICF does not change following exercise for males or females on either testing session. These data suggest that females show greater exercise-induced neuroplasticity compared to males, regardless of menstrual cycle phase.

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Poster

402. Voluntary Movements: Plasticity

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Program #/Poster #: 402.02/NN16

Topic: E.04. Voluntary Movements

Title: M1 tdcs does not influence performance of a fine-motor skill tweezer task

Authors: N. ZDANOWICZ, A. MEEK, D. GREENWELL, M. WOLFE, *Z. A. RILEY
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Abstract: Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique that has been applied to the primary motor cortex (M1) to facilitate learning of skilled, dexterous hand tasks. Recent studies have even used it to shorten training periods for learning precise neurosurgical skills with small instruments. However, it is unknown if the acquisition of the motor skill is due to consolidation, or learning over many days, or if skill proficiency can be observed in as little as a single practice session. The purpose of this study was to examine performance of a fine-motor skill tweezer task with the non-dominant hand while the subject received either anodal (facilitate learning), cathodal (inhibit learning), or sham stimulation conditions. 53 healthy young adults (age: 22.8 ± 3.3) were randomized into either anodal (n=16), cathodal (n=19), or sham (n=18) stimulation groups. The Edinburgh Handedness Inventory was used to determine their hand-dominance. Subjects completed a pre-test of the O'Connor Tweezer Dexterity Test followed by 20 minutes of practice with concurrent tDCS (based on their randomized condition) and then a post-test. The task consisted of using tweezers to place small metal pins into individual holes on a test board with the non-dominant hand. Pre- and Post-test performance was assessed by the time it took to place 50 pins. For practice, subjects filled one row of pins at a time followed by 30 seconds of rest after each completed row. tDCS electrodes

were placed over the M1 cortical area for the non-dominant hand and the contralateral supraorbital area. A current strength of 1mA was applied for 20 minutes in the anodal and cathodal conditions. Sham stimulation was applied according to established blinding procedures with a brief ramp in current followed by the current ramping back down. Practice performance was assessed by dividing the total practice into quartiles and assessing the average time per row for each quartile. All data were logarithmically transformed for parametric testing of the geometric means. There was a significant main effect of time ($p<0.001$) as all stimulation groups increased their speed to place 50 pins from pre- to post-test ($p<0.001$ to $p=0.025$). During practice, the average time spent placing each pin increased significantly from the first 25% to the last 25% of practice in the cathodal condition ($p<0.001$). Performance remained consistent across practice for Anodal and Sham conditions. The results indicate that anodal stimulation did not facilitate learning in the tweezer task, but cathodal stimulation can slow the acquisition of the skill at least in a single-session of practice.

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Poster

402. Voluntary Movements: Plasticity

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Program #/Poster #: 402.03/OO1

Topic: E.04. Voluntary Movements

Support: NSF GRFP 1342962

Title: Proprioceptive and sensorimotor neurophysiological changes associated with complex skill learning

Authors: *J. L. MIRDAMADI[1,2], C. R. SEIGEL[3], H. J. BLOCK[1,2]
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Abstract: Motor learning involves changes in behavior through practice and has been associated with plasticity in motor brain regions. It is well established that sensory information is important for motor control, yet its role in learning has only recently been investigated. Motor adaptation, a paradigm in which errors improve trial-by-trial in response to a perturbation, has been linked to plasticity in somatosensory cortex and shifted proprioception, or limb position sense. Further, individual differences in adaptation have also been linked to differences in somatosensory cortical activity. The importance of sensory processing in complex motor skill learning is less clear. Unlike adaptation, skill learning involves the acquisition of new movement patterns in the absence of a perturbation with performance limited by the speed-accuracy tradeoff. Here we

investigate the somatosensory contributions to motor skill learning at the behavioral and neurophysiological level in the right upper limb. Healthy young adults made visually-guided 2D reaching movements through an irregular-shaped track (20 cm x 20 cm horizontal workspace) as quickly and accurately as possible using a robotic manipulandum under null force. Subjects practiced movements over two consecutive days at a restricted speed range. Skill was quantified by measuring motor performance (accuracy inside track) across 5 speed ranges before practice on day 1 and again on day 3. Proprioception was measured across training and at retention using a passive two-alternative choice task to quantify bias (accuracy) and sensitivity (acuity). Short latency afferent inhibition, the reduction in motor evoked activity when a single pulse of transcranial magnetic stimulation (TMS) is preceded by a median nerve stimulus, was measured before and after practice. Based on previous findings linking the magnitude of SAI to somatosensory cortical (SI) activity, we hypothesized that changes in SAI would be related to proprioceptive changes and skill learning. Preliminary data (N= 7) indicate that proprioception largely improved, consistent with the literature. Excitability in primary motor cortex (M1) generally increased after practice, whether substantial skill learning occurred or not. We therefore speculate that M1 excitability changes result from motor execution rather than learning. However, SAI was reduced in some individuals, likely reflecting decreased S1 activity, and enhanced in others, likely reflecting increased S1 activity. This appears linked to individuals' amount of skill learning. Changes in SAI may be an important mechanism by which individuals learn new skills.

Disclosures: J.L. Mirdamadi: None. **C.R. Seigel:** None. **H.J. Block:** None.

Poster

402. Voluntary Movements: Plasticity

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 402.04/OO2

Topic: E.04. Voluntary Movements

Support: NHMRC Project Grant APP1078464

Title: Slow oscillating transcranial alternating current stimulation applied during a motor learning task enhances the effectiveness of learning

Authors: *M. V. SALE[1], A. KUZOVINA[2]
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Abstract: Repetitive training of a motor learning task can induce changes in motor performance arising from neuroplasticity. Retention of motor learning occurs via an encoding process, during which rapid neuroplastic changes occur in response to learning. Previous studies show that transcranial alternating current stimulation (tACS), a form of non-invasive brain stimulation, can

enhance encoding of declarative memories during wakefulness. However, the effect of tACS on *motor* learning in the awake brain is unknown. In this study, healthy 18-35 year old participants (n=28) received 0.75Hz tACS for 30 minutes during a ballistic thumb abduction motor learning task. Neuroplastic changes were quantified by measuring motor-evoked potential (MEP) amplitude of the *abductor pollicis brevis* muscle, and training-related behavioural effects were quantified by assessing changes in thumb abduction acceleration. These outcome measures were reassessed immediately after the motor learning task to quantify short-term changes, and approximately 24 hours later to assess longer-term changes. Participants in both active and sham stimulation conditions demonstrated significant increases in thumb abduction acceleration immediately after the motor learning. Further, participants in the active group maintained significantly higher thumb acceleration 24 hours later (t_{26}=2.0611, P=0.0494). There were no significant changes or inter-group differences in MEPs for both conditions. The results suggest that 0.75Hz tACS applied during motor learning enhances encoding, which manifests as enhancement in longer-term task consolidation. This has potential implications in neurorehabilitation where functional improvement relies on repetitive task re-learning, and may benefit from concurrent application of tACS to facilitate encoding.

Disclosures: M.V. Sale: None. **A. Kuzovina:** None.

Poster

402. Voluntary Movements: Plasticity

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 402.05/OO3

Topic: E.04. Voluntary Movements

Support: BMBF

Title: Optimized 10-cage video system for automated mouse phenotyping with open-source software

Authors: P. CHARLES[1], A. ISTUDOR[1], A. SCHATZ[1], *Y. WINTER[2]
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Abstract: The distribution and chaining of basic behavior elements throughout the 24h-day mirrors the state of an animal's behavior control circuitry. Thus, the analysis of undisturbed voluntary behavior can be used to identify a brain phenotype or indicate behavioral disturbances. Recording behavior over 24-hours results in big data. The only realistically feasible approach to collect and analyze such data is through computer automated methods. One standard approach is to use machine vision algorithms to extract data from video and then use trained neural networks and machine learning methods to automatically assign behavior categories to streams of image data. For mice, both proprietary and open-source software packages are available. They make

increasing use of both deep learning toolboxes and GPU parallel computing for boosting PC processing. In consequence, even on a larger scale video based phenotyping is becoming feasible. One caveat remains the initial quality of the images which greatly influences subsequent behavior processing. Image quality depends on the combination of cage setting, illumination, camera positioning, focal length, shutter speed and depth of field. We developed a multiple-cage platform for video phenotyping mice where we optimized each of these image acquisition parameters. The result is a compact and self-contained 10-cage video acquisition system suitable for long term usage in any environment. These videos can be used with any software for mouse behavior analysis, but it is optimized for our updated version of the (open source) software developed by the group of T. Poggio, M.I.T. (Jhuang et al., 2010, Nature Communications) that reliably detects the eight most common mouse behavior categories. With our platform, the parallel data acquisition and behavior analysis for 10 mice is possible in effectively real-time running three PCs in parallel. One PC acquires data and two PCs, with graphic card processing, perform post hoc analysis. This technology is setting the stage for the economical, large scale, automated video-phenotyping of mice.

Disclosures: P. Charles: None. **A. Istudor:** None. **A. Schatz:** None. **Y. Winter:** None.

Poster

402. Voluntary Movements: Plasticity

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 402.06/OO4

Topic: E.04. Voluntary Movements

Support: Department of Economy, Industry and Competitiveness
FEDER Grant SAF2016-80647-R
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The Michael J. Fox Foundation Grant ID: 9205

Title: Center-surround organization of short-term plasticity induced by transcranial static magnetic field stimulation of the human motor cortex

Authors: *C. AMMANN[1], M. DILEONE[1,2], A. OLIVIERO[3], G. FOFFANI[1,3]
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Abstract: Transcranial static magnetic field stimulation (tSMS) is a new non-invasive brain stimulation technique that decreases corticospinal excitability of the stimulated motor area (Oliviero et al., J Physiol 2011). Temporal features of tSMS effects indicate that the duration of tSMS-induced cortical plasticity is dependent on the duration of stimulation (Dileone et al.,

Brain Stimul 2018). However, spatial features of tSMS modulation have not been explored yet. The aim of this study was to examine the spatial organization of tSMS-induced effects. We recruited thirteen healthy subjects that participated in a crossover, double-blinded and counterbalanced study receiving 10 min tSMS through a cylindrical neodymium magnet of 45 mm diameter on the motor area corresponding to the first dorsal interosseous (FDI), in one session contralateral and in another session ipsilateral to the studied muscles of the left hand. Motor evoked potentials (MEP) induced by transcranial magnetic stimulation (TMS) were recorded from the FDI and extensor ulnaris carpis (EXT). For each session we determined two optimal 'hot spots' (mean distance between 'hot spots' = 26.8 ± 4.4 mm) for eliciting MEPs in the resting FDI and EXT. Before and after tSMS we recorded 80 MEPs randomizing 'hot spots' and TMS output intensities (110%, 120%, 130%, and 140% of resting motor threshold - RMT). As expected, tSMS significantly reduced corticospinal excitability of the FDI, when TMS output intensity that evoked a peak-to-peak MEP amplitude of ~ 1 mV was selected and at 110% RMT. Interestingly, most subjects that showed decreased excitability at the stimulated FDI 'hot spot' experienced an increased excitability at the nearby EXT 'hot spot'. Specifically, we found a significant positive correlation between the individual FDI-EXT cortical distance and the effect induced by tSMS on the EXT 'hot spot', i.e., the more distant the two 'hot spots' the greater the increase of EXT MEP amplitude. The interhemispheric modulation of corticospinal excitability was highly variable and will require further investigation. In conclusion, 10-min-tSMS induces short-term changes in corticospinal excitability with a specific spatial pattern of center-inhibition and surround-facilitation. These results uncover a center-surround organization of short-term plasticity in the human motor cortex.

Disclosures: C. Ammann: None. **M. Dileone:** None. **A. Oliviero:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurek SL. **G. Foffani:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurek SL.

Poster

402. Voluntary Movements: Plasticity

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 402.07/OO5

Topic: E.04. Voluntary Movements

Support: BBSRC Grant BB/N016793/1

Title: Corticospinal transmission in healthy humans is facilitated by repeated pairing of motor cortical and transcutaneous spinal stimulation

Authors: *R. HANNAH[1], P. BORGAS[1], L. DUFFELL[2], J. C. ROTHWELL[1]
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Abstract: Electrical stimulation of the spinal cord combined with volitional motor activity has been reported to improve motor control in individuals with spinal cord injury, but the mechanism for these improvements is unknown. Studies in primates suggest that timing spinal cord stimuli to coincide with descending motor commands enhances transmission at the corticospinal-motoneuronal synapse as a result of spike-timing dependent plasticity (STDP). Here we sought evidence for similar STDP-like mechanisms in the human spinal cord using non-invasive transcranial magnetic stimulation (TMS) and transcutaneous spinal stimulation (TSS). An advantage of this approach is that we can precisely control the timing of descending corticospinal and afferent (dorsal root) volleys, evoked by TMS and TSS respectively, converging on the spinal motoneurones. Eleven healthy volunteers (5 females, mean age 27 years) completed two sessions in which they received repeated pairing of TMS over the leg motor area and TSS over the lumbar region (100 pairs, 8 seconds apart). Corticospinal and afferent volleys were timed either to converge on spinal motoneurones at the same time, i.e. 0 ms delay between their arrival (Pstim0), or such that afferent volleys reached the spinal motoneurones 5 ms before the corticospinal volleys (Pstim5). Motor evoked potential (MEP) and spinal root reflex (SRR) amplitudes were recorded via surface EMG from the tibialis anterior and soleus muscles. In two individuals electrical stimulation was used to evoke thoracic MEPs (TMEPs) that activate corticospinal axons directly and therefore test transmission at the corticospinal-motoneuronal synapse. Agonist and antagonist EMG, peak force and peak rate of force development were recorded during ballistic dorsiflexion contractions as measures of motor control. Measurements were completed prior to and 5 minutes post the paired stimulation intervention. The Pstim0 protocol facilitated MEPs, whereas PStim5 had no effect. PStim0 also increased the amplitude of TMEPs (N = 2), implying that the changes were due to altered corticospinal-motoneuronal transmission rather than cortical excitability. SRRs, and performance and muscle activation during the ballistic contractions were unaffected by either protocol. The present data are consistent with the notion that spinal cord stimulation paired with descending corticospinal activity may induce STDP-like changes at the corticospinal-motoneuronal synapse. More sensitive measures of motor control may be required to uncover the functional consequences of this enhanced corticospinal transmission.

Disclosures: R. Hannah: None. **P. Borgas:** None. **L. Duffell:** None. **J.C. Rothwell:** None.

Poster

402. Voluntary Movements: Plasticity

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Topic: E.04. Voluntary Movements

Support: NIH 5R01NS064004 (JHM)
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Title: Theta burst electrical stimulation of the motor cortex produces functional reorganization of the corticospinal system in rats

Authors: *A. AMER[1,2,3], A. SARKAR[2,3], E. HARA[2,3], A. KHAN[3], Z. MIYFA[3], K. REN[4], L. RAMEAU[2,3], J. H. MARTIN[1,2,3]
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Abstract: Theta burst stimulation (TBS) of motor cortex (MCX) is a powerful neuromodulatory protocol for producing augmentation of MCX-evoked muscle responses and strengthening of synaptic transmission in healthy human and animal subjects and after motor systems injury. After a single epoch of TBS, stimulation-evoked motor responses (MEPs) are facilitated for approximately 30 minutes in humans and animals. Whereas acute TBS is well-studied, and its role in motor LTP has been characterized, the effects of chronic TBS are not-well understood. The aims of this study were to determine if there is day-to-day carryover of MEP augmentation produced by TBS and to further elucidate the anatomical and molecular underpinnings of this robust corticospinal system plasticity.
In this study, we activated MCX by intermittent TBS (iTBS) for varying times daily and for one or more days to drive the establishment of strong connections with spinal cord circuits in naïve rats. We aimed to identify MEP amplitude changes, corticospinal tract (CST) structural changes, and signaling pathway changes. We found that one epoch (3 minutes) of iTBS produced strong EMG potentiation lasting for up to 45 minutes. Five contiguous epochs of iTBS during a single session (27 minutes), prolonged EMG potentiation for 24-48 hours. Five epochs of iTBS daily, for 10 days, increased the slope of contralateral M1-to-muscle recruitment curves, and this augmentation persisted for at least 10 days after the end of stimulation. We next assayed CST structural changes that could support the enhanced motor responses. Chronic stimulation promotes outgrowth of the stimulated CST in the contralateral spinal cord intermediate zone. 3D reconstruction of CST synapses, defined by the co-expression of the pre- and post-synaptic markers; VGlut1 and PSD95 in labeled CST axons, revealed a significant increase in the total number and volume of PSD95 puncta as well as the total number and volume of colocalized VGlut1 and PSD95 clusters. Finally, we identified upregulation of mTOR and Jak/STAT signaling (Ps6 and PSTAT3) using Western blotting of MCX homogenates (whole cell and synaptosomal fractions) and immunohistochemistry of layer 5 pyramidal neurons.
Our findings suggest incremental carryover of MEP LTP after repeated epochs of iTBS and, remarkably, emergence of persistent MEP augmentation that associates with upregulation of known axon growth signaling and cortical plasticity markers. We hypothesize that iTBS both lays the groundwork for enhanced plasticity in MCX and activates an axon growth program. Together, these changes in spared corticospinal motor circuits could support recovery after injury.

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Poster

402. Voluntary Movements: Plasticity

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Topic: E.04. Voluntary Movements

Support: NIH R01 NS085167
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Title: Timing and intensity of vagus nerve stimulation influences motor cortex plasticity

Authors: *R. A. MORRISON, D. HULSEY, K. ADCOCK, Y.-Y. TSANG, A. KUO, J. JOHN, R. L. RENNAKER, M. P. KILGARD, S. A. HAYS
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Abstract: Stroke and traumatic brain injury (TBI) are two of the leading causes of long-term motor disability in the United States. While extensive rehabilitation after these injuries can lead to partial recovery, many still suffer chronic motor impairment afterward. Recovery is widely believed to be a result of plasticity in brain areas surrounding the injury. Recently, studies have suggested that administering vagus nerve stimulation (VNS) during rehabilitation can enhance recovery by increasing plasticity in activated motor circuits. If VNS-mediated recovery is dependent on enhanced synaptic plasticity, it is possible that optimizing VNS delivery for plasticity could yield even greater recovery. Here, we test the effect of varying the timing and intensity of VNS to characterize parameters that would yield the greatest enhancement of plasticity. To determine the effect of varying stimulation intensity, rats were trained to perform a skilled motor task in which a lever was pressed for a food reward. Once proficient, rats underwent five additional days of behavioral training in which low intensity VNS (0.4 mA), moderate intensity VNS (0.8 mA), high intensity VNS (1.6 mA), or sham stimulation was paired with forelimb movement. After completion of behavioral training, intracortical microstimulation (ICMS) was used to document movement representations in the motor cortex. We find that moderate intensity VNS significantly enhances reorganization of motor representations, while low and high intensity VNS fail to enhance motor cortex plasticity. Additionally, to ascertain whether the interval between stimulations influenced the degree of plasticity, a separate cohort of animals underwent five days of VNS pairing with short stimulation intervals (8 sec) or long stimulation intervals (30 sec). These results suggest that it may be critical to titrate the intensity of VNS that patients receive during rehabilitation, and highlight the importance of determining the optimal stimulation parameters for clinical implementation of VNS.

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Poster

402. Voluntary Movements: Plasticity

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 402.10/OO8

Topic: E.04. Voluntary Movements

Title: The use of tDCS to enhance learning of a dart throwing task

Authors: *A. W. MEEK[1], D. GREENWELL[1], M. WOLFE[1], B. J. POSTON[2], Z. A. RILEY[1]
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Abstract: Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique that is used to increase (anodal stimulation) or decrease (cathodal stimulation) skilled motor performance depending on the polarity of the applied current. Most studies using tDCS have focused on simple hand and finger based motor skills such as pinch force tasks or tracing tasks, though it is unknown if tDCS can influence learning of more complex unilateral motor skills such as throwing. The purpose of this study was to examine how tDCS influences the acquisition of dart throwing skills with the non-dominant hand in healthy subjects. 58 subjects (age: 23.3 ± 3.9) were randomized into either anodal (n=20), cathodal (n=19), or sham (n=19) stimulation groups. The Edinburgh Handedness Inventory was used to determine their hand-dominance. Subjects completed a pre-test of throwing accuracy followed by 20 minutes of practice with concurrent tDCS (according to their randomized condition) and then a post-test. The task consisted of throwing darts at the center (bulls-eye) of a dartboard from a distance of 10 feet. Accuracy was measured as the distance of each throw from the center of the bulls-eye. Gain scores (score - baseline) were calculated for each quartile of the practice throws and the post-test. tDCS electrodes were placed over the M1 cortical area for the non-dominant hand and the contralateral supraorbital area. A 1mA current was applied for 20 minutes in the anodal and cathodal conditions. Sham stimulation was applied according to established blinding procedures with a small ramp in current followed by the current shutting off. The results showed that anodal stimulation significantly improved performance between the first 25% and second 25% of practice throws ($p < 0.001$) and this improvement was maintained through the post assessment ($p = 0.013$). Cathodal and sham stimulation conditions showed no significant increase in gain scores across time. The gain scores for the anodal group were significantly higher than cathodal gain scores at the second 25% of practice ($p=0.036$) and remained significantly better through the post-test ($p=0.03$). There was a significant difference between the anodal and sham group gain scores in the last 25% of practice ($p=0.017$) with the anodal group showing greater

improvement. These results indicate that the acute application of anodal tDCS during a single short-term practice session can enhance the acquisition of a complex skilled motor task such as dart throwing, whereas the sham stimulation condition did not show improvement with practice. The results were polarity dependent as well, since the cathodal stimulation appeared to block learning.

Disclosures: A.W. Meek: None. **D. Greenwell:** None. **M. Wolfe:** None. **B.J. Poston:** None. **Z.A. Riley:** None.

Poster

402. Voluntary Movements: Plasticity

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 402.11/OO9

Topic: E.04. Voluntary Movements

Title: Kinesthetic illusion shapes motor cortex plasticity induced by action observation

Authors: *M. BOVE, M. BIGGIO, P. RUGGERI, L. AVANZINO, A. BISIO
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Abstract: Acquisition of new motor abilities depends heavily on movement execution. However, recent evidences pointed out the role played by action observation (AO) in acquiring new motor skills and evoking cortical plasticity. These phenomena could happen only when AO immediately precedes or is concurrent to movement execution, or when peripheral electrical stimulation congruent with the observed action are delivered during AO. In the present study, we combined action observation with the sensory afferent signals specific for movement execution, i.e. the proprioceptive information coming from the lengthening muscle, which was generated by means of a mechanical vibration able to evoke a kinesthetic illusion (KI) of movement. The proprioceptive stimulation was delivered by means of a vibrator positioned over the extensor pollicis brevis muscle, the latter generating an illusory sensation of thumb abduction. The experiment was composed of two conditions where the observed action and the afferent signal could be either congruent or incongruent. In the congruent condition (AO-KI CONGR) participants observed a 10-sec video showing the thumb abduction of the right hand (generated by the activation of the abductor pollicis brevis muscle - APB), whilst in the incongruent condition (AO-KI INCONGR) they observed a thumb adduction. The activity of the primary motor cortex (M1) in correspondence to the APB muscle area was evaluated by means of recruitment curves before, immediately after and 30 and 60 minutes after the end of the stimulation. The results showed a significant increase of the M1 excitability, as measured by the slope of the recruitment curves lasting until 60 minutes after AO-KI CONGR. No differences were observed before and after the administration of AO-KI INCONGR. Furthermore, in a control experiment we tested whether this effect was specific for the kind of sensory input, and a

tactile vibration was delivered during the observation of thumb abduction. This protocol did not evoke any changes in M1 excitability. Following these results, we proposed that AO, when combined to KI evoked by a proprioceptive stimulation, was able to evoke plastic changes in M1 activity. Since the illusory sensation of movement was crucial for this to occur, we might speculate that the locus where the two afferent signals combined was M1. These results candidate AO-KI as possible methodology to train the motor system without moving.

Disclosures: M. Bove: None. **M. Biggio:** None. **P. Ruggeri:** None. **L. Avanzino:** None. **A. Bisio:** None.

Poster

402. Voluntary Movements: Plasticity

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 402.12/OO10

Topic: E.04. Voluntary Movements

Title: Performance of a timing-based hand dexterity video game with M1 tDCS

Authors: *M. WOLFE[1], A. ZAKERESFAHANI[1], M. WILSON[1], A. W. MEEK[1], B. J. POSTON[2], Z. A. RILEY[1]
[1]Dept. of Kinesiology, Indiana University-Purdue Univ. Indianapolis, Indianapolis, IN; [2]Kinesiology and Nutr. Sci., Univ. of Nevada Las Vegas, Las Vegas, NV

Abstract: Transcranial direct current stimulation (tDCS) has been applied to the motor cortex (M1) to facilitate learning of skilled, dexterous hand tasks. However, when there is a timing component to the motor skill, tDCS is usually applied to the cerebellum. The purpose of this study was to determine if M1 tDCS could improve performance of a motor skill that required dexterous finger movements and timing. 34 healthy adults (age: 22.8 ± 2.7) were randomized into either anodal (n=19) or sham (n=15) stimulation groups. The Step Mania game is a timing-based video game that involves pressing the correct arrow keys on a keyboard at specific times when scrolling icons overlapped on a computer screen. Each subject practiced the task at least two times to obtain a pre-practice score. Each key press corresponded to a time relative to the optimal key pressing time. This put each key press into bins corresponding to an accuracy score. If the subject pushed the key optimally they were given a score of 1, and this score decreased as they were further away from the target (0.75, 0.5, 0.25, 0, and a complete miss, -1). Scores for each trial were an average these numbers. Each subject completed ~2 minutes of practice followed by ~2 minutes of rest and repeated this 5 times for 20 total minutes of practice. Post-test scores were obtained 5 and 10 minutes following practice. During practice, tDCS electrodes placed over the M1 cortical area for the non-dominant hand and the contralateral supraorbital area delivered a current of 1mA in the anodal condition. Sham stimulation was applied according to established blinding procedures with a brief ramp in current followed by the current ramping

back down. Gain scores (score – baseline) were calculated for each condition. Gain scores were similar for practice trials 1 and 2, but the anodal stimulation produced significantly greater gain scores during practice trials 3 and 4 (p=.047 and p=0.043, respectively). Gain scores did not significantly improve across time with sham stimulation, whereas anodal stimulation resulted in significantly higher gain scores at both 5 minutes (p=0.04) and 10 minutes (p=0.04) post-practice. These results show that after the initial few trials subjects not receiving anodal tDCS did not continue to improve for the duration of the practice, whereas the anodal stimulation resulted in continued improvements. In the context of motor learning, within a single session, it appears that anodal tDCS paired with practice can be more effective than practice alone for a timing-based video game task.

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Poster

402. Voluntary Movements: Plasticity

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Topic: E.04. Voluntary Movements

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Title: Movement-dependent electrical stimulation for promoting cortico-cortical plasticity

Authors: *S. MOORJANI, S. WALVEKAR, S. I. PERLMUTTER, E. E. FETZ
Physiol. and Biophysics, Univ. of Washington, Seattle, WA

Abstract: The strength of synaptic connections between motor-cortical sites in monkeys has been modified by spike-triggered stimulation[1] and electromyographic-activity-triggered stimulation.[2] We investigated a conditioning protocol in which electrical stimuli were triggered by movement to induce simultaneous activation of neurons at two motor-cortical sites. Such movement-triggered stimulation (MTS) led to strengthening of cortical connections that was strongly dependent on the behavioral context of the conditioning stimuli. Conversely, preventing co-activation led to weakening of connections.
Two monkeys were implanted with microwire electrodes placed bilaterally in the sensorimotor cortex. The strength of synaptic connections between sites was documented by the size of cortical evoked potentials (CEPs) recorded at one site after stimuli were delivered at the other site. Monkeys performed an alternating wrist flexion-extension target-tracking task involving a 1.6-s hold time. MTS was delivered for 90 min during flexion or extension holds while monkeys

performed the task. Movement-related activity at two motor-cortical sites (Nrec and Nstim) was inferred from the motor output elicited by stimulation at those sites. When MTS was delivered to Nstim during movements that activate neurons at Nrec, MTS produced dramatic strengthening of connections when paired with relevant activity, as seen by an increase in the size of CEPs evoked at Nstim by test stimuli at Nrec. In some cases, CEPs were over 300% larger after conditioning compared to preconditioning level. The resultant plasticity persisted for over 2 weeks during which the monkey's wrist activity dictated the strength of the connection, with larger gains coinciding with the monkey's higher activity levels. Importantly, neither activity nor conditioning alone produced similar effects, emphasizing the importance of behavioral context on the resultant plasticity. Conversely, when MTS was delivered to Nstim during movements that reduced activation of neurons at Nrec, the connection was depressed by 180%. We are investigating the mechanisms underlying MTS-induced plasticity, but our analyses to date suggest that modifications in CEP size were unlikely to have been produced by changes in general excitability.
Our data show that MTS can create conditions in which behavior drives conditioning-induced gains long after the stimulation has ended. Combining MTS with use-dependent physical therapies may enhance recovery outcomes after injury, a research direction we are pursuing in spinal-cord injured rats.
1. Jackson, A., et al. *Nature* **444** (2006).
2. Lucas, T. H. & Fetz, E. E. *J. Neurosci.* **33** (2013).

Disclosures: S. Moorjani: None. **S. Walvekar:** None. **S.I. Perlmutter:** None. **E.E. Fetz:** None.

Poster

402. Voluntary Movements: Plasticity

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 402.14/OO12

Topic: E.04. Voluntary Movements

Title: Prevalence of BDNF polymorphism in musicians: Evidence for compensatory motor learning strategies in music?

Authors: *T. L. HENECHOWICZ[1], J. L. CHEN[2], L. G. COHEN[3], M. H. THAUT[1]
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Abstract: Objective/Rationale. The study compared the prevalence of Val66Met BDNF SNP polymorphism (r6265) in musicians in professional training (N=30, M=15) to an ethnically matched general population sample from the 1000 Human Genome Project (N=424). The polymorphism is present in 25-30% of the general population and is associated with deficits in motor learning and neuroplasticity (Nooshabadi et al., 2016; Kleim et al., 2006; Morin-Moncet et

al., 2014). Therefore one may predict that musicians have a reduced polymorphism prevalence compared to the general population due to the high skill demands on motor learning and performance.
Methods. For genotypic and allelic frequency analysis DNA extraction and genotyping OG 500 kits from DNA Genotek were processed on the QIAsymphony SP magnetic bead DNA extractor using the 1.0 mL Oragene saliva protocol and QIAsymphony DSP DNA Midi Kit (QIAGEN). The samples were genotyped for the SNP rs6265 (BDNF; Val66Met) using a pre-designed TaqMan® SNP Genotyping Assay and were analyzed using the ViiA™ 7 Real-Time PCR System using ViiA™7 software. Fisher's exact test was used to assess difference in genotype frequencies between groups. Chi-square test assessed the difference between A allele frequencies (A allele = Met allele; G allele = Val allele).
Results. Genotypic and allelic frequencies in the musicians' sample were not statistically different from the general population sample. Genotypic Frequency: G/G 62.7% Controls vs 58.6 % Musicians; A/G 33.3% Controls vs 37.9 Musicians; A/A 4.0% Controls vs 3.5% Musicians ($p=0.88$). Allelic Frequency: G Allele 79.4% Controls vs 77.6% Musicians; A Allele 20.6 % Controls vs 22.4% Musicians ($p=0.75$). In the musician cohort no significant demographic differences were found for age and achievement except Met-Carriers had 4.4 more years in primary and 3.2 years in total training ($p<.01$).
Conclusion. Presence of Val66Met BDNF polymorphism did not bias against high-end motor skill learning in music. Learning strategies specific to music may exist to compensate for genotype predisposition. Motor learning in music is temporally 'overstructured', sonifies movement into sound patterns, and relies on high practice repetition rates. Interestingly in our cohort Met carriers had significantly more training years, indicating earlier entry ages into music training than Val carriers. Since Val66Met polymorphism is also associated with decreased rates of stroke recovery (Kim et al., 2016) data may have relevance for translations of music-based training strategies to stroke rehabilitation.

Disclosures: T.L. Henechowicz: None. **J.L. Chen:** None. **L.G. Cohen:** None. **M.H. Thaut:** None.

Poster

402. Voluntary Movements: Plasticity

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 402.15/OO13

Topic: E.04. Voluntary Movements

Support: VA Grant RX001640
NIMH Grant
NCIRE Grant
UCSF Neurology

NSF Graduate Research Fellowship

Title: Changes in spindle induced spiking activity after motor learning

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Abstract: Thalamo-cortical sleep spindles are thought to play an important role in the acquisition and consolidation of new motor skills. Spindles have been studied extensively using EEG and ECoG recordings, and this body of work has consistently reported a link between the rate of sleep spindles and improvements in performance. Moreover, spindles nested within cortical up-states have been implicated as a substrate for neural plasticity. However, the mechanisms tying sleep spindles to learning remain unclear. To better understand the role that sleep spindles play in motor learning, it is critical to understand the relationship between spindles and the fine-scale structure of spiking activity.

To that end, we recorded local field potentials (LFPs) and extracellular spiking activity from the primary motor cortex (M1) as four rats learned a skilled reach-to-grasp task. The task was split into four blocks: $Sleep_1$, $Task_1$, $Sleep_2$ and $Task_2$. Rats improved their performance (i.e. increased their accuracy and speed) during the reach-to-grasp task and showed further offline improvements in speed after sleeping. After task engagement we observed a significant increase in both the rate of sleep spindles in M1 and the degree of spindle nesting within slow oscillations.

We next analyzed the evolution of fine-scale spiking structure during spindles. Single neurons tended to spike at a preferred phase within the ongoing oscillation; importantly, this increase in phase-locking coincided with an increase in the pairwise cross-correlation (or *synchrony*) between neurons. Interestingly, both the phase-locking and spiking synchrony were larger for nested spindles compared to unnested spindles.

After learning a motor task, rats showed a marked increase in the rate of sleep spindles in M1, consistent with human and animal studies monitoring sleep after motor learning. We also observed changes in spindle-induced modulation of spiking activity and synchrony following learning. The spindle effects were magnified for spindles that occured within up-states, consistent with the idea that nested spindles play an important role in offline learning. Overall, our results suggest that spindles precisely modulate the timing of spiking activity and thereby support offline gains in motor performance.

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Poster

402. Voluntary Movements: Plasticity

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 402.16/OO14

Topic: E.04. Voluntary Movements

Support: NIH Grant 4R01HD073147-05

Title: tDCS over dorsolateral prefrontal cortex does not affect prefrontal-to-M1 interhemispheric inhibition

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Abstract: The lateral prefrontal cortex (PFC) plays a wide range of crucial roles in higher-order cognitive functions. Malfunction in this region has been associated with several mood disorders, and cognitive abnormalities. Previous studies have attempted to modulate PFC function by applying non-invasive transcranial direct current stimulation (tDCS) and demonstrated positive effects on behavioral functions. Although tDCS effects on cortical excitability have been well demonstrated when the primary motor cortex (M1) was targeted, neurophysiological evidence underlying PFC-tDCS effects remain poorly understood. In this study, we aimed to examine changes in PFC excitability by assessing inter-hemispheric inhibition (IHI) from the lateral PFC to M1 (PFC-M1 IHI) before and after anodal, cathodal, or sham tDCS. We hypothesized that the magnitude of PFC-M1 IHI would be modulated in a polarity-specific manner. We used a double-blinded, crossover and counterbalanced design. Each volunteer (n = 15) participated in three sessions for different tDCS conditions. For tDCS, one electrode was placed over the right lateral PFC and the other electrode was on the left supra-orbital area. For active (anodal, cathodal) or sham tDCS, the current was applied at 2 mA for 15 min or 30 s, respectively. PFC-M1 IHI was assessed using a paired-pulse TMS paradigm before and at 0, 10, and 20 mins after tDCS application. We also assessed corticospinal excitability by applying single pulse TMS over the left M1 to prove that potential modulation in PFC-M1 IHI was not accompanied with corticospinal excitability changes. Against our hypothesis, we found no modulation in the magnitude of PFC-M1 IHI and corticospinal excitability after anodal, cathodal, or sham tDCS. To rule out the possibility that PFC-M1 IHI could not be modulated by an intervention, we assessed the same measure while another group of participants (n = 15) performed on two different types of visuomotor reaction time tasks. Results showed that the magnitude of PFC-M1 IHI decreased during a pre-movement period (between visual cue appearance and initiation of physical reaction), suggesting that PFC-M1 IHI could change depending on movement contexts.

These findings indicate that PFC-M1 IHI may not be a powerful proxy to detect possible excitability changes in PFC modulated by tDCS application.

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Poster

402. Voluntary Movements: Plasticity

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Program #/Poster #: 402.17/OO15

Topic: E.04. Voluntary Movements

Support: NIH Grant R01NS030853
NIH Grant T32HD057850

Title: Neurophysiological correlates of maladaptive plasticity in the population code of the intact hemisphere of rats after an ischemic infarct of primary motor cortex

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Abstract: Behavioral impairment following acquired brain injury in primary motor cortex (M1) can often be improved with behavioral rehabilitation, as a result of neuroplastic reorganization of spared regions. Post-injury functional recovery has been observed to result in changes of motor map representation, redistribution of neuroanatomical connections, and shifts in the patterns of neural excitation relative to behavior, but the functional role of such changes remains unclear. Is reorganization in a given area a sign of maladaptive compensation, or a mechanism to adaptively overcome the loss of damaged tissue? We correlated the gross kinematic covariates of a reaching behavior with neurophysiological data collected in spared motor areas of neocortex in rats receiving either a sham or ischemic infarct in M1 contralateral to the preferred reaching forelimb. Regions of interest (ROIs) describing the extent of the reaching forelimb and paw were identified in 49,240 video image frames. A model was developed to estimate the relative contribution to accuracy in decoding the kinematic ROI using multi- and single-unit activity recorded from either M1 of the intact hemisphere, or premotor cortex (PM) of the injured hemisphere. Relative contributions to decoding accuracy from each area were then used to determine the likelihood of successful pellet retrieval in individual trials using a logistic regression model. The ability to predict trial outcomes based on relative accuracy contributions of the two areas suggests that population coding in M1 of the intact hemisphere initially plays an

adaptive role in control of behavior during the first week after injury, but gradually shifts to a maladaptive role by three weeks after the injury. Injured rats had an increased number of interhemispheric pairs of units with a roughly 5 Hz oscillatory cross-correlational structure, suggesting the increased involvement of a slow field potential coordinating activity in these regions. While no shift in this rhythmic correlation was evident over the course of 3 weeks, the phase offset between interhemispheric pairs of units is affected in injured rats, suggesting that the timing of coupled activity between intact M1 and PM is disrupted after injury.

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Poster

402. Voluntary Movements: Plasticity

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Program #/Poster #: 402.18/OO16

Topic: E.04. Voluntary Movements

Support: Wellcome Trust

Title: Real-time fMRI neurofeedback for bi-directional modulation of ipsilateral sensorimotor cortex

Authors: *Z.-B. SANDERS[1], C. SAMPAIO-BAPTISTA[1], K. DIOSI[1], M. LUEHRS[2], R. GOEBEL[2], H. JOHANSEN-BERG[1]
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Abstract: Sensorimotor activity patterns during executed movements are altered in stroke patients with hemiplegia, with patients showing increased bilateral activity compared to healthy individuals. Modulating these activity patterns may help improve motor functioning. However, different patients may benefit from either increased or decreased recruitment of ipsilateral sensorimotor cortex, depending on impairment level and corticospinal tract integrity. In this study, we investigated whether healthy individuals are able to use real-time functional magnetic resonance imaging (fMRI) over several days to bi-directionally alter brain activity in the ipsilateral sensorimotor cortex. We recruited 15 healthy right handed participants to participate in 6 sessions of 7T real time fMRI neurofeedback. During neurofeedback participants were asked to make unimanual movements with their left hand and were shown two bars representing activity from regions of interest (2x2x2cm) in each sensorimotor cortex. Each participant completed two different neurofeedback conditions separated by at least 1 month. In one condition, they were instructed to maximally co-activate the two sensorimotor cortices (Association), whereas in the other they were instructed to increase contralateral and decrease ipsilateral activity (Dissociation). Participants were scanned three times for each condition with

24 hours between the first two scans and 48 hours between the second and third scan. Participants were able to use neurofeedback to increase activity in the ipsilateral sensorimotor cortex during the Association condition compared to the Dissociation condition on all three days (whole brain voxel-wise paired sample t-test). To assess whether multiple sessions enhance the neurofeedback effect, we looked at within-condition changes over days. In the Association condition, ipsilateral activity was increased on Day 2 compared with Day 1 and contralateral activity was increased on Day 3 compared with Days 1 and 2. This suggests that participants initially learn to increase activity in the ipsilateral hemisphere, followed by increasing the contralateral hemisphere. In contrast, in the dissociation condition participants learned to decrease ipsilateral activity within each day, but not across days as no significant changes between days were found. These findings demonstrate that individuals are able to use neurofeedback to bi-directionally modulate brain activity in sensorimotor cortices and that multiple sessions can enhance these effects. This highlights the feasibility of tailoring neurofeedback interventions to patients depending on their characteristics.

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Poster

402. Voluntary Movements: Plasticity

Location: SDCC Halls B-H

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Program #/Poster #: 402.19/OO17

Topic: E.04. Voluntary Movements

Support: Zumberge Multi-school Interdisciplinary Award, University of Southern California

Title: Brain-behavior relationship: Interhemispheric inhibition and bimanual coordination in skilled musicians

Authors: *Y.-L. KUO, J. J. KUTCH, B. E. FISHER
USC, Los Angeles, CA

Abstract: Background: As interhemispheric inhibition (IHI) is essential for dexterous motor control, bimanual skill developed with instrument playing may result in increased IHI in musicians. However, it is unclear whether there is any difference in the relationship between interhemispheric inhibitory circuits and bimanual motor skills in skilled musicians compared to non-trained individuals. Therefore, the current study aimed to compare the relationship between IHI and bimanual coordination in skilled musicians compared to non-musicians. **Methods:** Thirty-six musicians and 36 non-musicians participated. An 8-element finger sequence task (FST) was used to test bimanual coordination. Speed, accuracy, and evenness of key pressing interval were recorded. The Purdue pegboard test was used to test asymmetric coordination, by

measuring the number of assembled objects. Using transcranial magnetic stimulation, IHI was measured as ipsilateral silent period (iSP), both in left (L) and right (R) hemispheres. Canonical correlation analysis (CCA) was used to identify linear relationships between the IHI and bimanual coordination measures. A general linear model was used to compare the IHI-bimanual coordination relationship between musicians and non-musicians. Permutation testing was used to validate whether the observed differences of IHI-bimanual coordination relationship between musicians and non-musicians occurred by chance. Variable reduction CCA was performed with only the highly-contributing variables based on multiple regression analysis. **Results:** Compared to non-musicians, musicians demonstrated significantly better bimanual coordination (faster speed, higher accuracy, and more evenness). No differences in iSP were observed between the two groups. However, canonical correlation analysis (CCA) showed that the composite IHI variables (iSP-L, iSP-R) were significantly related to bimanual coordination in musicians ($r = 0.52$), but not in non-musicians ($r = -0.06$). The strength of the relationship was significantly greater in musicians than in non-musicians ($p = 0.016$). Variable reduction CCA showed that greater L-to-R IHI was related to increased evenness but at the cost of reduced speed in musicians. **Discussion:** Musicians demonstrated superior bimanual coordination in an instrument-like task compared to non-musicians. Prolonged musical training strengthened the relationship between interhemispheric inhibitory circuits and bimanual motor skills. The trade-off between evenness and speed was dependent on IHI and may result from long-term musical training as reducing variability is an important skill in instrument playing.

Disclosures: J.J. Kutch: None. **B.E. Fisher:** None.

Poster

402. Voluntary Movements: Plasticity

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Program #/Poster #: 402.20/OO18

Topic: E.04. Voluntary Movements

Support: Canadian Institutes of Health Research
Natural Sciences and Engineering Research Council of Canada

Title: Changes in human white-matter architecture following sensorimotor adaptation

Authors: *A. J. DE BROUWER, J. Y. NASHED, D. STANDAGE, J. R. FLANAGAN, J. P. GALLIVAN
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Abstract: Sensorimotor adaptation is thought to reflect the resultant combination of two separate, but interacting processes: an implicit, error-based process linked to slow learning and an explicit, strategic process linked to fast learning and savings (i.e., faster re-learning). To

explore the neuroanatomical basis of these processes, we investigated the effects of a brief sensorimotor adaptation task on structural plasticity in human white matter using diffusion tensor imaging (DTI). Specifically, we examined whether individual differences in rates of (re)learning are related to short-term changes in structural organization of white matter. Participants performed two sessions (separated by 24 hours) of center-out cursor movements to visual targets using a force sensor, while adapting to an instantaneous 45° rotation of the visual feedback of the cursor. DTI scans were obtained at the beginning of each session. Preliminary analyses suggest that individual changes in diffusivity indices in regions in prefrontal cortex are related to faster relearning in the second session. Further tract-based analyses will be used to investigate the extent of these short-term changes and whether they are linked to individual differences in white matter connectivity.

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Poster

402. Voluntary Movements: Plasticity

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Topic: E.04. Voluntary Movements

Support: IMSS Grant FIS/IMSS/PROT/G11-2/1028

Title: Pharmacological lesion of the rat motor cortex induces spinogenesis in spinal cord motor neurons

Authors: *M. N. VÁZQUEZ HERNÁNDEZ[1], N. G. MARTÍNEZ-TORRES, 44340[2], D. GONZÁLEZ-TAPIA[3], M. E. FLORES-SOTO[1], H. SALGADO-CEBALLOS[4], I. GONZÁLEZ-BURGOS[1]
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Abstract: Motor function is impaired in multiple neurological diseases associated with corticospinal tract degeneration affecting communication between the cortical neurons and the spinal cord motor neurons. Motor impairments has been linked to plastic changes at both the presynaptic and postsynaptic levels. However, there is no evidence of changes in those postsynaptic structures mediating synaptic transmission between motor cortex and spinal cord, namely, dendritic spines. In this study, 26 Sprague-Dawley adult female rats were used. These were divided in two groups: experimental group (Exp) and control group (Ctrl). For the Exp group kainic acid was used to induce a pharmacological lesion to the primary motor cortex.

Fifteen days later, motor function was evaluated by the BBB scale and the Rota-rod device. Also, the density of thin, stubby, and mushroom spines was quantified in motor neurons from a thoraco-lumbar segment of the spinal cord, using a modification of the rapid-Golgi method. Spinophilin, synaptophysin, and β iii-tubulin content was semi-quantified. For the Ctrl group were used the same procedure, except that saline solution was used instead kainic acid. Pharmacological lesion resulted in inefective motor performance in both behavioral tests. Spine density and the proportion of thin and stubby spines were greater in group Exp in comparison with the Ctrl group. The proportion of mushroom spines was unchanged. Spinophilin, synaptophysin, and β iii-tubulin increased in the Exp animals. The clinical symptoms of neurological damage secondary to Wallerian degeneration of the corticospinal tract were associated with spontaneous, compensatory plastic changes at the synaptic level. Based on these findings, spontaneous plasticity is a factor to consider when designing strategies in the early phase of rehabilitation.

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Poster

402. Voluntary Movements: Plasticity

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Program #/Poster #: 402.22/PP2

Topic: E.04. Voluntary Movements

Support: NIH K12HD055931
Foundation for Physical Therapy
LSVT Global Small Student grant
AHA 00035638

Title: Targeted neuromodulation of interhemispheric connectivity after stroke

Authors: S. LIN, *J. A. PALMER, M. R. BORICH
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Abstract: Introduction: After stroke, atypical interhemispheric connectivity has been observed in stroke survivors with poor motor recovery and may affect post-stroke motor function. Specifically, findings from previous research have indicated the presence of excessive interhemispheric inhibition (IHI) from the contralesional to ipsilesional motor cortex, creating a model of interhemispheric competition after stroke that may contribute to motor recovery. However, the relationship between levels of IHI and motor function after stroke remain unclear. Further, it is unknown how modulation of IHI through transcranial magnetic stimulation (TMS)

might influence post-stroke motor function. The purpose of this study was to 1) investigate the relationship between IHI and post-stroke motor function and 2) determine the effect of a session of interhemispheric paired associative stimulation (ihPAS) on neuromodulation of IHI and motor performance in stroke survivors. Methods: Eight participants (3 male) with ischemic chronic (>6 mo.) stroke were tested during two separate sessions with two different ihPAS conditions: $ihPAS_{8ms}$ (interstimulus interval [ISI] of 8ms) and $ihPAS_{1ms}$ (ISI of 1ms). ihPAS consisted of 100 paired TMS pulses to contralesional M1 (cM1)followed by ipsilesional M1 (iM1)delivered at 0.25HZ. We assessed IHI bilaterally prior to and at three time points following ihPAS (POST 0', POST30', POST 24hrs) using the dual coil paired pulse paradigm with the conditioning pulse delivered 10ms prior to the test pulse on contralateral M1. Electromyography (EMG) was used to measure the motor evoked potential of first dorsal interosseous muscles bilaterally. Results: Significantly greater IHI (p=.004) from iM1-to-cM1 than cM1-to-iM1 was observed at baseline. Baseline iM1-to-cM1 IHI was positively correlated with paretic arm motor function. There was no relationship between cM1-to-iM1 IHI and paretic arm motor function. Following $ihPAS_{8ms}$, there was a decrease in iM1-to-cM1 IHI (p=.03) at POST 30'. Neither ihPAS condition modulated cM1-to-iM1 IHI. Discussion: The preliminary results suggest that greater iM1-to-cM1 IHI is present in individuals post-stroke and may be important for post-stroke motor recovery, a finding that contrasts the previous model of interhemispheric competition. Results also suggest that ihPAS may reduce iMi-to-cM1 IHI. Further analyses are needed to compare IHI values in stroke versus healthy individuals and to determine the relevance of this unanticipated directional change in IHI to change in motor performance and behavior following ihPAS.

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Poster

402. Voluntary Movements: Plasticity

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 402.23/PP3

Topic: E.04. Voluntary Movements

Support: National Endowment for the Arts

Title: Is there an instrument-dependent effect on interhemispheric inhibition and bimanual coordination in musicians?

Authors: Y.-L. KUO[1], J. E. GORDON[1], C. J. WINSTEIN[1], J. J. KUTCH[1], S. S. KANTAK[2], *B. E. FISHER[1]
[1]USC, Los Angeles, CA; [2]Moss Rehabil. Hosp., Elkins Park, PA

Abstract: Functional reorganization in musician's brain has been considered strong evidence of experience-dependent neuroplasticity. However, it is unclear whether musician's motor

performance was driven by, or independent of auditory process. Highly coordinated bimanual movements stem from intensive instrument training require abundant communication between both hemispheres. Interhemispheric inhibition (IHI) is one form of communication between hemispheres and changes with instrument type. Instrument type may also impact bimanual coordination given the dissimilar hand use with different instrument performance. However, it was yet to know whether bimanual skill developed with musical training in association with adapted IHI in musicians was dependent on instrument type. Therefore, we aimed to 1) investigate the impact of sound on the bimanual coordination; and 2) investigate instrument-dependent effects on the relationship between IHI and bimanual coordination in keyboard players compared to string players. An 8-element finger sequence task (FST) with and without auditory tones were used to test symmetric bimanual coordination. Speed, accuracy, and evenness were recorded as performance. Asymmetric bimanual coordination was measured by a force tracking task (FTT) with one hand performing sine wave tracking, while the other hand performing constant contraction. Error was measured as performance. Ipsilateral silent period (iSP) was obtained using transcranial magnetic stimulation to index IHI in both left (L) and right (R) hemispheres. Canonical correlation analysis was performed to identify linear relationships between the IHI and bimanual coordination outcomes. Keyboard and string players did not show significantly different performance in FST and FTT, nor in the iSP outcomes. In FTT, better performance with the R hand tracking sine wave and the L hand performing constant contraction was observed in both groups, compared to the L hand tracking sine wave and the R hand performing constant contraction. Canonical correlation analysis showed that better bimanual coordination performance was related to increased IHI from the L hemisphere as well as decreased IHI from the R hemisphere in both groups. Musicians were able to transfer their bimanual coordination acquired from instrument training to novel laboratory motor tasks without the presence of auditory tones as part of the performance outcome. Adding both symmetric/asymmetric tasks washed out differences in IHI-bimanual coordination relationship between instruments. Better L hand control through enhanced interhemispheric communication may be critical for advanced bimanual coordination.

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Poster

402. Voluntary Movements: Plasticity

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Program #/Poster #: 402.24/PP4

Topic: E.04. Voluntary Movements

Title: Changes in cortical excitability during ischaemic nerve block

Authors: *S. NAULLS, P. SARAI, P. H. STRUTTON
Imperial Col. London, London, United Kingdom

Abstract: Following injury to the peripheral nervous system (PNS) there is a period of neuroplasticity associated with alterations in cortical excitability. The phenomenon of deafferentation, where there is a loss of afferent input to the brain which often arises following injury to the PNS, can be modelled using ischaemic nerve block (INB). Topographic changes to the representation of muscles within the motor cortex and changes in corticospinal excitability are thought to mediate this plasticity. Motor evoked potentials (MEPs) proximal to the site of the block have been observed to increase and we recently demonstrated that MEPs on the contralateral side to the block are also increased, but the mechanisms underlying these changes are poorly understood. Using non-invasive transcranial magnetic stimulation (TMS) over the motor cortex, we examined MEPs in left and right abductor pollicis brevis (L/RAPB) in healthy participants using single pulse TMS. We also used paired-pulse TMS to explore the extent to which short-interval intracortical inhibition (SICI) and facilitation (SICF) contribute to the increase in MEP amplitudes contralateral to the site of occlusion. INB induced by a tourniquet inflated around the left forearm resulted in a reduction in MEP amplitude in LAPB, distal to the site of occlusion. However, MEPs in RAPB (contralateral to the occlusion site) increased in amplitude. We also observed a reduction in the degree of SICI and an increase in SICF. Our findings suggest that alterations in intracortical inhibitory and facilitatory circuits contribute to the increase in cortical excitability during INB. The functional relevance of the increases in corticospinal excitability to contralateral muscles remains unclear.

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Poster

402. Voluntary Movements: Plasticity

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Program #/Poster #: 402.25/PP5

Topic: E.04. Voluntary Movements

Title: Mechanisms underlying changes in cortical excitability following ischaemic nerve block of the upper limb

Authors: *M. CONSTANTINESCU[1], P. SARAI[2], P. H. STRUTTON[2]
[1]Musculoskeletal Laboratory, Dept. of Surgery and Cancer, Fac. of Medicin, [2]Imperial Col. London, London, United Kingdom

Abstract: Reorganisation of the motor cortex due to injuries to the peripheral nervous system, such as limb amputation, is in part mediated by alterations in cortical excitability. Such injuries cause loss of afferent input to the brain and induce plasticity. Ischaemic nerve block (INB) is a

model of acute reversible deafferentation and results in changes in the motor cortex of both hemispheres. This causes a decrease in motor evoked potential (MEP) amplitudes in muscles distal to the site of occlusion and an increase in MEP amplitudes in muscles proximal to the occlusion and in the contralateral limb muscles. The mechanisms underlying the contralateral changes are not well understood and were the focus of this study. A cuff was inflated around the left forearm for 30 minutes and measures of corticospinal excitability, intracortical inhibition and facilitation were assessed using single and paired-pulse TMS in healthy subjects. Electromyographic activity was recorded from left and right abductor pollicis brevis (L/RAPB) muscles. Paired-pulse TMS was used to examine the contribution of long-interval intracortical inhibition (LICI) and intracortical facilitation (ICF) on changes in corticospinal excitability contralateral to the side of occlusion. INB resulted in a reduction in MEP amplitudes in LAPB, distal to the occlusion. MEP amplitudes increased in the muscle contralateral to the side of occlusion (RAPB). We observed an increase in ICF of RAPB and a reduction in LICI towards the end of the occlusion period. These findings suggest that alterations in intracortical faciliatory and inhibitory circuits within the motor cortex may mediate the increase in cortical excitability of muscles contralateral to the occlusion. The functional relevance of INB-induced changes to corticospinal pathways controlling contralateral muscles remains unclear, but greater understanding may aid the development of rehabilitative strategies to improve hand function following stroke or incomplete spinal cord injury.

Disclosures: M. Constantinescu: None. **P. Sarai:** None. **P.H. Strutton:** None.

Poster

402. Voluntary Movements: Plasticity

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 402.26/PP6

Topic: E.04. Voluntary Movements

Title: Use of ischaemic nerve block to assess the potential of transcranial magnetic stimulation to monitor spinal cord perfusion

Authors: *A. M. WATSON, P. SARAI, P. H. STRUTTON
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Abstract: Spinal cord injury due to prolonged ischaemia is a major complication of vascular surgery and current methods of monitoring are insufficient in detecting injury intra-operatively. During vascular procedures such as thoraco-abdominal aortic aneurysm (TAAA) repair, iatrogenic blood supply restriction to lower limbs results in deafferentation, thereby reducing motor evoked potentials (MEPs) from the ischaemic limbs. Previous work using ischaemic nerve blocks (INB) as a model of deafferentation has shown increases in transcranial magnetic stimulation (TMS)-induced MEPs in muscles proximal and contralateral to the block site

reflecting changes in corticospinal excitability. In order to use TMS as an intra-operative monitor of spinal cord perfusion, a greater understanding of the effects of ischaemia is required. The use of TMS to monitor MEPs during INB provides a comparable model for changes likely to occur to MEPs during TAAA surgery. However, the extent to which the degree of deafferentation influences cortical excitability remains unexplored and was the aim of this study.
We used non-invasive TMS and INB in the left upper limb for 30 minutes to investigate corticospinal excitability in left and right abductor pollicis brevis (L/RAPB) and left and right biceps brachii (L/RBB) in healthy subjects. The INB was applied, using inflation of a cuff, on two separate days to different locations on the limb (forearm and arm) to induce deafferentation of varying degrees. INB resulted in a reduction in the amplitudes of MEPs distal to the occlusion and increases in MEPs proximal and contralateral to the block. The greater degree of deafferentation, however, was associated with smaller increases in MEP amplitudes in the RAPB, LBB and RBB, suggesting that greater limb deafferentation results in smaller changes in corticospinal excitability. This may be, in part, due to the influence of pain, since greater degree of INB was associated with higher average pain scores. The results of this study may help interpretation of intra-operative TMS-induced MEPs used to monitor spinal cord perfusion during TAAA surgery.

Disclosures: A.M. Watson: None. **P. Sarai:** None. **P.H. Strutton:** None.

Poster

402. Voluntary Movements: Plasticity

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 402.27/PP7

Topic: E.04. Voluntary Movements

Title: Effect of acute exposition to Toluene on cortical excitability, neuroplasticity, and cognitive functions in healthy humans

Authors: *M.-F. KUO[1], F. YAVARI[2], C. VAN TRIEL[3], M. NITSCHE[2]
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Abstract: Toluene is a well-known organic neurotoxicant and a major component of many industrial and commercial products such as adhesives, paint thinners and gasoline. Many workers come into daily contact with toluene in working environment. Due to heterogeneity of the findings from existing animal and human studies about its behavioral and mechanistic effects, toluene occupational and short-term exposure limits (OEL and STEL) vary from 14 to 300 ppm across countries. Furthermore, its acute effects, especially in humans, remain poorly understood. The purpose of this study was to investigate the effects of acute exposure to toluene on cortical

excitability, plasticity, and implicit motor learning in a population of healthy humans. Seventeen subjects were assessed with different transcranial magnetic stimulation measurements: motor thresholds, short-latency intracortical inhibition and intracortical facilitation, and short-interval afferent inhibition (SAI) before and after placebo or toluene (single peak of 200 ppm) administration. Furthermore, we evaluated long term potentiation-like neuroplasticity induced by anodal transcranial direct current stimulation over the motor cortex, and the participants conducted a motor sequence learning task, the serial reaction time task (SRTT). Our findings revealed that toluene abolished the plasticity induced by anodal tDCS, attenuated intracortical facilitation, and increased inhibition in the short-latency afferent inhibition measure, while cortico-spinal excitability and intracortical inhibition were not affected. On the behavioral level, toluene did not alter performance of the motor learning task. These results suggest that toluene might act by modulating NMDA receptor activity, and cortical glutamatergic and cholinergic systems in the human brain. This study encourages further research to obtain more knowledge about mechanisms of action and effects of toluene on both naïve and chronically-exposed populations.

Disclosures: M. Kuo: None. **F. Yavari:** None. **C. van Triel:** None. **M. Nitsche:** F. Consulting Fees (e.g., advisory boards); NeuroElectrics.

Poster

402. Voluntary Movements: Plasticity

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 402.28/PP8

Topic: E.04. Voluntary Movements

Support: BMBF GCBS grant 01EE1501

Title: Titrating the neuroplastic effects of cathodal transcranial direct current stimulation (tDCS) over the primary motor cortex

Authors: *M. A. NITSCHE[1,2], M.-F. KUO[3], D. AGBOADA[3], A. JAMIL[3], M. MOSAYEBI[3,4]
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Abstract: Background: Transcranial direct current stimulation (tDCS) non-invasively induces polarity-dependent excitability alterations in the human motor cortex lasting for more than an hour after stimulation. Clinical applications with encouraging results have been reported in several pilot studies, but the optimal stimulation protocols remain to be determined. This is also important because the efficacy and directionality of tDCS effects follow non-linear rules

regarding neuroplastic effects for the stimulation parameters duration and intensity. Methods: In this study, we systemically explored the association between tDCS, these parameters and induced after-effects on motor cortex excitability. Cathodal tDCS was applied in four different intensities (sham, 1, 2 and 3mA) and three durations (15, 20 and 30mins) in 16 young healthy subjects and the after-effects were monitored with TMS-induced motor evoked potentials (MEP) until the next day evening after stimulation. Results: The results revealed nonlinear after-effects: 1mA-15min, 1mA-30min and 3mA-20 min cathodal tDCS induced LTD-like plasticity, while LTP-like plasticity was observed after 2mA-20min stimulation. Conclusions: Our study thus provides further insights on the dependency of tDCS-induced neuroplasticity from the stimulation parameters, and therefore delivers crucial information for future applications.

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Poster

403. Brain-Machine Interface: Grasp

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 403.01/PP9

Topic: E.05. Brain-Machine Interface

Support: SNSF grant 205321_170032

Title: Cortical representations of sensorimotor control of fine grasping in non-human primates

Authors: *S. M. WURTH[1], M. BADI[1], M. KAESER[2], B. BARRA[2], F. MOREILLON[3], G. COURTINE[4,5], M. CAPOGROSSO[2], J. BLOCH[5], E. M. ROUILLER[2], S. MICERA[1,6]
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Abstract: Kinesthetic information is critical to plan and adapt movements to behavioral goals and environmental constraints. For instance, we not only use our hands to manipulate objects, but also to communicate with and understand our environment. While touch as feedback modality has been extensively studied, the specific contribution of proprioception in the control of upper limb movements is less understood. To address this question, we established a series of electrophysiology and behavioral experiments to examine the integration of touch, proprioception, and motor execution in the cerebral cortex of a chronically implanted human primate (NHP). Specifically, we periodically map individual finger joint and wrist movements as well as touch stimuli applied to the hand to cortical activation maps in M1 and S1 area 2. In addition, single unit activity is recorded while the animal is performing a reach, grasp, and pull

task with objects of varying types and shapes that require the use of different grip patterns. The task involves proprioception and touch sensory modalities in addition to the motor execution. These multimodal recordings of sensorimotor intracortical activity, arm and hand joint kinematics and grip forces during well-controlled behavioral manipulations will increase our fundamental understanding of the processes through which kinesthetic information in integrated into cortical networks to produce task-specific movements.
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Poster

403. Brain-Machine Interface: Grasp

Location: SDCC Halls B-H

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Program #/Poster #: 403.02/PP10

Topic: E.05. Brain-Machine Interface

Support: Swiss National Science Foundation Grant NeuGrasp [205321_170032]
Wyss Center for Bio and Neuroengineering
Bertarelli Foundation

Title: Neuroanatomical, computational, and experimental evidences for the use of intraneural peripheral nerve stimulation to induce fine hand movements

Authors: *M. BADI[1], S. WURTH[1], M. KAESER[3], P. ČVANčARA[5], T. STIEGLITZ[5], G. COURTINE[2,6], M. CAPOGROSSO[4], J. BLOCH[7], E. ROUILLER[3], S. MICERA[1,8]
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Abstract: Cervical spinal cord injury (SCI) and stroke severely impact grasping movements required for activities of daily living. Intraneural peripheral nerve stimulation enables specific activation of passing fibers. This paradigm has restored precise leg movements in animal models of SCI and selective sensation in human amputees. Intraneural peripheral nerve stimulation may also restore fine grasping in paralyzed hands, but this possibility has not been investigated. Here, we assess the feasibility of using intrafascicular electrical stimulation of peripheral nerves to

produce precise hand movements in the non-human primate (NHP). We first extensively characterized the branching points of the median, radial, and ulnar nerves to their target muscles in the adult macaca fascicularis in order to identify the optimal implantation site for intraneural electrodes. We then reconstructed the tridimensional structure of the identified portion of each nerve in order to analyze the fascicular organization within the nerve at this level. Additionally, we assessed the distribution of motor fibers within the fascicles using immunohistochemistry. The obtained data was used to build realistic computational models of intraneural peripheral nerve stimulation for each nerve. The simulations confirmed the advantages of using intrafascicular electrodes to induce precise hand movements, demonstrating the possibility to selectively recruit patches of motor fibers without a priori knowledge of the electrode placement. We validated these results during electrophysiology experiments using transverse intrafascicular multichannel electrodes (TIMEs) implanted in the nerves of anesthetized NHPs. The stimulation achieved a selective recruitment of wrist and finger flexors and extensors in a reproducible manner across animals. We exploited these results to determine stimulation sequences that aimed at reproducing the muscle activation patterns underlying different grasping movements. For this, we mapped the obtained muscle recruitment maps to continuous EMG recordings during behavioral experiments and automatically optimized the selection of stimulation channels for each phase of the movement such as to reproduce the desired EMG pattern. Taken together, these results suggest encouraging evidences for the usability of the TIMEs to restore fine hand control after paralysis.

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Poster

403. Brain-Machine Interface: Grasp

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Topic: E.05. Brain-Machine Interface

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Wyss Center for Bio and Neuroengineering

Title: Probing the cortical sensorimotor network of nonhuman primates during reaching and grasping

Authors: *M. G. PERICH[1], S. CONTI[2], B. BARRA[2,3], M. KAESER[2], G. SCHIAVONE[4], N. GREINER[2,3], M. HARVEY[2], J. BLOCH[5], S. P. LACOUR[4], G. COURTINE[3], E. M. ROUILLER[2], M. CAPOGROSSO[2], T. MILEKOVIC[1]
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Abstract: For people with high cervical spinal cord injury (SCI), restoring the ability to reach and grasp is a high priority. Epidural electrical stimulation (EES) of the spinal cord has been shown to improve voluntary control of the lower limbs by modulating spinal motor circuits through direct activation of afferent fibers. Brain-controlled EES neuromodulation may also be applied to the cervical spinal cord to restore lost hand and arm function. Neural signals in the primary motor cortex (M1) are commonly used for neuroprosthetic control. Yet, movement-related neural activity is also strongly influenced by the sensory feedback from the primary somatosensory cortex (S1). This sensorimotor loop is perturbed following upper limb paralysis. Furthermore, signals from the afferent fibers recruited by EES directly activate the sensorimotor cortex. The functional effect of spinal EES on natural cortical dynamics during movement planning and execution is currently unknown. Recovery of dexterous hand and arm movements will require a technique that can obtain accurate and comprehensive control signals robust to both the effects of paralysis and EES-based therapy on the cortical signals. Here, we recorded neural population activity from multiple areas of the sensorimotor cortex to study sensorimotor coordination during reaching and grasping. We trained two facicularis macaque monkeys to perform a reach, grasp, and pull task while interacting with a robotic arm. We then surgically implanted two Utah electrode arrays in the contralateral hemisphere of each monkey. The first monkey received arrays in the primary motor and dorsal premotor (PMd) cortices, while the second was implanted in primary motor and somatosensory cortices. We recorded neural activity during the unconstrained reaching, along with arm kinematics and EMG signals from proximal and distal muscles of the arm. We studied the interactions between neural populations within and between the implanted brain areas. We used sensorimotor cortical responses to cervical EES during reaching to probe the effect of direct afferent input on cortical sensorimotor activity. Ultimately, we aim to apply these techniques to understand how the sensorimotor cortical signals are disrupted following cervical SCI and how sensorimotor cortical dynamics is affected by subsequent EES-based rehabilitation.

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Poster

403. Brain-Machine Interface: Grasp

Location: SDCC Halls B-H

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Program #/Poster #: 403.04/PP12

Topic: E.05. Brain-Machine Interface

Support: SNSF Ambizione to MC(PZOOP2_167912 / 1)
Foundation Bertarelli Catalyst Fund
Wyss center
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Title: Spinal reflexes elicited by continuous epidural electrical stimulation of the intact cervical spinal cord during reaching and grasping in awake non-human primates

Authors: *B. BARRA[1], S. CONTI[1], M. G. PERICH[2], M. KAESER[1], N. GREINER[3,1], G. SCHIAVONE[4], T. MILEKOVIC[2], J. BLOCH[5], S. P. LACOUR[4], G. COURTINE[3,5], E. M. ROUILLER[1], M. CAPOGROSSO[1]
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Abstract: Recovery of reaching and grasping is a priority for people suffering from cervical spinal cord injury (SCI). Following up on the encouraging results obtained with Epidural Electrical Stimulation (EES) in improving motor control of lower limbs, several groups are exploring the applicability of EES to the recovery of arm and hand function after cervical SCI. However, the neural control of arm and hand is markedly different from the patterned and reflex-based control of locomotion. Indeed, EES enhances locomotion by recruiting large primary afferent fibers thus increasing the excitability of the reflex circuits that are prominently active during locomotion. The efficacy of cervical EES may be limited by the reduced importance of spinal reflexes in the planning and execution of voluntary three-dimensional reaching movements. To address this question, here we studied the muscle responses elicited by continuous EES of the cervical spinal cord in intact, awake, and behaving non-human primates. We implanted a macaque monkey with a tailored soft spinal implant with eight stimulation contacts targeting C6 to T1 dorsal roots and n=8 bipolar EMG electrodes in the left arm and hand muscles. The animal was trained to perform a three-dimensional reaching and grasping task with a robotic interface while we recorded arm joint kinematics and EMG signals, along with the interaction forces of the robot. We delivered continuous supra-threshold EES at different rostro-

caudal locations and stimulation frequencies. We found that each stimulation pulse elicited muscle responses locked to the stimulation frequency. As for lumbar EES, the amplitude of the evoked responses was strongly modulated during behavior, suggesting a pre-synaptic recruitment of arm motoneurons. Stimulation delivered at distinct rostro-caudal locations recruited muscles innervated by the corresponding dorsal roots mostly during their naturally active phases. For instance, stimulation of the C7 root, strongly potentiated the EMG of Triceps muscles but only during the elbow extension phase. However, the activity of non-targeted muscles was affected as well, showing both potentiation and inhibition phase-dependent effects, contingent to the stimulation parameters. These non-functional muscle activations induced by continuous EES, impaired the normal execution of the subsequent grasping and pulling phases. Consequently, time-varying stimulation profiles at different spatial locations will be required to selectively potentiate functional muscle activity for three-dimensional reaching and grasping after severe SCI.

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Poster

403. Brain-Machine Interface: Grasp

Location: SDCC Halls B-H

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Program #/Poster #: 403.05/PP13

Topic: E.05. Brain-Machine Interface

Support: Ministry of Science and ICT (MSIT), Brain Research Program, 2016M3C7A1904986

Title: Three dimensional reaching to grasp task for brain machine interface in rhesus monkey

Authors: *S.-M. KIM, S.-Y. HYUN, J.-R. CHOI, I.-H. JEONG, S.-J. LEE, B.-H. LEE, S. OH, B. KANG, J.-W. SOHN
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Abstract: Reaching to grasp is natural movement and it happens frequently for serving useful daily functions in human life. For this reason, it is important that external device in brain-machine interface (BMI) provide high-precision movement like actual arm to paralyzed or amputated patients. To accomplish this goal, we set up behavioral task to investigate neural mechanism of three dimensional (3D) reaching and grasp in rhesus monkey (*Macaca mulatta*). In this task, a monkey was required to reach and grasp targets presented by robot arm (Manipulator-H, Robotis). The target position was pseudo randomly selected out of eight positions. The experiment was composed of blocks in which trial of target of same position was presented five times (40 trials/block). When the monkey successfully grasped the presented

target, it was rewarded with a drop of water. If not, the target was presented at the same position again until the monkey acquired the target without giving water. All the position of arm and hand was recorded with motion capture system (Optotrak 3020, Northern Digital, Inc.). For 11 days, the monkey performed 4450 trials and achieved 86% of success rate (3845/4450 trials). After training, we implanted two 96-channel intracortical microelectrodes (Utah array, Blackrock® Microsystems) in the primary motor cortex (M1, limb area) and posterior parietal cortex (5d), respectively. Recording began one week after implantation. We have recorded both M1 and 5d while the monkey performed 3D reaching and grasp task. For 7 days, the number of neural units were 124 and 31 per session on average from M1 and 5d, respectively. We are planning to use behavioral task result and the collected neural data for developing 3D movement of BMI system near in the future.

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Poster

403. Brain-Machine Interface: Grasp

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Topic: E.05. Brain-Machine Interface

Support: Swiss National Science Foundation programs Ambizione
Foundation Bertarelli Catalyst Fund
Wyss Center for Bio and Neuroengineering in Geneva

Title: Impact of macro and microanatomy of the cervical spinal cord on the recruitment of arm motoneurons in primates and humans

Authors: *N. GREINER[1,2], B. BARRA[1], G. SCHIAVONE[3], S. BORGOGNON[1], E. PRALONG[4], S. P. LACOUR[3], J. BLOCH[4], G. COURTINE[2], M. CAPOGROSSO[1]
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Abstract: After spinal cord injury (SCI), the communication between supraspinal centers and spinal sensorimotor circuits below the lesion is impaired, leading to paralysis. However, these circuits remain functional. Epidural electrical stimulation (EES) of the lumbar spinal segments can modulate the motoneuronal activity of these spared circuits via the direct recruitment of their afferent component. This modulation enabled volitional control of lower limb muscles in animal models and humans with SCI. Similar circuits at the cervical level of the cord contribute to coordinating upper-limb movements, suggesting that EES could also improve arm and hand

function in tetraplegia. However, the ability of cervical EES to target individual or groups of upper-limb muscles is poorly understood. To address this question, we coupled an anatomically realistic Finite-Element model of the cervical spinal cord with realistic microanatomy of Ia-fibers and alpha-motoneurons. Specifically, the Ia-fibers running in the dorsal roots branched in the dorsal columns and spawned collaterals towards the ventral horn of the grey matter where they innervated realistic models of alpha-motoneurons. Simulations indicated that targeting EES to somatotopically relevant dorsal roots could activate specific motoneuron pools. However, muscles innervated with a large number of Ia-fibers were recruited more often and at lower thresholds than muscles with low Ia-fiber count, significantly impacting the stimulation specificity. We compared these results with electromyographic activity recorded in arm and hand muscles in three nonhuman primates and three humans who received single pulses of EES at various rostro-caudal levels of the cervical cord. Experiments confirmed the ability of EES to engage specific groups of muscles reproducibly across subjects. Nonetheless, variability across subjects suggested a muscle- and subject-dependent nature of the Ia-mediated motoneuronal excitability; likely applying also to the motoneuronal modulations mediated by Ib- and II-pathways, implicated during EES. Taken together, these results highlight the importance of further quantitatively document the composition and connectivity of the proprioceptive feedback circuit to deepen our mechanistic understanding and refine the design of EES therapies.

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Poster

403. Brain-Machine Interface: Grasp

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Title: Grasp representation in posterior parietal cortex and premotor cortex in a tetraplegic human

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Abstract: Tetraplegia from spinal cord injury leaves patients paralyzed below the neck and unable to perform many activities of daily living. Brain-machine interfaces (BMIs) could give tetraplegic patients more independence by reading signals out of the brain directly and using these signals to control external devices such as robotic arms or hands. Object manipulation is of foremost importance for tetraplegic persons, as this is a critical aspect of human independence. Regaining arm and hand function is therefore ranked the highest priority in a survey among this population (Anderson, Journal of Neurotrauma, 2004). In this work, two tetraplegic patients participated in a clinical trial of a BMI in which motor intentions for grasping were read-out from high-level areas of the cortical grasp circuit including premotor cortex and posterior parietal cortex. Patient EGS suffered spinal cord injury at level C3-4 and has microelectrode arrays implanted in anterior intraparietal cortex (AIP) and Broadmann's Area 5 (BA5). Patient FG has a C5-C6 spinal cord injury and is implanted in the supramarginal gyrus (SMG) and in the area of the ventral premotor cortex (PMv). While this work builds on prior work studying grasp-related activity in AIP and BA5, SMG and PMv have never been studied before using intracortical recordings from microelectrode arrays in human. A task was designed to examine the neural activity related to imagined grasps in the implanted areas. Each trial began with a brief inter-trial interval, followed by a visual cue for one of five specific grasps. Then, after a delay period, the subject was instructed to imagine performing the cued grasp. In a variation of this task, a subset of trials, pseudorandomly interleaved throughout the task, instructed the subject to do nothing during the last phase of the trial. We found that grasps were well represented by firing rates of neuronal populations recorded from each of these cortical areas. We observed that neuronal signals recorded during grasp cues were most predictive of motor imagery in SMG, suggesting that this area is involved in planning of grasp activity early in the visual-motor control circuit. Importantly, the data collected from AIP and BA5 for this work were recorded more than four years after implantation and provide strong evidence for viability of the electrodes years after implantation. Together, these findings suggest that grasp signals can be robustly decoded from high-level areas of cortex in human over multiple years of implantation, and further demonstrate the potential for BMIs to provide increased independence through grasp capabilities for persons living with tetraplegia.

Disclosures: S.K. Wandelt: None. **S. Kellis:** None. **M. Armenta Salas:** None. **L. Bashford:** None. **M. Jafari:** None. **H. Jo:** None. **K. Pejsa:** None. **D. Kramer:** None. **B. Lee:** None. **C. Liu:** None. **R.A. Andersen:** None.

Poster

403. Brain-Machine Interface: Grasp

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 403.08/PP16

Topic: E.05. Brain-Machine Interface

Support: Office of Research and Development, Rehabilitation R&D Service, Department of Veterans Affairs (N9288C, A2295R, B6453R)
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Title: Multiple grasp types can be reliably decoded from the precentral gyrus of people with ALS with progressive levels of motor impairment

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Abstract: Background: Intracortical brain-computer interfaces have the potential to improve functional independence for individuals with motor impairment from disorders such as amyotrophic lateral sclerosis (ALS). Previous work has demonstrated that individuals with tetraplegia can control robotic limbs using neural signals decoded from electrodes implanted in the precentral gyrus. However, it is not known whether the severity of paralysis affects the encoding of motion-related neural features in motor cortex. To address this question, we characterized how well we could decode 4 commonly used hand movements (power, pinch, and key grasps, and forearm supination/turn) from intracortical signals recorded from the precentral gyrus of people with ALS who had varying degrees of motor impairment.
Methods: Three research participants with ALS participated in this study. Participant T6 had an ALS Functional Rating Scale (ALSFRS) score of 14 and was able to visibly perform all 4 hand movements; T7 had an ALSFRS score of 17 and had no visible residual hand movement; and T9 had an ALSFRS score of 12 and had trace residual movement of only the thumb. All participants had intracortical arrays placed in their dominant motor cortex as part of the ongoing BrainGate2 pilot clinical trial. On each trial, participants were asked to “perform, or attempt to perform” one of the above 4 actions while neural signals were recorded. Each action was cued using a text display, followed by a visual go command after a variable delay period.
Results: A total of 719 trials were analyzed between the 3 participants over 6 sessions (2 sessions

per participant, with 45 - 155 trials per session). In offline analysis, data was spike-sorted using an automated spike sorter, yielding between 114 and 303 spiking units per session. 45 trials and 114 units were pseudorandomly sub-sampled from each session to control for signal quality and the amount of data used for decoding. These were used to train a linear discriminant analysis classifier, whose accuracy was tested on hold-out sets using Monte Carlo cross-validation without replacement. This was repeated across 100 different subsamples per session. Mean decoding accuracy during the action epoch was 75%, 78%, and 87% for T6, T7 and T9, respectively (chance = 25%), which did not correlate with either ALSFRS scores or degree of observable residual hand motion.
Conclusion: Multiple functionally relevant grasp types could be decoded from the intracortical signals of the precentral gyrus in people with ALS with differing levels of motor impairment. However, from this limited sample, no obvious relationship was observed between level of impairment and decoding accuracy.

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Poster

403. Brain-Machine Interface: Grasp

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 403.09/PP17

Topic: E.05. Brain-Machine Interface

Support: Internal funds at the Feinstein Institute for Medical Research

Title: A feedback controller for sustained functional grasps using neuromuscular electrical stimulation

Authors: *J. G. CIANCIBELLO[1], S. PADMANABAN[4], K. KING[2], M. STRAKA[3], C. BOUTON[5]
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Abstract: Motor neuroprostheses using neuromuscular electrical stimulation (NMES) must be able to evoke movements in a predictable manner, especially for sustained grasps. Current NMES-based devices suffer from rapid muscle fatigue and variable muscle force generation

given electrical stimulation parameters. To account for these variations in flexion strength, we implemented a traditional proportional-integral-derivative (PID) controller, which has an input of the movement force, to adapt the NMES stimulation current in closed-loop. We evoked finger movements in a participant with a C5 spinal cord injury using an NMES system that delivered biphasic, charge-balanced pulses transcutaneously on the forearm. The force of individual finger flexion was measured with thin-film FlexiForce sensors and used for feedback to the PID controller. Trials consisted of enabling the PID controller to match a desired force for a given time (6-15 s). We observed a compensatory effect from the PID controller, since muscle fatigue is present after stimulating for a long period of time (>6 s). Furthermore the controller matched the desired force relatively quickly (<1 s), and can be applied to individual finger movements. Future directions will support functional isometric grasps that incorporate several PID controllers. These PID controllers could be used in conjunction with a brain-computer interface to allow volitional, sustained movements in a paralyzed limb.

Disclosures: J.G. Ciancibello: None. **S. Padmanaban:** None. **K. King:** None. **M. Straka:** None. **C. Bouton:** None.

Poster

403. Brain-Machine Interface: Grasp

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 403.10/PP18

Topic: E.04. Voluntary Movements

Title: Behavioural and neurophysiological effects of concurrent transcranial direct current stimulation and motor training in young and older adults

Authors: *R. A. MOONEY[1,2], J. CIRILLO[1,2], W. D. BYBLOW[1,2]
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Abstract: Healthy ageing is typically accompanied by a reduced capacity for both structural and functional plasticity which has important implications for the acquisition and retention of novel motor skills. The application of transcranial direct current stimulation (tDCS) to primary motor cortex presents as a promising intervention to enhance skill acquisition and retention in older adults. However, the underlying neurophysiological effects of concurrent anodal tDCS and motor training has received little systematic investigation in this population. Fourteen neurologically healthy young and twelve older adults participated in the present study. To determine skill, the speed-accuracy function for a sequential visual isometric wrist extension task was assessed by measuring error at fixed execution speeds. Participants then completed discrete training blocks at a self-selected pace during which they received real or sham anodal tDCS in a repeated measures double-blinded crossover design. The speed-accuracy function was reassessed immediately after, 24 hours post and 7 days post training. Threshold-hunting paired-pulse

transcranial magnetic stimulation protocols were used to examine corticomotor excitability, short- and long-interval intracortical inhibition and short-interval intracortical facilitation in the non-dominant extensor carpi radialis before and after training. Overall skill was reduced in older adults compared with young ($P < 0.001$). Both age groups exhibited skill improvements after training which were retained at the 24 hour and 7 day follow up sessions (all $P < 0.05$). Corticomotor excitability increased ($P < 0.001$) and short-interval intracortical inhibition decreased ($P = 0.033$) similarly in both age groups after training. There was no modulation of long-interval intracortical inhibition or short-interval intracortical facilitation (all $P > 0.12$). Anodal tDCS had no effect on training-induced skill improvements or retention ($P = 0.16$), or the modulation of neurophysiological variables (all $P > 0.20$). These findings suggest that older adults maintain the ability to acquire and retain a novel motor skill, although absolute performance is lower than young adults. Furthermore, training-induced skill improvements are accompanied by an increase in corticomotor
excitability and reduction in intracortical inhibition within primary motor cortex which are not modified by concurrent anodal tDCS.

Disclosures: R.A. Mooney: None. **J. Cirillo:** None. **W.D. Byblow:** None.

Poster

403. Brain-Machine Interface: Grasp

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 403.11/PP19

Topic: E.05. Brain-Machine Interface

Support: Internal funds at the Feinstein Institute

Title: Using inertial measurements to automatically identify and calibrate neuromuscular stimulation patterns for restoring movement in paralysis

Authors: *K. KING, J. G. CIANCIBELLO, C. BOUTON, M. STRAKA
Feinstein Inst., Manhasset, NY

Abstract: Neuromuscular electrical stimulation (NMES) can be used to activate muscles and generate movements in individuals living with paralysis from spinal cord injury (SCI), stroke, or other conditions. To restore isolated and synergistic finger and wrist movements, NMES systems require a large number of electrodes placed on the forearm. Given the possible combinations of stimulation patterns as well as the variety of movement outcomes possible, sensors that detect movements would significantly decrease calibration times while also quantifying the evoked movements. Moreover, as the position of the muscle bodies relative to the skin surface changes with hand orientation (e.g. pronated, supinated), an effective clinical device would need to detect this and deliver appropriate stimulation. To characterize and validate movements, we developed

wearable devices, placed on the fingertips and on the wrist, that incorporated inertial measurement units. With these devices, we measured linear and angular motion while evoking hand movements in two participants with C5 SCI as well as one able-bodied participant. Individual finger movements and coordinated grasps were evoked by delivering current transcutaneously with biphasic, charge-balanced pulses. We recorded acceleration and angular velocity of finger tips and wrist and applied a complementary filter to interpret the orientation of the hand in real time. We demonstrate that these devices can detect and classify forearm orientation, evoked finger and hand movements. These devices were also used in preliminary calibration experiments, where various finger movements were automatically identified while stimulating randomized electrode pairs. Future dsirections include incorporating these devices to calibrate neuromuscular stimulation patterns while restoring volitional movement via a brain-computer interface.

Disclosures: K. King: None. **J.G. Ciancibello:** None. **C. Bouton:** None. **M. Straka:** None.

Poster

403. Brain-Machine Interface: Grasp

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 403.12/PP20

Topic: E.05. Brain-Machine Interface

Support: Schaefer School of Engineering and Science, Stevens Institute of Technology

Title: Effects of a compliant surface on cognitive agency during hand grasp loading

Authors: *R. NATARAJ, S. SANFORD, A. SHAH
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Abstract: Individuals needing restoration of hand grasp function due to spinal cord injury or upper-limb amputation may employ a prosthesis device. Identifying operational parameters such that an individual feels greater cognitive integration may optimize performance of object manipulation with such a prosthesis. How grasp loads are generated by the device onto objects such that users feel more agency, the perception of being the “true author” of one’s movements, could provide the cognitive link between user and device for better functional grasp. Furthermore, this link may not only be predicated on the loads applied to an object, but how hand kinematics are generated due to object surface deformation. In this study, we investigate how agency and grasp performance depend on perceived task-specific loading, provided through visual feedback, for a compliant object surface. These results are directly compared and contrasted against those utilizing a rigid object surface. Understanding the repercussions of hand force operations and proprioceptive shifts with a deforming surface may be a strong platform for better prosthesis design. How loading is triggered and postures are formulated for this complex

manipulation task should be key considerations in identifying parameters of feedback control systems driving device operation. Our long-term goal is to continue developing a virtual reality system to design better controller operation of prostheses that co-maximize cognitive integration and performance. We have previously employed motion and rigid-surface force protocols to observe relationships across virtual prosthesis operation, agency, and successful task performance. In the next phase, we aim to create complex manipulation tasks that challenge user-device abilities and refine device operation for better cognitive integration. Such a test-bed can be extended as a concept platform for more efficient movement rehabilitation following neuromuscular pathologies such as spinal cord injury, stroke, or upper-limb amputation. Related findings should identify more optimal parameters for controllers, generate greater motor gains with fewer practice repetitions, and better incorporate the user in device operation. Foundational methods could be employed towards improved development of exoskeletons, approaches using functional electrical stimulation, and sensory-feedback prostheses.

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Poster

403. Brain-Machine Interface: Grasp

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Program #/Poster #: 403.13/PP21

Topic: E.05. Brain-Machine Interface

Support: NIH Grant R01 NS053603

Title: The stabilization of M1 neural modes for the control of muscle activity through a Brain Computer Interface

Authors: *X. MA[1], J. A. GALLEGO[2], A. FARSHCHIAN[1], K. L. BODKIN[1], S. A. SOLLA[1], L. E. MILLER[1]
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Abstract: A Brain Computer Interface (BCI) that uses functional electrical stimulation (FES) to activate paralyzed muscles under the control of signals recorded from primary motor cortex (M1) should provide users with a stable tool for implementing their intended motion. However, changes in the neurons recorded by implanted arrays can cause undesired variations in the actions produced by the BCI. Fortunately, the encoding of motor commands across the millions of neurons in M1 is highly redundant, and motor intent can be represented by a much smaller number of collective signals, the “neural modes”. These modes span a low-dimensional manifold embedded in the high-dimensional space of activities of recorded neurons; neural modes are likely to provide more stable signals for the control of muscle activation.
We consider M1 recordings while monkeys perform a variety of wrist and reach-to-grasp tasks,

and compare linear methods for dimensionality reduction, typically principal component analysis (PCA), to nonlinear dimensionality reduction achieved through deep neural network autoencoders. For an isometric wrist flexion task, a PCA based dimensionality reduction from the D ~100 neural space into a d=16 manifold captures 64% of the variance; the d=16 manifold found by the autoencoder captures a comparable 65% of the variance. Data collected from a week-long series of sessions allow us to analyze the stability of the manifold signals. If the autoencoder is not retrained, the variance accounted for (VAF) drops below 10% after three days. This deterioration in performance, due to loss of existing neural signals or capture of new ones, needs to be compensated by a retraining of the neural autoencoder. While a full retraining of the autoencoder on a daily basis maintains the initial 65% VAF, we find that partial training suffices to maintain VAF within 5%. This partial retraining involves only the initial autoencoder layer, from neural inputs onto the first layer of hidden units, and the final autoencoder layer, from the last hidden layer onto the reconstructed neural outputs. This partial retraining is achieved with less than 40% of the data required for full retraining.
The activation of neural modes can be used as decoder inputs to predict muscle activations (EMGs), with performance within 90% of that achieved when using all neural signals. We find that although the partial retraining of the autoencoder does not suffice to maintain this level of EMG prediction for a full week, if this partial retraining is coupled to the retraining of the module that maps neural modes onto EMGs, performance is sustained, providing a stable BCI over this time scale.

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Poster

403. Brain-Machine Interface: Grasp

Location: SDCC Halls B-H

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Program #/Poster #: 403.14/PP22

Topic: E.04. Voluntary Movements

Support: Marie Sklodowska-Curie Global Fellowship 658868
NIH R37NS21135

Title: Electrocorticographic dissociation of alpha and beta rhythmic activity in the human sensorimotor system

Authors: *A. STOLK[1], L. BRINKMAN[2], M. VANSTEENSEL[3], E. J. AARNOUTSE[4], R. T. KNIGHT[5], F. LEIJTEN[3], C. DIJKERMAN[3], F. DE LANGE[6], I. TONI[7]
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Med. Cent, Utrecht, Netherlands; [5]UC Berkeley, Berkeley, CA; [6]Donders Insitute, Nijmegen, Netherlands; [7]Donders Inst., Nijmegen, Netherlands

Abstract: Alpha (8-12 Hz) and beta (15-25 Hz) rhythmic activities over the sensorimotor cortex are prominent and functionally relevant [Brinkman et al., 2014; 2016]. However, it remains unclear whether alpha and beta rhythms build on the same neuronal ensembles, and whether those ensembles actually contribute to computing a forthcoming movement. Complicating the issue is the fact that rhythmic activity rides on top of concurrent power-spectral 1/*f* modulations, making it difficult to yield robust insights into the fundamental properties of oscillatory activity. A further challenge is to disentangle movement-related computations from somatosensory reafferent signals associated with the execution of a movement.

Here we assess the cortical and functional specificity of those sensorimotor rhythms by using electrocorticographic (ECoG) data acquired during the performance of a psychophysically controlled movement imagery task. The preferred manner in which participants imagined grasping an object depended on the object's orientation and followed the biomechanical constraints of the body. We used irregular-resampling auto-spectral analysis to distinguish subject-specific rhythmic effects from arrythmic 1/*f* components of the ECoG signal [Wen & Liu, 2016]. Mean and spread of alpha and beta spectral distributions were individually defined with a two-term Gaussian model. We also considered (arrythmic) high-frequency activity (60-120 Hz) and the 1/*f* slope of the ECoG power-spectrum. The latter is a putative index of excitation-inhibition balance shown to closely track fluctuations in consciousness states in anesthetized macaques [Gao et al., 2017].

Both sensorimotor rhythms displayed effector-specific trial-by-trial modulation and were each spatiotemporally correlated with arrhythmic high-frequency activity. However, alpha and beta rhythms were weakly correlated and differed in their cortical and functional properties. Peaks of sensorimotor alpha were distributed on the postcentral gyrus, with the majority of electrodes yielding predominantly somatosensory sensations during electrical stimulation. In contrast, sensorimotor beta was strongest at electrodes directly above the central sulcus, with electrical stimulation yielding both movements and somatosensory sensations. Beta rhythmic activity closely tracked fluctuations of the 1/*f* slope in both time and space across sensorimotor cortex. Alpha rhythms were not influenced by that index of excitation-inhibition balance.

These findings suggest that sensorimotor alpha and beta rhythms involve different neuronal ensembles, and support different computations.

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Poster

403. Brain-Machine Interface: Grasp

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Program #/Poster #: 403.15/QQ1

Topic: E.05. Brain-Machine Interface

Support: T&C Chen Brain-machine Interface Center at Caltech
Boswell Foundation
NSF 1533589
NIH U01NS098975

Title: Spatiotemporal analysis of longitudinal spike recordings in human cortex

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Abstract: Chronic experiments exploring the human cortex produce enormous datasets that offer a unique opportunity to study neural circuits over extended periods of time. In these data sets neural information is recorded from the same location, in similar experimental contexts, over several years. These recordings may therefore benefit from current innovations in the analysis of large datasets which, applied to other data types, are offering novel and exciting possibilities to identify high-order correlations in data. What therefore could be revealed in neural data where single and multiunit activity is recorded over multiple time scales from multiple electrodes?
In a clinical trial a single human subject has been implanted with two Blackrock Neuroport arrays in the posterior parietal cortex: the anterior intraparietal cortex (AIP) and Brodmann area 5 (BA5). The arrays were implanted in April 2013, at which point the subject began a range of tasks designed to investigate the control of a Brain-Machine Interface (BMI). One task in particular has continued, almost unaltered, since the beginning of the clinical trial--a center out training paradigm for cursor control. In this task, a cursor, presented on a display screen, moves from the center to one of eight targets located around the start point. Data collected while the cursor moved under computer control was used to train a decoder that would then be used in an online control task. Across sessions the decoder varies, slightly changing the relationship between the neural signals and the control; however, during training periods, nothing alters the relationship between the observation and the imagined movement signal. This subset therefore provides a data set of over ten thousand trials, with a consistent task, collected across four years. We are exploring the stability of the signals in these recordings as metrics to measure the long-

term viability of BMI use and investigating the spatial, temporal and cross-channel relationships that evolve between neurons and behavior over time. We have identified features in the neural signals which we interpret as learning over multiple time scales, in addition to features which correlate with measurements in the viability of the electrode-tissue interface. Further work aims to identify the formation of stereotyped activity that result from task familiarization over many trials.

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Poster

404. Brain-Machine Interface: Somatosensory

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Topic: E.05. Brain-Machine Interface

Support: NIH U01NS098975
The Center for Sensorimotor Neural Engineering at the Univ. of Washington
Tianqiao and Chrissy Chen Brain-machine Interface Center at Caltech
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Title: Functional connectivity analysis of primary somatosensory cortex with posterior parietal cortex and ventral premotor cortex

Authors: *H. JO[1], S. KELLIS[1,2,3,4], M. ARMENTA SALAS[1], L. BASHFORD[1], M. JAFARI[1], K. PEJSA[1], D. J. KRAMER[2,4], B. LEE[2,4], C. LIU[2,4], E. E. FETZ[5], R. A. ANDERSEN[1,3]
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Abstract: Functional connectivity of the brain, defined as the temporal dependency in neural activity between different brain areas, has long been studied as a mechanism to explore neuronal circuits and systems. Many different approaches have been used to quantify connectivity using spikes and/or local field potentials (LFPs) as inputs. In this study, we analyzed functional connectivity between primary somatosensory cortex (S1) and either the supramarginal gyrus (SMG) or ventral premotor cortex (PMv). Our goal is to establish a baseline network structure that will allow us to evaluate changes due to learning and cortical plasticity in future experiments. We recorded from SMG, PMv, and S1 of a 34-year-old tetraplegic subject with Neuroport microelectrode arrays (Blackrock Microsystems). From these data we quantified

functional connectivity using spike correlations. We identified pairs of channels whose correlation was more than three times the median absolute deviations (MADs) away from the median correlation of all possible pairs of channels. On average, we found 5% and 7% of channel pairs with significant correlations in S1-PMv, and S1-SMG, respectively. These correlations were relatively stable in three sessions recorded over a period of a month, with 1% and 4% of the channel pairs in S1-PMv and S1-SMG respectively exceeding the threshold of three MADs in at least two sessions. These results demonstrate one measure of functional connectivity between areas of cortex involved in sensorimotor processing. Future analysis and data collection will allow us to compare the above functional connectivity result with other standard measures such as spike-field coherence and field-field coherence.

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Poster

404. Brain-Machine Interface: Somatosensory

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Program #/Poster #: 404.02/QQ3

Topic: E.05. Brain-Machine Interface

Support: NIH U01NS098975
T&C Chen Brain-Machine Interface Center at Caltech
Boswell Foundation
Della Martin Foundation

Title: Effect of varying stimulation parameters on elicited sensation with intracortical microsimulation in human somatosensory cortex

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Abstract: We recently demonstrated that Intracortical Microsimulation (ICMS) in the primary somatosensory cortex of a human subject can elicit natural sensations, both proprioceptive (the sense of movement) and cutaneous (touch on the skin) in nature. We seek to understand in more detail the relationship between the stimulation parameters of ICMS and the sensations they produce in order to accurately deliver task-relevant feedback. Furthermore, we continue to explore the range of potential sensory responses produced under different stimulus configurations. We delivered ICMS to primary sensory cortex (S1) in a human clinical trial

participant. The subject has a C5-C6 level spinal cord injury resulting in quadriplegia. Implant surgery was performed approximately two years post-injury, two Blackrock stimulation microarrays were positioned in S1 and two recording microarrays in premotor and parietal areas. Recording and stimulation began in December 2016, two weeks post-surgery, and has continued on a regular basis. We have extended previous mapping results by beginning to vary new features of the stimulation: discriminating between sets of stimulations from a wide range of amplitude and frequency values, stimulating at multiple electrode sites concurrently and varying the timing and duration of the stimulation. We note the sensory outcomes of ICMS by recording features of the percepts (i.e. the subject's verbal description of the character, duration, intensity, and location). Preliminary evidence suggests that stimulation at multiple electrode sites elicits sensations that are much stronger in intensity than when the same stimulation is delivered to the electrodes individually. Additionally, these stimuli often produce sensations different in description to the sensations elicited by stimulation to the individual electrodes, and biases the sensations to be more proprioceptive in nature. Stimulation discrimination amongst a subset of electrodes shows a stable threshold for detection at 22-25μA. The aim of the current work remains to reliably produce percepts that will be useful in the context of brain controlled systems with direct somatosensory feedback. Future experiments are planned to explore the neural mechanisms of S1 that govern the formation of sensory percepts as well as combining sensations with the decoding of brain behavior to produce a bidirectional brain-computer interface.

Disclosures: L. Bashford: None. **M. Armenta Salas:** None. **S. Kellis:** None. **H. Jo:** None. **M. Jafari:** None. **K. Pejsa:** None. **B. Lee:** None. **C. Liu:** None. **R.A. Andersen:** None.

Poster

404. Brain-Machine Interface: Somatosensory

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Program #/Poster #: 404.03/QQ4

Topic: E.05. Brain-Machine Interface

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NSF DGE-1256082
NIH 1T32CA206089
WRF UWIN Fellowship

Title: Spatiotemporally overlapping haptic and direct cortical stimulation in humans results in simultaneous perception with a range of delays predicted by the response time delay to cortical stimulation

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Abstract: Objective: Direct cortical stimulation (DCS) may help close the loop in brain computer interfaces. Recent work by our group has shown a significant delay in response times to DCS of primary somatosensory cortex (S1) compared to natural sensory stimulation in humans. In the current on-going study we extend this finding to examine psychometric response timings and perception, and conduct spectro-temporal analyses of human electrocorticographic (ECoG) signals during near-simultaneously delivered DCS of hand area of S1 and natural haptic stimulation to equivalent areas of the subject's hand.
Methods: A subject was implanted with subdural electrodes for epilepsy monitoring. We provided haptic stimulation through digital touch probes to the same cutaneous region where sensation was perceived during DCS of S1 hand cortex. DCS was applied in close temporal proximity to natural haptic touch with varying time lags. The subject was asked after each trial which stimulus was perceived first, and also responded via button press to stimulus onset.
Results: Only in trials where haptic touch trailed DCS by over 200 ms did the subjects reliably perceive DCS as arriving first. From haptic touch trailing DCS by 200 ms to approximately both arriving concurrently, there was ambiguity in the subject's perception of which stimulus arrived first (Fig. A, left). As previously seen, responses to DCS (when perceived first) were slower than trials when haptic was perceived first (Fig. A, right). The subject described perception of both sensations as distinct in all conditions, indicating that perception of both stimuli was not masked by simultaneous application.
Conclusions: There is a range of latencies for which overlapping spatial and temporal DCS and natural stimuli are perceived as arriving simultaneously. This has implications for future brain-computer interfaces, where both cortically-delivered feedback and natural feedback may arrive synchronously. Furthermore, our results demonstrate that spatially and temporally overlapping natural feedback and DCS can be isolated as distinct sensations.

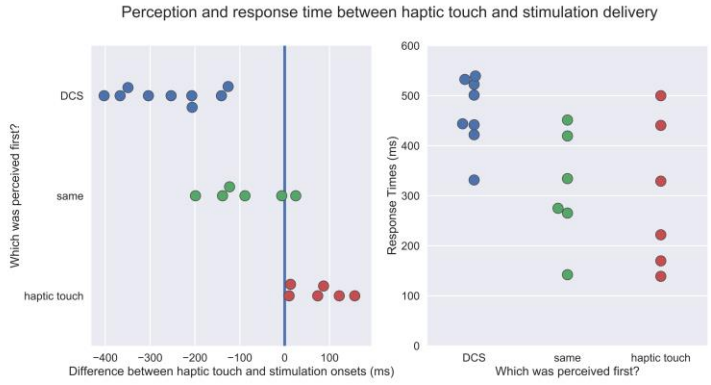

Figure A: With DCS delivered before haptic stimulation with a range of time deliveries from 400 ms to -200 ms, all trials were distinctly perceived as DCS arriving first. Between -200 ms and 25 ms, with 25 ms representing DCS onset 25 ms after haptic touch onset, the subject reported the stimuli arriving at approximately the same time. With DCS arriving between 75 ms to 150 ms after haptic touch, the subject always reported perceiving haptic touch first (Left panel). The plot shows a distribution of response times, where following a Kruskal-Wallis test and Nemenyi's multiple comparisons test, the haptic touch condition stochastically dominates the DCS condition, consistent with our previous results that haptic touch results in faster reaction times than DCS (right panel).

Disclosures: D.J. Caldwell: None. **J.A. Cronin:** None. **K.E. Weaver:** None. **A.L. Ko:** None. **R.P. Rao:** None. **J.G. Ojemann:** None.

Poster

404. Brain-Machine Interface: Somatosensory

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Topic: E.05. Brain-Machine Interface

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NSF DGE-1256082
NIH 1T32CA206089-01A1
WRF Fund for Innovation in Neuroengineering

Title: Perception of and parameters for direct cortical stimulation in human somatosensory cortex

Authors: *J. A. CRONIN[1,6], D. J. CALDWELL[1,6], G. M. BOYNTON[2], K. E. WEAVER[3,6], R. P. RAO[4,6], J. G. OJEMANN[5,6]
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Abstract: Introduction: Direct cortical stimulation (DCS) of primary somatosensory cortex (S1) may provide a means of delivering somatosensory feedback in a brain-computer interface. Previous work from our group has demonstrated that human subjects can perceive and discriminate the intensity of DCS based on varied current amplitude or frequency, use DCS as feedback in a motor task, and respond more slowly to DCS of S1 than to natural sensory stimulation. There remains, however, a sizeable gap in our understanding of the stimulation parameters that may be used to convey a wide range of uniquely discernable percepts. Here we report results from our ongoing work exploring human subjects' perception of DCS by varying stimulation parameters and computing psychometric functions for DCS.
Methods: Human subjects were implanted with subdural electrodes for epilepsy monitoring. We used Tucker-Davis Technologies hardware to deliver bipolar, constant-current stimulation using trains of biphasic square pulses. We varied the current amplitude using a staircase method to quantify subjects' perceptual thresholds. Five subjects completed the experiment with staircases converging on a 50%, 89%, and 79% threshold for Subjects 1-3, respectively. Subjects 4 and 5 also converged on a 79% threshold using a double-interleaved staircase in which two 1-up, 3-down staircases were randomly interleaved to discourage pattern recognition. The resultant data were fit with a Weibull function using a maximum likelihood method.
Results: Using 200 ms DCS trains with 200 Hz pulses, we found perceptual current thresholds

of 0.77, 1.38, and 1.31 mA for Subjects 1-3, respectively, in single experiments with 205 µs pulse widths (PWs). Subject 4 completed the experiment on two consecutive days with 205 µs PWs and thresholds of 1.29 mA on the first day and 0.88 mA on the second day. Subject 5 also completed the experiment twice; once with 205 µs PWs resulting in a 3.76 mA threshold and once with 819 µs PWs resulting in a 2.56 mA threshold. Figure 1 illustrates the results of the staircase procedure and psychometric function for Subject 5.

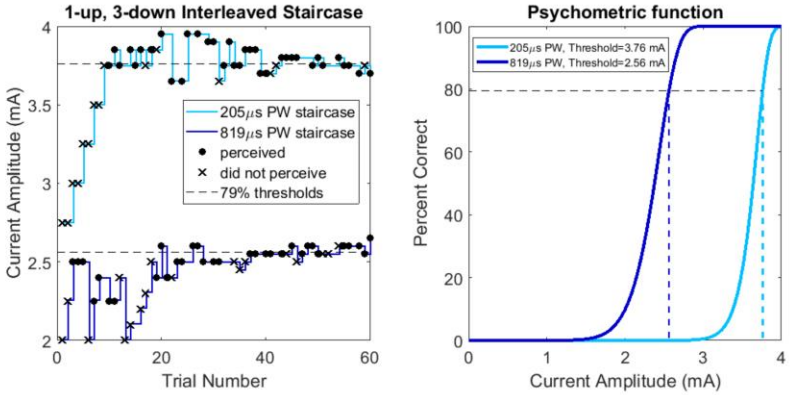

Figure 1. Comparison of perceptual thresholds (79%) and psychometric function for Subject 5. Subject 5 completed two 1-up, 3-down double-interleaved staircases both with 200 ms DCS train durations and a 200 Hz pulse frequency. Each experiment's two staircases included 25 test and 5 catch trials, randomly interspersed. In a given experiment, the two staircases were randomly interleaved to discourage pattern recognition, but are displayed as a single merged staircase here. The first staircase experiment used a pulse width (PW) of 205 µs (light blue), while the second staircase used a PW of 819 µs (dark blue). **Left panel:** Subject 5's responses to each of the non-catch trials. This subject completed 10 catch trials, with no stimulation delivered, to estimate the subject's guessing rate. He did not perceive any of the catch trials, so his guess rate was estimated as 0%. **Right panel:** Estimated psychometric function with 79% thresholds overlaid. The subject's guess rate (0%) dictated the lower bound of the psychometric function. Using the fitted psychometric function, we estimate Subject 5's perceptual current thresholds to be 3.76 mA and 2.56 mA for the 205 and 819 µs PWs, respectively.

Disclosures: J.A. Cronin: None. **D.J. Caldwell:** None. **G.M. Boynton:** None. **K.E. Weaver:** None. **R.P. Rao:** None. **J.G. Ojemann:** None.

Poster

404. Brain-Machine Interface: Somatosensory

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Topic: E.05. Brain-Machine Interface

Support: DARPA
SSC Pacific
ARCS

Title: Human perception of biomimetic intracortical microstimulation in somatosensory cortex

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Abstract: Brain-computer interfaces are powerful technologies that may allow for the restoration of motor function to individuals with damage to the brain, spinal cord, or limbs. A critical component of restoring this function, and indeed of natural motor control, is somatosensory feedback. In an ongoing experiment, we are studying the use of intracortical microstimulation (ICMS), delivered through Utah arrays implanted in somatosensory cortex, to restore tactile percepts in a human participant with a C5/C6 spinal cord injury. ICMS can elicit sensations of the contralateral hand ranging from less natural percepts, such as tingle, to more natural percepts, such as pressure and touch. However, it is not well understood what leads to these different qualities and reported levels of naturalness. One technique that may elicit more naturalistic percepts is to stimulate with "biomimetic" pulse trains that mimic normal neural activity. Although these pulse trains only represent a small component of what occurs in the cortex in response to natural peripheral stimuli, we hypothesized that single-channel pulse trains, where the pulse timing was based on recorded spiking activity, would be distinguishable from fixed-frequency pulse trains and further that these biomimetic pulse trains would feel more natural to the participant.
We began by building a biomimetic pulse train from neural data recorded from non-human primate cortex during finger indentation. We then used a two-alternative forced choice task in which the participant had to determine which of the two presented pulse trains, either the biomimetic or a fixed-frequency (100 Hz) pulse train, felt more natural. Each pulse train was presented at 60 μA for 1-s. The participant was not made aware of which pulse train was normal or biomimetic and the ordering of the trains was varied randomly throughout a set. On eight of the sixteen electrodes tested, there was a significant difference in the pulse train selection (z-test with Benjamani-Hochberg correction for multiple comparisons, Q=0.05) with six of the eight rated as being more natural with biomimetic pulse trains. Although improved naturalness does not guarantee improved function for bidirectional BCI technology, and further testing will be required to establish functional gains from biomimetic feedback, naturalistic artificial touch will likely improve the user experience and embodiment of the device. Understanding which stimulation features or electrode properties drive improved naturalness will inform the development of more natural and possibly more useful stimulation regimes.

Disclosures: C.L. Hughes: None. **J.M. Weiss:** None. **S.J. Bensmaia:** None. **R.A. Gaunt:** None.

Poster

404. Brain-Machine Interface: Somatosensory

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Support: MOTU INAIL PPR-AI 1/2
FeelAgain ERC starting grant 2017

Title: Sciatic intraneural stimulation evokes selective sensations from the phantom leg of trans-femoral amputees

Authors: *F. M. PETRINI[1,2], G. VALLE[3], M. BUMBASIREVIC[4], F. BARBERI[3], D. GUIRAUD[5], T. STIEGLITZ[6], S. MICERA[1], S. RASPOPOVIC[2]
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Abstract: Leg amputation destroys the communication between brain and environment during walking. Leg amputees rely on practically inexistent and often uncomfortable haptic feedback from the stump-socket interaction to monitor ground and obstacles contact, climb stairs, or walk in challenging environments. The lack of sensory feedback causes specific impairments to subjects that do not perceive the prosthesis as part of their body and risk falls, have decreased mobility, increased cognitive burden during walking resulting in prosthesis abandonment. In hand amputees, to restore the bidirectional communication, nerve interfaces have directly linked sensors readout from robotic hands to direct stimulation of nerves above injury. We believe that this strategy could also restore sensations from missing legs, with many scientific and technological barriers to overcome. It has never been proved that the electrical stimulation of the leg nerves by implantable neural interfaces can induce reliable sensations from missing leg and foot. In this work we developed a leg prosthesis restoring sensory feedback by means of direct nerve stimulation injected through transversal intraneural electrodes (TIME) implanted in the sciatic nerve. The stimulation was driven by the readout of pressure sensors placed under the prosthetic foot, and an encoder embedded in the prosthetic knee. We assessed the capability of 3 transfemoral amputees to recognize, blindfolded and acoustically insulated, the location where the prosthetic foot was touched and the degree of flexion of the prosthetic knee. The subjects were asked to recognize only touch, only flexion and then both conditions at the same time. We compared the performance when sensory feedback was restored and when no nerve stimulation was delivered. We found that single and double condition-tasks were executed on average respectively with a success rate of about 85% and 75%, when sensory feedback was provided. Without nerve stimulation the average success rate dropped to 25%.

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Poster

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Topic: E.05. Brain-Machine Interface

Support: NIH Grant 5R01EY015545-12
Tianqiao and Chrissy Chen Brain-Machine Interface Center at Caltech
Boswell Foundation

Title: A vision of touch

Authors: *S. CHIVUKULA[1], T. AFLALO[1], C. Y. ZHANG[1], M. JAFARI[1], E. ROSARIO[2], D. OUELLETTE[3], K. PEJSA[1], N. POURATIAN[4], R. A. ANDERSEN[1]
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Abstract: Growing evidence suggests that the brain integrates sensory information across modalities in proportion to its predictive value in estimating properties of the body and its surroundings. Within this framework, the lack of one sensory modality may at least partially be compensated by alternate modalities. A powerful example is the ventral visual stream recruitment of tactile and auditory information in the congenitally blind (Ptito et al., 2012). In a unique opportunity, we examined the neural encoding of seen (but not felt) touch on insensate arm regions of a tetraplegic human subject arising from visual feedback alone. We recorded from 2179 single neurons in the posterior parietal cortex (PPC) of a C3/4 spinal cord injured subject. Three task paradigms involving observed brush strokes to bilateral, insensate arm regions were used to 1) investigate the existence of, and limb coverage of, visual receptive fields (RFs), 2) explore their sensitivity to touch versus non-contact stimuli, and 3) determine their specificity to the native limb versus substitute objects. Single units (25.2%) were identified with significant response to seen touch. Bilateral visual RFs were encoded, and RFs collectively covered the entirety of the limbs. Visual RFs were encoded in a mix of reference frames (gradient analysis; Pesaran et al., 2006). In 39.5% of units, RFs were anchored to a region of the limb, independent of gaze (i.e., field or body-part centered). In most others (57.0%), the response depended on the relative gaze location and stimulated body part (i.e., eye-centered units). Next, touch was compared to non-contact stimuli. Response intensity was strongest for touch, with little responsiveness to stimuli above (visually overlapping) the limb, adjacent to the limb, or distant from the limb ($p<0.001$). In separate experiments, we examined the specificity of neural responses to the native limb by comparing them against touch to substitute objects. The population responsiveness varied across tested objects. Response patterns were selective (greater

specificity for the native limb (p<0.001) and distinct (multiple objects discriminably encoded). Moreover, the reference frames encoding touch responses to a substitute effector were less field centered than for the native limb (p=0.024). Taken together, our results suggest a neural basis within the human PPC for the processing of seen (but not felt) contact with oneself. Shared neural mechanisms also appear to selectively and discriminably encode contact-based interactions between objects in our sensory environment, hinting at a role for the PPC in a broader, visuospatial interpretation of our sensory surroundings.

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Poster

404. Brain-Machine Interface: Somatosensory

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Rehabilitation Research and Development Service, Department of Veterans Affairs (B6453R, N9288, A2295R)
Executive Committee on Research (ECOR) of Massachusetts General Hospital
MGH-Deane Institute for Integrated Research on Atrial Fibrillation and Stroke
NINDS (UH2NS095548)

Title: Representation of face, head and leg movements in “arm/hand” area of human motor cortex

Authors: *F. WILLETT[1,2], P. REZAII[1], L. R. HOCHBERG[8,9,11,12,10], J. M. HENDERSON[1,3,4], K. V. SHENOY[2,5,6,7,3]
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Abstract: Neural activity in motor cortex is thought to have a mixed somatotopic organization, in which face, arm and leg movements are represented in distinct areas of cortex but individual muscles within any one body part may be mixed (e.g. wrist and finger movements may overlap within arm area). However, these conclusions have been supported mainly by electrical

stimulation, intracortical recordings of arm movements only, or recording technologies with low spatial resolution (e.g. fMRI). Here, we revisit motor somatotopy using intracortical recordings from “hand knob” area of motor cortex while a participant in the BrainGate2 pilot clinical trial (T5) attempted a variety of face, head, leg and arm movements. Since T5 has a C4 level spinal cord injury, he could make face and head movements freely but could only attempt to make arm and leg movements.

Surprisingly, we found that face, head, and attempted leg movements are robustly represented in arm/hand area of motor cortex. Surveying 33 different movements, we found that changes in firing rate evoked by non-arm movements was 27% (face), 56% (head), and 46% (leg) as large as that evoked by attempted arm movement. This modulation was movement-specific and enabled accurate decoding of individual face, head and leg movements (94% accuracy across 25 different movements). We also found substantial representation of the ipsilateral limbs, with ipsilateral leg and arm movements generating modulation 60% and 55% as large as the contralateral limb. Next, we investigated how neural activity changed if two movements were executed simultaneously. Surprisingly, we found that modulation for the “secondary” effector (the effector with the least modulation when measured in isolation) was substantially attenuated during simultaneous movement (67% attenuation), while modulation for the “primary” effector remained mostly intact (15% attenuation). If these results are due to plastic reorganization after spinal cord injury, they suggest a larger reorganization than once thought (especially given the substantial leg-related activity). If not, the results suggest an update to the conventional somatotopy, given that we found overlapping representations even for widely separated areas of the body (e.g. face vs. leg).

For neuroprosthetics, the results show that while a variety of isolated movements can be decoded from a single cortical area, recording from multiple areas may help when decoding multiple effectors (to mitigate attenuation of the secondary effector). The results also imply that concurrent face / head movements could interfere with (or contribute to) arm movement decoding if not explicitly decorrelated.

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Poster

404. Brain-Machine Interface: Somatosensory

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Topic: E.05. Brain-Machine Interface

Support: NIH-R01-EB008578
DARPA & ARO (W911NF-17-1-0022)
FIU Coulter Eminent Scholar Endowment

Title: Assessment of functional benefits afforded by sensory-enabled prostheses to upper-limb amputees

Authors: *J. J. ABBAS[1], S. S. KUNTAEGOWDANAHALLI[2], K. HORCH[2], L. RINCON GONZALEZ[2], A. E. PENA[2], A. K. THOTA[2], B. K. HILLEN[2], D. AGUILAR[2], R. JUNG[2]
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Abstract: Recent developments in prosthetic systems have been directed at providing sensory feedback to enhance the functional capacity of users. These systems aim to improve the ability to manipulate objects and/or reduce the attentional demands by providing sensory feedback that is synchronized with signals derived from prosthesis-mounted sensors. Some systems utilize electrodes implanted either near peripheral nerve fibers or central nervous system structures; others use skin-mounted vibrotactile actuators or electrocutaneous stimulation. We have developed a battery of tests to be performed with sensory-enabled prostheses in order to characterize the ability of the sensory stimulation to elicit graded percepts and to characterize the impact of sensory feedback on the ability of the user to control the prosthesis in a graded manner and use it to perform functional tasks. To assess the ability of stimulation to elicit graded percepts, we have developed two perceptual modality matching paradigms: one in which the subject uses the intact hand to report sensations elicited by stimulation on the side with the amputation and one in which the subject first uses the intact hand to reach a target level of the modality being tested (e.g. hand opening, force) and then adjusts stimulation on the side with amputation to match the percept on the intact side. To assess the ability to control the prosthesis in a graded manner, we have developed two graded control tasks: one in which the subject uses the prosthesis to match a target level of the output (e.g. hand opening, force) and one in which the subject first uses the intact hand to match the target level and then uses the prosthetic hand to match the contralateral percept. To assess the user's ability to perform functional tasks, we have developed two fragile object tasks (one in which the subject picks up fragile cookies and one in which they pick up a 'mechanical egg', which is an adjustable device designed to break when too much force is applied but can be readily reset after breaking), a controlled squeeze task (in which the subject uses a squeeze bottle to fill small cups with water), and a sensorimotor exploration task (in which the subject uses the prosthetic hand to ascertain the size and stiffness of an object). To assess the impact of stimulation or attention, the individual prosthesis control tests can be performed with or without stimulation and either in a single-task or dual-task paradigm. These tests have been designed to be used in conjunction with standardized clinical outcome measures and surveys to assess the functional benefits afforded by sensory-enabled prostheses.

Disclosures: J.J. Abbas: None. **S.S. Kuntaegowdanahalli:** None. **K. Horch:** None. **L. Rincon Gonzalez:** None. **A.E. Pena:** None. **A.K. Thota:** None. **B.K. Hillen:** None. **D. Aguilar:** None. **R. Jung:** None.

Poster

404. Brain-Machine Interface: Somatosensory

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Topic: E.05. Brain-Machine Interface

Support: NIH-R01-EB008578
DARPA & ARO (W911NF-17-1-0022)
FIU Coulter Eminent Scholar Endowment

Title: Neural-enabled prosthetic hand system to restore sensation in upper-limb amputees

Authors: *R. JUNG[1], S. S. KUNTAEGOWDANAHALLI[1], A. K. THOTA[1], A. E. PENA[1], K. W. HORCH[1], J. PATRICK[2], J. J. ABBAS[3]
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Abstract: Today's upper limb amputees have needs that are not being met by current prosthetic technology because these systems do not provide effective sensory feedback from the prosthesis. This results in reduced quality of life and limitations in performing duties that require manipulation and high-quality grasp control. To address this problem, we have developed the Neural Enabled Prosthetic Hand (NEPH) system to provide amputees with sensations that are synchronized with sensor-derived signals from the prosthetic hand. The system includes an implanted electronic neurostimulator to deliver stimulation via fine-wire longitudinal intrafascicular electrodes (LIFEs) implanted in peripheral nerves of the residual limb and an external, prosthesis socket-mounted module that utilizes a wireless link to communicate stimulation commands to the implanted electronics. The NEPH system has been designed for daily use; donning, doffing, daily operation and maintenance are all similar to use of a commercially-available myoelectric prosthetic hand. The system has been approved as an investigation device by the FDA for evaluation in a first-in-human early feasibility clinical trial in transradial amputees and has a CMS Category B4 classification, thereby facilitating Medicare coverage. Detailed surgical procedures have been developed to implant multiple LIFEs for targeted stimulation of fibers in the median and ulnar nerves, routing the lead and passing the electronic housing from the medial to the lateral aspect of the upper arm and implanting the electronic housing with attached antenna in a subcutaneous pocket. The implanted components are all fabricated at a commercial manufacturing facility. There is no implanted battery and a single prosthetic frame-mounted battery powers the commercially available instrumented prosthetic hand, external electronics and implant electronics (via RF). The external electronics, which are embedded in the prosthetic hand frame, allow real-time wireless delivery of stimulation parameters to the implanted neurostimulator based on sensors in the prosthesis. The

user can select from stored, customized stimulation mapping functions and adjust stimulation intensity during use.

Disclosures: R. Jung: None. **S.S. Kuntaegowdanahalli:** None. **A.K. Thota:** None. **A.E. Pena:** None. **K.W. Horch:** None. **J. Patrick:** None. **J.J. Abbas:** None.

Poster

404. Brain-Machine Interface: Somatosensory

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 404.11/QQ12

Topic: E.05. Brain-Machine Interface

Support: Keio Institute of Pure and Applied Sciences (KiPAS) research program

Title: Acquisition of body schema to control a virtual tail via EEG-based brain-computer interface

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Abstract: Body schema refers to self-body representation that is considered to be acquired through integration of multimodal information. Previous studies suggest that body schema can be modulated as extension of the existing body. However, it remains unknown whether adult humans can acquire body schema of a novel body part that they do not innately have. In the current study, to evaluate body schema of a "tail" after training by using electroencephalogram (EEG) based Brain-Computer Interface (BCI) technology, we assessed kinematical, psychological, and neurophysiological data throughout successive 3-days interventions.
Nine healthy adults conducted BCI control training (100 trials/day). One trial consisted of the rest (with eye opening), the preparation, the task (motor imagery), and the interval phase. Participants were instructed to find a free image by which the tail was well controlled without regard to tail-related images. Participants were allowed to freely change the images every 10 trials, and they verbally reported what they would image in the next 10 trials. The scalp EEG signal was derived from the Cz of the International 10-20 System, and α-band (8-13 Hz) event related desynchronization (ERD) was calculated every 100 ms with a 90% overlapped 1-s sliding time window processed. The virtual tail movement was determined by ERD value. If more than 20% of ERD was detected during the task phase, the tail reached the target and that trial was defined as succeeded. Participants were not informed by what algorithm the tail movements are controlled. Body ownership of the tail was measured by Body Ownership and Agency Questionnaire (Osimo et al., 2015) which answers with 7-levels of Likert scale in every 10 trials.

We found significant increase in the success rate ($p < 0.05$) and score of the body ownership ($p < 0.05$) after 3-days of training. Since participants changed their images exploratorily in day 1, the number of image variation was larger than day 3 ($p < 0.05$). In the middle days of training, the success rate improved 16 % on average, however they rarely imagined the tail movement. Finally, their imagined movements converged on the tail movement in six subjects and body ownership of the tail enhanced 1.8 points on average. These results suggest that the capability of "controlling of the tail" improved, then body ownership of the tail acquired.
Our finding demonstrated for the first time that BCI training had a potential to acquire body schema of a novel body part in adult human. The present study would not only contribute to understanding of neural mechanisms of body schema, but also to rehabilitation of patients who have distorted self-body perception due to neurological disorders.

Disclosures: S. Kimura: None. **S. Kasuga:** None. **N. Mizuguchi:** None. **J. Ushiba:** None.

Poster

405. Sensory Input to Respiratory Control

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 405.01/DP07/QQ13

Topic: E.08. Respiratory Regulation

Support: MRC Discovery Award (MC-PC-15070)

Title: Central circuits for CO_2 chemosensory regulation of breathing in freely behaving mice

Authors: *A. M. BHANDARE, R. T. R. HUCKSTEPP, N. E. DALE
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Abstract: The regulation of breathing by blood PCO_2 is critical for survival in animals. Central CO_2 chemosensory circuits contribute to the regulation of breathing, however their respective contributions remain unclear in freely behaving animals. In the retrotrapezoid nucleus (RTN), neurons and astrocytes are sensitive to CO_2/H^+ and their activation may increase breathing frequency and tidal volume. This has been shown: *in vitro* in slice preparations; *in vivo* under anaesthesia; and via molecular detection of activated cFos. As anaesthesia depresses neural and glial activity, the response of RTN neurons and astrocytes to hypercapnia in freely behaving animals remains unknown, though optogenetic stimulation/inhibition of RTN neurons in conscious rats would suggest that its contribution is significant. We have investigated dynamic cellular activity of putative chemosensitive RTN neurons and glia in conscious animals by means of genetically-encoded calcium indicators and implanted gradient refractive index optic fibres (GRIN lens). Our novel microendoscopic approach has allowed us to record Ca^{2+} transients *in vivo* from individual RTN neurons and astrocytes in mice (12-20 weeks old of either sex) during hypercapnic and hypoxic challenges. We find that RTN neurons and astrocytes are transiently

activated by changes in inspired CO_2. A transient drive for breathing from chemosensory RTN neurons and astrocytes during hypercapnia suggests that other central circuits may be required for the full respiratory response to hypercapnia in freely behaving animals.

Disclosures: A.M. Bhandare: None. **R.T.R. Huckstepp:** None. **N.E. Dale:** None.

Poster

405. Sensory Input to Respiratory Control

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 405.02/QQ14

Topic: E.08. Respiratory Regulation

Support: Grant-in-Aid for Scientific Research on Innovative Areas (17H05540)

Title: Cardiorespiratory regulation by glutamatergic neurons in the caudal solitary nucleus

Authors: *S. YOKOTA[1], K. TAKEDA[2], M. LAZARUS[3], Y. ARIMA[1], M. FUJITANI[1], Y. OKADA[4]
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Abstract: The caudal solitary nucleus (cNTS) is the site receiving general visceral afferent, which conveys information on arterial gas partial pressures and blood pressure from the carotid body and carotid sinus, respectively. Glutamatergic cNTS neurons are activated under hypoxia and hypercapnia. However, the role of these neurons in cardiorespiratory regulation is not completely understood. In this study, we chemogentically activated glutamatergic cNTS neurons to examine the role of these neurons in cardiorespiratory regulation. For this purpose, we injected Cre-dependent adeno-associated viral vectors expressing a mutated M3-muscarinic receptor into the cNTS of genetically modified mice that expressed Cre recombinase under the control of the endogenous vesicular glutamate transporter 2 gene (VGLUT2-Cre mice). We then measured respiratory parameters by whole body plethysmography and blood pressure using a non-invasive sphygmomanometer after intraperitoneal injection of clozapine-N-oxide. As a result, chemogenetic activation of glutamatergic cNTS neurons increased tidal volume and minute ventilation with no significant change in respiratory rate and slowed heart rate with no significant change in blood pressure. Next, we demonstrated, by using Fos expression combined with retrograde tracing or tyrosine hydroxylase (TH) immunohistochemistry, that some phrenic nucleus-projecting neurons and many TH-immunoreactive neurons in the ventrolateral medulla expressed Fos protein after chemogenetic activation of glutamatergic cNTS neurons. We further showed, by using Cre-dependent conditional anterograde tracing combined with retrograde tracing or TH immunohistochemistry, that axon terminals of glutamatergic cNTS neurons were

apposed to phrenic nucleus-projecting neurons and TH-immunoreactive neurons in the ventrolateral medulla. These results suggest that glutamatergic cNTS neurons may regulate the tidal volume of respiration via projections to neurons that send axons to the phrenic nucleus, whereas glutamatergic cNTS neurons regulate heart rate via projections to TH-positive ventrolateral medullary neurons.

Disclosures: S. Yokota: None. **K. Takeda:** None. **M. Lazarus:** None. **Y. Arima:** None. **M. Fujitani:** None. **Y. Okada:** None.

Poster

405. Sensory Input to Respiratory Control

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 405.03/QQ15

Topic: E.08. Respiratory Regulation

Support: Damon Runyon Postdoctoral Research Fellowship (Y.L.)
M.A.K. is an investigator of the Howard Hughes Medical Institute

Title: Molecular and functional diversity of pulmonary sensory neurons revealed by single-cell RNA sequencing

Authors: *Y. LIU, A. DIAZ DE ARCE, M. A. KRASNOW
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Abstract: Pulmonary sensory neurons monitor the state of the lung and communicate with the brain to regulate breathing and pulmonary physiology. In pulmonary diseases, the activity of pulmonary sensory neurons is altered, leading to debilitating symptoms including chronic cough, dyspnea, and airway hyperresponsiveness. To understand the molecular and cellular mechanisms underlying these symptoms, a comprehensive characterization of pulmonary sensory neurons in health is required. Over the past several decades, the electrophysiological properties of pulmonary sensory neurons have been characterized, which revealed intriguing heterogeneity. However, the full molecular and functional diversity of these neurons was poorly understood. Here, we obtained genome-wide transcriptome of mouse vagal sensory neurons that are retrogradely labeled from the lung using single-cell RNA sequencing technology. By unbiased cluster analysis, we defined eight molecularly distinct subtypes of these neurons and identified molecular marker genes for each subtype. Detailed analyses on expression patterns of genes involved in sensory function and cell-cell communication enable predictions on which endogenous and exogenous stimuli each subtype of neurons can sense, what neurotransmitters and neuropeptides they can release, and their potential interactions with non-neuronal cells. Following the initial classification, we further quantified the number of each identified subtype and characterized their terminal morphologies and locations in the lung by combining antibody

staining, in situ hybridization, and genetic labeling. This work provides a systematic and comprehensive molecular and anatomical characterization of vagal pulmonary sensory neurons, enabling subtype-specific manipulation of these neurons to interrogate their physiological functions.

Disclosures: Y. Liu: None. **A. Diaz de Arce:** None. **M.A. Krasnow:** None.

Poster

405. Sensory Input to Respiratory Control

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 405.04/QQ16

Topic: E.08. Respiratory Regulation

Title: Respiratory load compensation and perception during submaximal exercise in collegiate athletes

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Abstract: Respiratory perception is complex and combines the emotional state of the subject, as well as, the magnitude and duration of the respiratory load. Emotion affects respiratory load perception regardless of physiological ventilatory changes (Tsai et al., 2013). A respiratory load results in inadequate respiratory compensation with high loads but may not show an effect at mild loads (Lind and Hesser, 1984). It is not known if respiratory compensation and perception are compromised during submaximal exercise with a mild respiratory load in healthy collegiate athletes. **PURPOSE:** To determine respiratory function and perception with a respiratory load in submaximal exercise post-concussion. **METHODS:** Healthy male collegiate athletes (age 19-23) performed a VO_2 maximal graded exercise test (Bruce protocol) followed a week later by 60% VO_2 submaximal (submax) exercise with/without respiratory load (10% restriction). Athletes were assigned to responder (R) or non-responder (NR) groups: R (n=4) and NR (n=4). Restricted load (RL) physiological and perception parameters were compared using T-test measurements. **RESULTS:** R during submax RL exercise had increased VO_2 13% (28.02±0.64 ml/min/kg SEM, p=0.01) compared to NR (24.90±0.83 ml/min/kg SEM), HR 13% (133.22±3.78 bpm SEM, p=0.001) compared to NR (118.24±3.21 bpm SEM), RPE 23% (12.1±0.87 SEM, p=0.35) compared to NR (9.85±0.1.32 SEM), Urge to Stop (UTS) 52% (11.46±0.72 SEM, p=0.02) compared to NR (7.55±0.38), Rating of Breathing Exertion (RBE) 28% (11.95±0.55 SEM, p=0.18) compared to NR (9.30±1.11 SEM), but decreased Ve 11% (48.37±5.33 SEM, p=0.26) compared to NR (54.57±1.65 SEM) and Rf 8% (32.30±0.86 bpm SEM, p=0.31) compared to NR (34.96±2.46 bpm SEM). **CONCLUSION:** Compensation was evidenced by increased oxygen consumption, heart rate, and perception to respiratory load in R athletes. The NR athletes had

blunted responses to the respiratory load. Future research including anxiety and stress measurements pre- and post-respiratory load are warranted.

Disclosures: S. Adams: None. **D.C. Malloy:** None.

Poster

405. Sensory Input to Respiratory Control

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 405.05/QQ17

Topic: E.08. Respiratory Regulation

Support: JSPS KAKENHI Grant 18K17783
JSPS KAKENHI Grant 26460311
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Title: Significance of astrocytic activation in hypoxic respiratory responsiveness in the *in vitro* medulla-spinal cord preparation of the newborn rat

Authors: *I. FUKUSHI[1], M. UCHIYAMA[2,1], Y. KURITA[2], I. YAZAWA[3], S. OKAZAKI[4,1], Y. HASEBE[5,1], Y. KONO[6,1], S. YOKOTA[7], K. TAKEDA[8,1], Y. MORI[9], H. ONIMARU[10], Y. OKADA[1]
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Abstract: Hypoxia transiently increases ventilation via the excitation of peripheral chemoreceptors. However, peripheral chemoreceptor-denervated animals increase ventilation in response to hypoxia when unanesthetized and awake, indicating the existence of the central respiratory hypoxia-sensing mechanism. Recently, it has been elucidated that astrocytes play active roles in various brain functions, and we have hypothesized that astrocytes are involved in modulation of respiration in a hypoxic condition. To investigate this hypothesis, we conducted experiments using arundic acid, an inhibitory modulator of astrocytic function. Firstly, we examined the astrocyte/neuron specificity in cell activation blockade action of arundic acid, by comparing its effects on high potassium-induced activation in cultured cortical astrocytes and cerebellar granule neurons of the mouse by ratiometric calcium imaging. Arundic acid inhibited activation of astrocytes in a dose-dependent manner. However, arundic acid did not suppress

activation of neurons. Secondly, we examined hypoxic responses of astrocytic activities in the ventrolateral medulla and respiratory frequency in the isolated medulla-spinal cord preparation of newborn rats, which is ideal for the study of the purely central mechanism of respiratory control. Each preparation was superfused first with control oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (aCSF), followed by brief hypoxic exposure by replacing the superfusate with a hypoxic (95% N_2, 5% CO_2) aCSF. Astrocytic activity in the ventrolateral medulla was measured by confocal calcium imaging. Neural respiratory output was recorded from ventral roots of the 4th cervical cord. We found that a number of astrocytes were activated in a hypoxic condition in the ventrolateral medullary respiratory region. Without pretreatment with arundic acid, respiratory frequency increased in a hypoxic condition. However, increases in respiratory frequency of the preparation pretreated with arundic acid tended to be smaller during hypoxic exposure. We conclude that astrocytes in the medulla play an important role in facilitation of respiration in a hypoxic condition.

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Poster

405. Sensory Input to Respiratory Control

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 405.06/QQ18

Topic: E.08. Respiratory Regulation

Support: HL108609 (D.A.B)
HL074011 (P.G.G)

Title: PACAP expression in RTN chemosensory neurons contributes to CO_2-stimulated breathing

Authors: *Y. SHI, D. STORNETTA, A. SAHU, K. LI, Y. WABARA, A. DHAKAL, E. PEREZ-REYES, R. STRONETTA, P. GUYENET, D. BAYLISS
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Abstract: CO_2/H^+-sensitive neurons of the retrotrapezoid nucleus (RTN) are a nexus for integration of multiple respiratory-related inputs, providing homeostatic regulation of blood gases and arterial pH by driving the networks that determine the rate and depth of breathing. Firing activity in RTN neurons is a critical determinant of respiratory output. These excitatory glutamatergic RTN neurons also express a number of neuroactive peptides, for which a physiological function remains enigmatic. Among these, recent single cell RNA-Seq and *in situ* hybridization studies revealed that pituitary adenylate cyclase-activating peptide (PACAP) is

expressed universally in mouse RTN neurons. In global knockout mice, PACAP deletion is associated with reduced whole animal CO_2 sensitivity and a sudden infant death syndrome (SIDS)-like phenotype. Here, by using single cell qRT-PCR and *in situ* hybridization, we show that PACAP expression is developmentally regulated in mouse RTN neurons, with low levels in the late embryonic period, and a prominent peak in the days immediately after birth that tapers to moderate levels that are maintained into adulthood. In adult mice, viral shRNA-mediated knockdown of PACAP selectively in RTN neurons decreased ventilatory stimulation by CO_2 assessed by whole-body plethysmography. After PACAP knockdown, activation of RTN neurons *in vivo* by CO_2, determined by Fos expression, was unaffected; by contrast, CO_2-dependent Fos expression activation was decreased in neurons of the preBötzinger complex (preBötC), which contains respiratory rhythm-generating neurons targeted by the RTN. In anesthetized mice, direct injection of PACAP into the preBötC region increased the rate and amplitude of diaphragmatic activity, consistent with stimulation of central respiratory output. Conversely, viral shRNA-mediated knockdown of PAC1, the PACAP receptor, in the preBötC region reduced CO_2-stimulated breathing. Collectively, these data indicate that PACAP expression by RTN neurons is necessary for normal ventilatory responses to CO_2, likely via actions in the preBötC. The particularly prominent expression of PACAP around the time of birth, an especially vulnerable period, may provide a critical mechanism to facilitate and maintain the transition to air breathing required for extrauterine life.

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Poster

405. Sensory Input to Respiratory Control

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 405.07/QQ19

Topic: E.08. Respiratory Regulation

Support: NIH/NHLBI HL104101 (DKM, MLO) and HL137094 (DKM)
Dravet Foundation AG180243

Title: Baseline activity and CO_2/H^+-sensitivity of neurons in the retrotrapezoid nucleus are increased in a mouse model of Rett syndrome

Authors: *C. M. GONÇALVES[1], M. L. OLSEN[2], D. K. MULKEY[1]
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Abstract: Rett Syndrome (RTT) is an X-linked neurodevelopmental disorder that is caused by loss-of-function mutations in the methyl-CpG-binding protein 2 (MeCP2) gene. A profound and life-threatening symptom of RTT is disordered breathing, characterized by forced or irregular breathing, hyperventilation, and frequent episodes of apnea. Mouse models of RTT recapitulate this respiratory phenotype and suggest disruption of the mechanism by which the brain regulates breathing in response to changes in CO_2/H^+ (i.e., respiratory chemoreception) may be a contributing factor. The retrotrapezoid nucleus (RTN) is an important chemoreceptor region; however, the functional consequence of MeCP2 deficiency yet to be explored in RTN neurons. To make this determination, we made cell-attached recordings of RTN chemoreceptor activity in brainstem slices (230μM) from male $MeCP2^{-/y}$ and wild type littermate ($MeCP2^{tm1.1Jae}$) mouse pups between 9 and 12 days of age. Chemosensitive RTN neurons in slices from wild type mice had a baseline activity of 0.19 ± 0.2 Hz under control conditions (5% CO2; pH=7.30) and showed a robust increase in activity (Δ 1.58 ± 0.4 Hz; N = 7) in response to 15% CO2 (pH 6.9). RTN neurons from $MeCP2^{-/y}$ mice had a higher basal firing rate (1.48 ± 0.40 Hz; N = 9) ($p < 0.05$) and responded more vigorously to 15% CO2 (Δ 2.90 ± 0.4 Hz; $p < 0.05$). This is interesting because enhanced chemoreceptor gain may cause periodic or irregular breathing; therefore, these results support the possibly that MeCP2 deficiency disrupts RTN chemoreceptor function and contributes to disordered breathing in RTT.

Disclosures: C.M. Gonçalves: None. **M.L. Olsen:** None. **D.K. Mulkey:** None.

Poster

405. Sensory Input to Respiratory Control

Location: SDCC Halls B-H

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Program #/Poster #: 405.08/QQ20

Topic: E.08. Respiratory Regulation

Support: NIH/NHLBI HL104101
HL137094
Dravet Foundation AG180243

Title: Adenosine/A1 receptor modulation of chemosensitive neurons in the retrotrapezoid nucleus and *in vivo* in A1 receptor knockout mice

Authors: *S. D. JAMES[1], S. A. MASINO[2], D. N. RUSKIN[3], T. S. MOREIRA[4], D. K. MULKEY[5]
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Abstract: The brain regulates breathing in response to changes in tissue CO_2/H^+ by a process termed central chemoreception. Neurons and astrocytes in a brainstem region known as the retrotrapezoid nucleus (RTN) function as respiratory chemoreceptors. The role of astrocytes in this process involves CO2/H+-dependent release of ATP to enhance activity of chemosensitive RTN neurons. Considering that in most brain regions extracellular ATP is rapidly broken down to adenosine by ectonucleotidase activity, we wondered whether adenosine signaling contributes to RTN chemoreceptor function. To explore this possibility, we characterized the effects of exogenous adenosine on the activity of chemosensitive RTN neurons in slices from rat pups (7-12 days postnatal). Cell-attached recordings from RTN chemoreceptors show that bath application of adenosine (1 μM) strongly inhibited neural activity under control conditions and during high CO2. Adenosine-mediated inhibition of chemoreceptor activity was blunted by prior incubation with the A1 receptor antagonist DPCPX (30nM). We also found in whole-cell voltage clamp mode (Vhold=-60 mV, 1μM TTX) that exposure to adenosine increased outward current 10.42 ± 0.68 pA ($p < 0.001$) and conductance 0.26 ± 0.02 ($p < 0.01$) by activation of an inward rectifying K+ conductance. Furthermore, exposure to adenosine also decreased the frequency of excitatory post-synaptic currents recorded from chemosensitive RTN neurons ($p < 0.05$). These results suggest adenosine/A1 receptor signaling inhibits RTN neurons by pre- and post-synaptic mechanisms. To test this possibility in vivo, we characterized respiratory activity in A1 receptor knockout mice. Contrary to our expectations, A1 receptor knockout mice showed a reduced respiratory frequency under room air conditions ($p < 0.05$) and showed a reduced ventilatory response to CO2 (balance O2) compared to litter mate control mice ($p < 0.05$). Although it is not clear why ablation of inhibitory A1 receptors results in a hypoventilatory phenotype, we consider the most likely possibility to be that A1 receptor knockout mice are also seizure prone and seizure activity can suppress breathing. Nevertheless, at the level of the RTN we conclude that adenosine signaling through A1 receptors inhibits chemoreceptor activity.

Disclosures: S.D. James: None. **S.A. Masino:** None. **D.N. Ruskin:** None. **T.S. Moreira:** None. **D.K. Mulkey:** None.

Poster

405. Sensory Input to Respiratory Control

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Program #/Poster #: 405.09/QQ21

Topic: E.08. Respiratory Regulation

Support: NIH/NHLBI HL104101
NIH/NHLBI HL137094
Dravet Foundation AG180243

Title: $P2Y_2$ receptors contribute to specialized CO_2/H^+ dependent regulation of arteriole tone in the retrotrapezoid nucleus

Authors: *C. CLEARY, D. MULKEY
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Abstract: Regulation of cerebral blood flow is critical for normal brain function; the accumulation of CO_2/H^+ causes vasodilation leading to enhanced delivery of O_2 and removal of CO_2/H^+, thus helping to match blood flow with tissue metabolic needs. Tissue CO_2/H^+ also functions as the primary stimulus for breathing by activating respiratory chemoreceptors. Interestingly, recent evidence suggests that CO_2/H^+-dependent regulation of blood flow in a chemoreceptor region, known as the retrotrapezoid nucleus (RTN), is opposite to the rest of the cerebrovascular tree; exposure to high CO_2/H^+ decreases arteriole tone by a mechanism that may involve $P2Y_2$ receptors. However, it is not clear which cells at the astrocyte-arteriole interface express $P2Y_2$ receptors. Making this determination is important since regulation of blood flow in the RTN influences respiratory behavior. Therefore, the goals of this study are to confirm *in vitro* that $P2Y_2$ receptors are important determinants of RTN vascular tone and, using qRT-PCR, to characterize $P2Y_2$ mRNA expression within each component of the astrocyte-arteriole interface in chemoreceptor regions, including the nucleus tractus solitarius (NTS), midline raphe, and RTN as well as non-respiratory brainstem regions. Brainstem slices (150 υm thick) were isolated from adult C57BL6/J mice and maintained under control conditions (5% CO2, balance air) plus thromboxane A2 receptor agonist U46619 (125 nM) to induce a partial constriction. While monitoring vessel diameter with fluorescent video microscopy, we found that bath application of a selective P2Y2/4 agonist UTPyS (100 υM) constricted arterioles by 10.5% +/- 0.41%, whereas exposure to a selective $P2Y_2$ antagonist AR-C 118925XX (10 υM) blocked the CO_2/H^+-evoked vasoconstriction. Next, using cell type specific reporter lines (smMHCCre/eGFP, Aldh1l1Cre/TdTomato, Tie2-Cre/TdTomato) we isolated astrocytes, endothelial cells, and smooth muscle cells from each region of interest (NTS, Raphe, RTN) for subsequent FACS and qRT-PCR. Consistent with our hypothesis, we found that $P2Y_2$ mRNA was highly expressed in smooth muscle cells from RTN arterioles but was not detectable in endothelial cells across all regions of interest. $P2Y_2$ mRNA did not appear to be enriched in astrocytes from either region. Since activation of $P2Y_2$ in smooth muscle is known to constrict arterioles, whereas activation of these receptors in endothelial cells does the opposite, these results identify $P2Y_2$ receptors in smooth muscle cells as a likely candidate for the CO_2 dependent regulation of vascular tone in the RTN seen *in vitro* and *in vivo*.

Disclosures: C. Cleary: None. **D. Mulkey:** None.

Poster

405. Sensory Input to Respiratory Control

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 405.10/QQ22

Topic: E.08. Respiratory Regulation

Support: NIH HL108609

Title: A Trpm4-like subthreshold oscillation underlies repetitive firing in mouse chemosensitive RTN neurons

Authors: K. LI[1], Y. SHI[1], *D. A. BAYLISS[2]
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Abstract: The firing activity of CO_2/H^+-chemosensitive neurons of the retrotrapezoid nucleus (RTN) provides a critical drive to brainstem respiratory networks that regulate breathing. These neurons display a highly regular repetitive firing pattern when recorded *in vivo*, or when depolarized beyond action potential threshold *in vitro* (e.g., by lowering pH or current injection). The ionic basis for this steady firing pattern has not been established. Here, we demonstrate a role for the Trpm4 channel that underlies pacemaker-like firing activity in other neurons. By single cell RNA-Seq and *in situ* hybridization, we found that a majority of mouse RTN neurons express *Trpm4* transcripts (~60-70%). In the presence of tetrodotoxin (TTX), RTN neurons in mouse brainstem slices exhibited a prominent subthreshold oscillation that increased in amplitude with membrane depolarization. Consistent with a role for the Ca^{2+}-sensitive and Na^+-permeable Trpm4 channel, TTX-resistant membrane oscillations in RTN neurons were inhibited by blocking calcium currents (with Cd^{2+}) or by replacing bath sodium (with NMDG); moreover, they were also blocked by the general Trp channel blocker, flufenamic acid, and by the Trpm4-selective inhibitor, 9-phenanthrol. Under conditions of bath acidification in the presence of TTX, the typical pH-dependent membrane depolarization of RTN neurons was associated with a marked enhancement of oscillatory subthreshold activity. The firing activity of RTN neurons, measured by cell-attached recordings *in vitro*, was strongly diminished by 9-phenanthrol, although the stimulatory effect of pH on action potential discharge from this lower baseline firing rate was unaffected. Together, these data implicate Trpm4 in determining the repetitive firing activity of RTN neurons; these channels influence RTN neuron firing responses by enhancing membrane oscillatory behavior during acidification-induced depolarization in a manner seemingly independent of the basic intrinsic neuronal pH-sensing mechanisms.

Disclosures: K. Li: None. **Y. Shi:** None. **D.A. Bayliss:** None.

Poster

405. Sensory Input to Respiratory Control

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 405.11/QQ23

Topic: E.08. Respiratory Regulation

Support: FONDECYT 1171434
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IBRO International Travel Grant 2018

Title: Metabolic inhibition of astrocytes reduces the respiratory response induced by hypercapnic acidosis of the raphe nucleus

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Abstract: Homeostatic regulation of breathing is achieved through feedback information provided by peripheral and central respiratory chemoreceptors. Peripheral chemoreceptors sense $O_2/CO_2/H^+$ in the arterial blood and central chemoreceptors CO_2/H^+ in the interstitial fluid of the CNS and cerebrospinal fluid. In the brainstem, respiratory chemoreceptors are found, among other sites, in the retrotrapezoid nucleus, nucleus tractus solitarius, ventral lateral medulla, preBötzinger complex and raphe nucleus obscurus (RNO). Astrocytes play a key role as chemosensory interoceptors in the brainstem, they can release ATP, D-serine and glutamate in response to hypercapnia (increased levels of CO_2). We addressed here whether astrocytes from the RNO can modulate the respiratory response to hypercapnia *in vitro* and *in vivo*. In caudal brainstem slices obtained from neonatal CF1 mice (P0-P4), the fictive respiration was recorded from the ventral respiratory column (VRC) with glass suction electrodes while were superfused with artificial cerebrospinal fluid (aCSF) equilibrated with O_2/CO_2 = 95%/5%, (pH 7.4, 30 ± 1°C) or during hypercapnia, by gassing aCSF with O_2/CO_2 = 90%/10%, (pH 7.2). The local acidosis at the RNO was obtained by microinjection of aCSF-Pipes buffer (pH 6,5). The metabolic inhibition was done by superfusing the slices with fluoroacetate (5 mM, FA) with glutamine (1.5 mM, Gln) for 30 min before and during each hypercapnic test. Tidal volume (VT), minute volume (VE) and frequency (fR) were obtained by plethysmography from adult CF1 mice which were implanted with a cannula into the raphe obscurus nucleus 4 days before. We evaluated their ventilatory responses to hypercapnia before and after FA-Gln and saline solution microinjections into the RNO on the awake animals.Hypercapnia in slices increased the fR in about 30% whereas local acidosis of the RNO with aCSF-Pipes increased this fR in about 21%. Application of FA-Gln reduced both the basal fR and the hypercapnia-induced increase in fR in about 20%. In conscious adult mice microinjections of FA-Gln, decreased the hypercapnia-

induced ventilatory response in VT and VE. These results are compatible with the notion that caudal medullary astrocytes from RNO are interoceptors mediating central chemoreception.

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Poster

405. Sensory Input to Respiratory Control

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Topic: E.08. Respiratory Regulation

Support: NIH/NIBIB U01EB021960
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Title: Brainstem mechanisms of cardio-ventilatory coupling

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Abstract: The respiratory and cardiovascular systems are integrated physiologically to oxygenate tissues and remove carbon dioxide. Brainstem neural circuits control cardio-respiratory functions, generate rhythms spontaneously and integrate central and sensory input to maintain gas homeostasis. Respiratory and cardiovascular outputs are partially synchronized/modulated by each other, and the respective brainstem neuronal networks have reciprocal synaptic connections. However, no quantitative mechanistic description was suggested to explain specific aspects of the cardio-respiratory interactions and their alterations in certain pathophysiological conditions.The influences of respiration on heart rate and blood pressure are well-recognized attributes of cardio-respiratory coupling; however the influence of a heart beat on respiration, known as cardio-ventilatory coupling (CVC), is just being recognized. CVC is a form of partial synchronization between cardiac and respiratory rhythm that is characterized by an increased probability of a heartbeat occurring at specific phases of the respiratory cycle. The neuronal circuitry that mediates CVC is not well understood.Previously, we suggested that baroreceptor input facilitates the activity of an inhibitory neuronal population of the respiratory central pattern generator (rCPG) that is active during the expiratory phase. Here, we introduce a closed loop model of the integrated respiratory and cardiovascular control system to describe our hypothesized mechanism for CVC. In this model, CVC is mediated by the pulsatile inputs from

arterial baroreceptors to neurons of the respiratory central pattern generator. Projections from 2nd order baro-sensitive neurons of the nucleus of solitary tract (NTS) excite a population of expiratory neurons in the rCPG. Excitation of these rCPG expiratory neurons makes the onset of inspiration less likely to occur right after the heartbeat thus reproducing a characteristic structure of the heartbeat probability distribution. To validate our *in silico* design, we utilized a dataset of single-unit ensemble recordings acquired from *in situ* rat preparation. The neurons were located primarily in the ventrolateral medullary cardio-respiratory column and were characterized by their firing patterns, by connectivity to other neurons and motor nerve recorded simultaneously and by their response to acute increases in perfusion pressure. These recordings captured the central coordination of cardio-respiratory network during the baroreflex and supported neural connectivity proposed in our model.

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Poster

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Program #/Poster #: 405.13/QQ25

Topic: E.08. Respiratory Regulation

Support: Supported by R00-HL 111215, NIH HL 103415, and 1OT20D001983.

Title: Effect of laryngeal adductor reflex and hypercapnia on the swallow-breathing relationship in cats

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Abstract: Swallow-breathing coordination is critical for maintaining a clear airway during the act of swallowing. The initiation of a swallow is driven from afferent feedback to swallow pattern generators that coordinate with respiratory pattern generators for appropriate timing. As feedback plays a central role in swallow generation, swallowing is subject to many reflexes and feedback responses impacting the upper airway and respiration such as the pulmonary stretch feedback and the laryngeal adductor reflex (LAR). While the laryngeal adductor reflex has largely been thought of as a reflexive close of the vocal folds upon laryngeal mechanical stimulation, we are beginning to uncover its role in affecting pattern generation of both swallowing and respiration. Here we examined the role of the laryngeal adductor reflex on swallowing-breathing coordination in room air and 10% CO2. Swallowing was induced in

anesthetized spontaneously breathing cats by infusion of water into the oropharynx and experiments were conducted under conditions of room air or 10% CO2. Electromyogram activity was measured using bipolar electrodes inserted into upper airway muscles (mylohyoid, thyroarytenoid, and thyropharyngeus), inspiratory muscles (parasternal muscles and costal diaphragm), and expiratory muscles (external oblique). Previous work investigating the changes in swallow-breathing coordination during hypercapnia demonstrated swallows occurred more frequently during inspiration and the transition from expiration to inspiration. Accompanying these changes, we report a decreased occurrence of the LAR, and a change in its recruitment pattern during hypercapnia. In all cases, initiation of the LAR produced an abrupt stop of respiratory activity and in many cases a series of sequential swallows. These preliminary results inform on the potential connections of the laryngeal feedback to swallow and respiratory pattern generators, and imply an enhanced role of the larynx in swallow-breathing coordination.

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Poster

405. Sensory Input to Respiratory Control

Location: SDCC Halls B-H

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Topic: F.03. Neuroendocrine Processes

Support: Ruth L. Kirschstein NRSA Individual Predoctoral Fellowship (A.D.)
Damon Runyon Postdoctoral Research Fellowship (Y.L.)
M.K. is an investigator of the Howard Hughes Medical Institute

Title: Anatomical characterization of Olfr78-expressing pulmonary sensory neurons

Authors: *A. J. DIAZ DE ARCE, Y. LIU, M. KRASNOW
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Abstract: Pulmonary sensory neurons monitor the physiological state of our respiratory tract and regulate breathing to maintain homeostasis. This process is disrupted in many common respiratory diseases, for example asthma, COPD, and respiratory tract viral infections. Altered activation levels of these sensory neurons can cause chronic cough and other respiratory symptoms, and they may contribute to disease progression. Classical studies have divided these sensory neurons into four classes: mechanosensitive rapidly adapting receptors, slowly adapting receptors, cough receptors, and chemosensitive C-fibers. However, recent single cell genome-wide expression profiling studies in mice have revealed greater diversity among these neurons. One newly discovered class of pulmonary sensory neurons expresses olfactory receptor 78 (Olfr78), a lactate receptor that is part of the acute blood oxygen-sensing pathway in the carotid

body. We used a Cre-driver mouse line and an adeno-associated virus that expressed a fluorescent reporter in a Cre-dependent manner to selectively label Olfr78-expressing neurons. Each neuron terminated at multiple sites in the lung. The terminals were either free nerve endings in the alveolar region or in neuroepitheilial bodies, clusters of neuroendocrine cells located at branch junctions along the bronchial tree that are activated by hypoxia. Together, expression of Olfr78 and the innervation of neuroepithelial bodies suggest that these neurons may be part of a pulmonary hypoxia sensing pathway. Further characterization is necessary to understand the role of these neurons in lung physiology and pulmonary diseases.

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Poster

406. Advances in the Neural Basis of Birdsong

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 406.01/RR1

Topic: F.01. Neuroethology

Support: MEXT/JSPS KAKENHI Grant 17H06380
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Title: Relation between song preference and song learning in male Bengalese finches

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Abstract: Juvenile songbirds memorize song of an adult bird (song tutor) and match their own vocalizations to it through subsequent sensorimotor learning. They can also discriminate song of their tutor from songs of other individuals, both as juveniles in the early phase of sensorimotor learning and as adults long after song crystallization (Clayton, 1988; Gobes and Bolhuis, 2007). In these studies, such discrimination ability was tested by measuring responses to playback of two different songs. Selective approach to a tutor song over an unfamiliar song indicates both recognition and preference for the tutor song. Whether such song preference has any function in ongoing song learning has not been directly investigated. In this study, we quantified tutor song preference of juveniles, and evaluated their song learning performance after song crystallization. We used male Bengalese finches for song tutoring and preference tests. The birds were raised by both parents until around 2 weeks of age, but afterwards kept only with their mother and siblings until around 40 days old. They were then moved to tutoring cages and housed with an unrelated male (tutor) for 2-3 weeks. This procedure led to varied song similarity between the tutee and tutor. We tested song preference 3 times; at 60-70 days (soon after tutoring finished), 90 days, and 120 days old. In the preference test, subjects were exposed to alternate playbacks of tutor

song from one end of a test chamber and novel song from the other. We measured the duration they spent in proximity to the speakers that broadcasted songs. We evaluated song learning performance by the percentage of tutor song elements that a subject eventually sang. So far, we tested 11 birds and found that tutor song preference measured at 60-70 days, but not at 90 nor 120 days, was positively correlated with song similarity. This raises the possibility that tutor song preference during song learning has some impact on the accuracy of learning. Currently we are testing another set of birds with different conditions of tutoring and test stimuli to examine this hypothesis more strictly.

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Poster

406. Advances in the Neural Basis of Birdsong

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Program #/Poster #: 406.02/RR2

Topic: F.01. Neuroethology

Support: Seed Fund from Big Ideas Generator (BIG) at the University of Chicago
NSF-BCS-16326465

Title: Functional connectivity signatures accurately distinguish male zebra finches who have been isolated from song during the sensitive period for vocal learning

Authors: *E. A. LAYDEN, K. E. SCHERTZ, M. G. BERMAN, S. E. LONDON
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Abstract: Juvenile male zebra finch (*Taeniopygia guttata*) songbirds learn to sing from an adult male tutor during a sensitive period spanning Posthatch (P) days P30 to P65. If male zebra finches do not have tutor experience during this period, tutor song learning capability extends past P65. Tutor song learning is linked with the juvenile's emerging song stereotypy, often high at P90. Here we used longitudinal fMRI functional connectivity (FC) analyses to investigate neural signatures of learning capability and song stereotypy in males scanned at P25, P45, P65, and P90. Birds were randomly assigned to one of three conditions: normal (Norm, $N = 5$) birds were aviary-reared with multiple potential adult male tutors; tutored (Tut, $N = 7$) birds were housed with two adults, 1 male tutor and 1 female; and isolated (Iso, $N = 6$) birds were housed with 2 adult females. We partitioned the brain into a set of six bilateral song regions of interest (ROIs: Area X, LMAN, RA, HVC, Auditory Forebrain (NCM, CMM), and Field L) and measured the Pearson correlation between the fMRI signals of each ROI pair during a resting state period. Additionally, we recorded song bouts at P60 and P90 and computed measures of stereotypy. Implementing a linear mixed-effects model, we found that Iso birds exhibited lower FC between symmetrical bilateral ROI pairs (i.e., homotopic FC) from P45-P90 than either

Norm (B = -0.130, $t(2895)$ = -3.88, $p < 0.001$) or Tut (B = 0.067, $t(2895)$ = 2.06, p = 0.040). Importantly, we noted strong associations between average homotopic FC at P65 and song stereotypy measures at P60: %similarity ($r(7)$ = 0.71, p = 0.033), accuracy ($r(7)$ = 0.66, p = 0.055), and %sequential ($r(7)$ = 0.31, $p > 0.40$). We next implemented linear discriminant analyses to examine whether FC could successfully distinguish between conditions across ages P45-P90. Norm vs. Tut could not be accurately classified. However, a linear discriminant incorporating just three connections (L HVC-L RA, L Aud. Forebrain-L HVC, and R HVC-L HVC) distinguished between a combined Norm/Tut group and Iso with 88.6% accuracy. Iso showed lower FC for L HVC-L RA ($t(42)$ = -2.94, p = 0.005), higher FC for L Aud. Forebrain-L HVC ($t(42)$ = 2.72, p = 0.009), and marginally lower FC for HVC homotopy ($t(42)$ = -1.79, p = 0.081). These results highlight putative neural signatures of vocal learning capacity in zebra finches, including L HVC-LRA FC and L Aud. Forebrain-L HVC FC, two ipsilateral connections with known anatomical connectivity and previously established importance for song. We also find novel evidence supporting the role of interhemispheric coordination, in the form of homotopic FC, for the development of song stereotypy.

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Poster

406. Advances in the Neural Basis of Birdsong

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 406.03/RR3

Topic: F.01. Neuroethology

Support: BELSPO IAP P7/17
FNRS Senior research Associate
FNRS Research Fellow

Title: Correlation between song learning and perineuronal nets development in song control nuclei of juvenile canaries

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Abstract: Perineuronal nets (PNN) are aggregations of extracellular matrix components surrounding the soma of specific neurons, mainly GABAergic interneurons expressing the calcium binding protein parvalbumin (PV+). In mammals, the development of PNN limits

synaptogenesis around PV+ neurons and PNNs have been identified as a marker of the end of sensitive periods for brain plasticity in several neuronal systems. In songbirds, vocal learning requires exposure to conspecific vocalizations by male tutors during a sensitive period. This is followed by a sensorimotor period when birds progressively match their vocalizations with the memorized tutor's song until the fully mature song is crystallized. In a closed-ended learner, the zebra finch, PNN expression in select song nuclei is higher in males than in females and higher in adults than in juvenile males. The timing of PNN appearance in the developing zebra finch brain correlates with the timing of sensitive periods for song learning. We also showed that PNN are more densely expressed in the song control system of zebra finches than in two open-ended learners, the European starling and the canary and that testosterone (T) induces song crystallization in early spring and increases the number of PNN in castrated canaries. Together these data suggest that PNN might regulate the end of the sensitive period(s) for vocal learning during ontogeny. To elucidate this question further, we quantified PNN expression at critical time points during the first year of life in canaries and correlated these data with their song development. Brains were collected from groups of males (n=6-8/group) at time points corresponding to specific vocal developmental stages: first spring (subsong), summer (early plastic song), fall (plastic song), winter (ongoing song crystallization), and second spring (fully crystallized song/adults). In winter, one additional group received T implants to test whether this accelerates PNN development and song crystallization. The number of PNN reached their maximum in the fall in HVC but only in the winter in RA and Area X. In the group treated with T there was no further enhancement of PNN expression over what was observed in untreated birds at the same age. Total song duration and song developmental score only reached their maximum in the spring and were enhanced by T in the winter. Other song characteristics such as the energy or bandwidth were already at the adult levels during the winter. Most of these song measurements correlated with the number of PNN in the song nuclei suggesting that PNN contribute to song crystallization that starts in the winter and is completed at the onset of spring.

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Poster

406. Advances in the Neural Basis of Birdsong

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Program #/Poster #: 406.04/RR4

Topic: F.01. Neuroethology

Title: Single cell resolution and seasonal distribution of neuronal phenotypes in the song control system of canaries

Authors: C. FRANKL-VILCHES[1], P. ALCAMI[1], *S. LEITNER[2], N. WILHELMI[1], A. BAKKER[1], M. L. GAHR[1]
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Abstract: Plasticity in brain-behaviour relationships can best be investigated in seasonal breeding species. However, conclusions on the degree of seasonal plasticity of the neural circuits such as the song system of songbirds may depend on the neuronal characterisation method used. Comparative studies have typically investigated the relationships between neuronal morphology and neuronal markers using immunohistochemistry or in situ hybridization. Both techniques are difficult to implement for a number of reasons. First, a relatively small number of antibodies are available for immunohistochemistry in birds. Second, conventional in situ hybridization is time demanding, offering limited flexibility and sensitivity. In this report, we describe a fast, highly sensitive, and specific detection of RNAs using confocal imaging of RNAscope® technology in frozen brain slices collected year-round from domesticated canaries; a species that shows seasonal variation in song behaviour. This method allowed the accurate location of mRNAs at the single cell level, and at the same time allowed their co-localization with neuron type-specific markers. Through the use of this technique, we detected the presence of androgen and estrogen receptors. These observations allow us to postulate which neuron types are responsive to hormones.

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Poster

406. Advances in the Neural Basis of Birdsong

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Program #/Poster #: 406.05/RR5

Topic: F.01. Neuroethology

Support: MWU Faculty Startup

Title: Age and sex differences in vitamin A signaling across the songbird vocal circuit

Authors: *C. R. OLSON, V. PRASAD, R. M. POTTER
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Abstract: Vitamin A is a diet-derived hormone that is converted to retinoic acid in the brain to potentiate a number of memory-related brain functions. When exposed to deficits or excesses during the juvenile vocal learning critical period, zebra finches experience detrimental effects on the learning of their songs. Through differential display and in situ hybridization, previous

studies have found expression of ALDH1A2, the terminal enzyme for retinoic acid synthesis, in the forebrain vocal nuclei HVC (a proper noun) and LMAN (the lateral magnocellular nucleus of the nidopallium), but not expressed in surrounding tissue or other nuclei in the vocal circuit. This view suggests a restricted role of vitamin A signaling in a subset of the vocal circuit. Here we report the immunohistochemical labeling of ALDH1A2, and show more wide-spread vitamin A signaling across the vocal circuit. We found this enzyme present in axonal projections and target vocal nuclei outside its areas of expression, including the striatal nucleus Area X and RA (the robust nucleus of the arcopallium). This represents an important mechanism by which complex learned behaviors are regulated through vitamin A signaling across distant functional domains of the nervous system. Furthermore, sex and age-related changes in the ALDH1A2 transport and signaling may be related to the establishment of vocal brain nuclei during development or to critical periods of juvenile vocal learning.

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Poster

406. Advances in the Neural Basis of Birdsong

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Topic: F.01. Neuroethology

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Title: Changes inSCN4B and the resurgent sodium current during a critical period may be important for the production of crystalized song in adult zebra finches

Authors: *B. ZEMEL[1], P. V. LOVELL[2], S. R. FRIEDRICH[3], C. V. MELLO[2], H. P. VON GERSDORFF[4]
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Abstract: How the brain develops the ability to acquire and produce learned vocalizations is not understood. Zebra finches, unlike rodents and non-human primates, produce learned vocalizations, and thus are a model for studying this phenomena. Like other vocal learners, finches acquire their vocalizations in two phases: a sensory phase, in which a tutee memorizes a tutor song, and a sensory-motor phase, in which the tutee refines it's vocalizations to match those of the tutor. Finches are initially non-vocal, but later enter a critical period in which vocalizations are increasingly refined from the babbling of unstructured notes known as subsong, to a more

structured, yet plastic song that becomes increasingly stable and reproducible in a process known as crystallization. In order to produce crystallized song the vocal nuclei must develop and maintain reliable and robust electrical signals. Little is known about the molecular determinants underlying the intrinsic excitability of these vocal nuclei. In this study, we examined the developmental expression of ion channel subunits that we hypothesize enable fast and reliable firing of action potentials in nucleus RA, a nucleus that is required for the production of learned vocalizations in songbirds. We found that the voltage-gated sodium channel beta 4 subunit, Navβ4, undergoes dramatic developmental changes in expression. This subunit is associated with resurgent sodium currents in several systems where it enables neurons to fire at high frequencies. Thus, we hypothesized it might be critical for maintaining fast and reliable firing in RA neurons. We performed *in situ* hybridizations and patch-clamp slice recordings in RA. *In situ* hybridization reveals expression of Navβ4 mRNA in the RA of adult male finches, with little to no expression in the surrounding arcopallium. In sharp contrast, this subunit shows no expression in the RA of post-hatch day 20 juvenile males, which cannot vocalize. Whole-cell voltage clamp recordings in adult males revealed resurgent sodium currents from cells within RA, but not the surrounding arcopallium, which demonstrate the functional expression of Navβ4 protein. In addition, whole-cell current clamp recordings reveal that RA projection and interneurons fire APs at higher frequencies than neurons from the surrounding arcopallium. These results indicate that Navβ4 is not only developmentally regulated, but may also contribute to intrinsic firing properties of adult, but not juvenile RA neurons. Together, these findings reveal evidence for a development program of regulated gene expression that is implicated in the maturation of cellular properties important for producing adult birdsong.

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Poster

406. Advances in the Neural Basis of Birdsong

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MEXT/JSPS JP17K19629

Title: Cell type specific investigation of corticalstriatal premotor neurons in song learning

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Abstract: Complex vocalizations like human speech are formed by a sequence of precise vocal movements, both acoustically and in temporal order. The learning of such vocal motor sequences depends in mammals on the motor cortex and basal ganglia. Such areas develop parallel temporal sequence representations over learning practice, but it remains unknown how necessary for vocal motor skill learning the coordination between cortical and basal ganglia activity is. Studying this question has suffered several drawbacks, as mammalian motor cortices serve multiple motor skills and most experimentally tractable mammals do not exhibit vocal learning. To solve this issue, songbirds can be used as a model, as they develop their songs along easily quantifiable vocal motor skill learning and possess a specialized neuronal circuit for song learning. This circuit has cortical-like (nucleus HVC) and basal ganglia (Area X) components, with a neuronal type connecting from the cortical to the basal ganglia areas: the HVCx neuron population. We investigated the function of the cortical-basal connection by ablating the HVCx cells and analyzing the effects on the young birds' song learning process. In order to cell-specifically ablate the HVCx neurons, a Cre-dependent toxin system was expressed in the target cells using a combination of Adeno-Associated Vectors. This cell-specific ablation was performed on juvenile Zebra finches before the start of subsong and exposed them to playback of the same tutor song in sound isolation boxes alongside control virus-injected juveniles. Severe HVCx ablation, with one third of HVCx cells remaining, caused a failure of the song to crystallize its sequence and acoustics both remaining highly variable until 200 days post hatch, a time point when normal birds have crystallized their songs. Less severe ablation, with one half remaining, allowed for the emergence of stereotyped syllables and motif sequence, albeit with reduced acoustic similarity to the tutor song. The results suggest that HVCx neurons are important for the robustness of song learning, understood as the development of acoustically and sequentially stable birdsongs.

Disclosures: M. Sánchez-Valpuesta: None. **Y. Shibata:** None. **N.C. Asogwa:** None. **K. Wada:** None.

Poster

406. Advances in the Neural Basis of Birdsong

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 406.08/RR8

Topic: F.01. Neuroethology

Support: HHMI
Rockefeller University

Title: DNA methylation: A contributing mechanism for specializing vocal learning brain circuits

Authors: *C. GILBERT[1], G. GEDMAN[1], L. CANTIN[1], T. CARROLL[1], E. D. JARVIS[1,2]
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Abstract: Vocal learning is the ability to produce novel vocalizations based on an auditory input, and it forms the basis of human language acquisition. Not surprisingly, vocal learning is a rare trait, as only five groups of mammals (cetaceans, pinnipeds, elephants, bats, humans) and three groups of birds (songbirds, parrots, and hummingbirds) have evolved it independently. Strikingly, when examined, the behavior, neuroanatomy, and specialized gene regulation in vocal learning brain circuits are convergent. Microarray and RNA-seq comparative studies between vocal learning and vocal non-learning birds with humans and vocal non-learning non-human primates in our lab have found over 100 convergent differentially expressed genes in the vocal learning circuits relative to non-vocal learning circuits and vocal non-learners. These genes are enriched in neural connectivity, neural protection, and synaptic plasticity functions. I hypothesize that one potential driver for these brain specializations in these genes is DNA methylation, a known mechanism to cause differential gene expression between brain regions and cell types, in mammals. To test this hypothesis, we examined whether differential DNA methylation exists in zebra finch (a songbird) vocal learning brain regions. We used whole-genome bisulfite sequencing (WGBS) to analyze the methylation patterns of cells in the RA song nucleus relative to the adjacent non-vocal motor learning regions (lateral arcopallium, LAI). In concert with ongoing RNA-Seq and ChIP-Seq studies, pilot WGBS studies show that some of the differentially expressed genes in RA versus LAI are also differentially methylated. Notably, the specialized DNA methylation patterns were often present in a non-CG methylation (mCH) context, which is known to be important for mammalian neuronal development and cellular differentiation. These findings indicate the differential DNA methylation could be contributing to specialization of gene regulation and thereby circuitry and function of vocal learning brain circuits.

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Poster

406. Advances in the Neural Basis of Birdsong

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Program #/Poster #: 406.09/RR9

Topic: F.01. Neuroethology

Support: F31MH110209

Title: AAV-siRNA targeting miR-128 enhances learned vocal communication

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Abstract: Currently, no pharmacological interventions exist for vocal communication deficits arising from autism spectrum disorder (ASD) or intellectual disability. This is largely due to the lack of pre-clinical models with construct validity, since learned vocal communication cannot be modeled in rodents or cell culture. Fortuitously, recent advances in songbird genetics show that gene expression in the striatum of songbirds is highly similar to that in humans, indicating evolutionary convergence of the molecular mechanisms that govern vocal learning.
Within a recently published data set from our lab we identified ~1000 human autism and intellectual disability-related genes that are expressed in the striatal song control nucleus, Area X, during the developmental critical period for song learning. These genes are part of larger transcriptional modules that are correlated to key features of learned vocal behavior, including several that are significantly enriched for high confidence autism-related genes.
We have identified a compound that remediates learned vocal communication deficits in songbirds as follows: We used a social isolate paradigm to generate songbirds with impaired vocalization. Isolated birds were returned to their social environment well after the normal critical period closure then treated with oral ginsenoside Rh2 (GRh2) or vehicle for four weeks. Birds that received vehicle failed to organize their syllables into stable sequences, whereas GRh2 treatment enhanced syllable sequencing. Moreover, acute administration of GRh2 altered expression of multiple autism-related genes in Area X including *KMT2C, CACNA2D3,* and others.
In cultured human glioma cells, GRh2 treatment alters levels of miR-128, a brain-enriched microRNA that increases *in vivo* over development in songbirds and humans. Aberrant elevation of miR-128 is associated with autism in humans, suggesting a potential role in social communication. We used a siRNA sponge designed to decrease miR-128 levels in Area X during the critical period for song learning. Strikingly, bilateral injection of the sponge into Area X enhanced learned vocal sequencing in young songbirds relative to scramble controls. Ongoing work will characterize the molecular effects of the AAV constructs. These preliminary results suggest that the molecular mechanisms underlying speech and language can be pharmacologically and genetically targeted to accelerate the development of novel therapeutics for disorders like autism and intellectual disability.

Disclosures: C.M. Aamodt: None. **S.A. White:** None.

Poster

406. Advances in the Neural Basis of Birdsong

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Program #/Poster #: 406.10/RR10

Topic: F.01. Neuroethology

Support: HHMI

Title: “Enhancing" vocal learning: Gene regulatory specializations in song circuits

Authors: *L. CANTIN[1], M. WIRTHLIN[2], J. CAHILL[1], G. GEDMAN[1], C. GILBERT[1], A. PFENNING[2], E. D. JARVIS[1,3]
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Abstract: Vocal learning is a rare trait shared by a small number of distantly related species, including songbirds, parrots, and hummingbirds among birds, and humans, cetaceans, bats, elephants and pinnipeds among mammals. As a result of convergent evolution, these species (at least as studied in birds and humans) share similar vocal learning behavior development and analogous vocal learning brain circuitry with similar specialized gene expression profiles. A previous study from our groups identified a distinct set of ~50-70 convergent specialized genes between the human laryngeal motor cortex (LMC) and the songbird robust nucleus of the arcopallium (RA), and between the human anterodorsal striatum and the songbird Area X when normalized to their surrounding non-song/speech motor regions. When disrupted, some of these genes in humans are associated with communication disorders. Non-coding regulatory regions usually control the expression of genes. Identifying and comparing these regions across vocal learning species may provide clues into the molecular basis for evolution of this complex trait and into the genetic causes of the associated disorders. We performed Native ChIP-seq, ATAC-seq, bisulfite-seq and comparative genomic experiments to identify non-coding regulatory regions of these gene in the song learning nuclei of songbirds. We found several hundred open chromatin H3K27ac peaks with differential activity between the songbird RA or Area X and the surrounding motor regions, including those associated with specialized genes identified in our RNA-seq experiments. We aligned the genomes of avian vocal learning species and their closest vocal non-learning relatives to test for possible convergent accelerated non-coding regions that may be playing a role in the gene specializations of avian vocal learning lineages. We found accelerated regions overlap with the differential chromatin activation peaks of genes with specialized gene expression in song learning nuclei. Future studies will focus on similar analyses of post-mortem human brains. Our findings suggest that convergent changes in regulation of specific sets of genes in vocal learning brain regions may have been associated with convergent accelerated changes in gene expression enhancers of those genes.

Disclosures: L. Cantin: None. **M. Wirthlin:** None. **J. Cahill:** None. **G. Gedman:** None. **C. Gilbert:** None. **A. Pfenning:** None. **E.D. Jarvis:** None.

Poster

406. Advances in the Neural Basis of Birdsong

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Topic: F.01. Neuroethology

Support: NIH R01NS104925
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Nvidia GPU to lab of Samuel Sober

Title: A combined convolutional-recurrent deep neural network for accurate annotation of large birdsong datasets

Authors: *D. A. NICHOLSON[1], T. J. GARDNER[2], Y. COHEN[2]
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Abstract: Songbirds provide an excellent model system for investigating how the brain learns and controls motor skills. Because they sing spontaneously, songbirds produce terabytes of behavioral data. However, many analyses require labeling the elements of song, called syllables. Labeling syllables by hand consumes many hours, and labeling all the song is often infeasible, preventing full analysis of this data.
This bottleneck calls for the development of automated tools (Kogan Margoliash 1998, Tchernichovski et al. 2001, Wu et al. 2008). Many analyses are easily automated when applied to song that contains highly stereotyped syllable sequences, such as that of the commonly studied zebra finch. However such methods are challenged by songs with variable syllable sequences, such as the songs of Bengalese finches or canaries. Several groups have demonstrated accurate labeling of Bengalese finch syllables with supervised machine learning (Troyer 2012, Tachibana et al. 2014, Nicholson 2016). These methods depend on features extracted from syllables, which are segmented from song by threshold crossings of amplitude. Such methods are impaired when segmentation fails because of background noise or experimental manipulations.
One remedy to this issue is to use an algorithm that both segments song into syllables and then classifies those syllables, such as a neural network (Koumura Okanoya 2016). Here we present a neural network architecture which uses convolutional layers to learn features of song syllables from spectrograms, and uses recurrent layers to capture temporal correlations in song. Using a repository of Bengalese Finch song to benchmark (https://figshare.com/articles/BirdsongRecognition/3470165), we show our network approximately halves the previously-reported lowest error rate, using half the training data. With another repository (https://figshare.com/articles/Bengalese_Finch_song_repository/4805749), we

show the network's predictions are accurate on thousands of songs recorded across days. Current tests of the network with canary song suggest it also accurately labels thousands of songs from individuals of this species, in spite of the species' lengthy song bouts and large repertoire of song syllables. To make the network easy to install, test, and use, we have developed a Python package built on Tensorflow, and made the code available here: https://github.com/yardencsGitHub/tf_syllable_segmentation_annotation. We conclude that we have provided an easy-to-use method that is robust across species of songbird. Beyond birdsong, our method may also be applicable to other vocalizations, like those of bats, dolphins, and marmosets.

Disclosures: D.A. Nicholson: None. **T.J. Gardner:** None. **Y. Cohen:** None.

Poster

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Topic: F.01. Neuroethology

Support: NIH Grant R24 GM120464
NSF Grant 143602
NIH Grant R21 DC014432

Title: Redefining the convergent brain transcriptome for human speech and birdsong

Authors: *P. V. LOVELL, C. V. MELLO
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Abstract: Song learning birds and humans share the rare ability to acquire their vocal sounds by imitation. In humans, this trait (vocal learning) subserves the acquisition and production of speech and language. Remarkably, humans and three orders of birds (i.e. songbirds, hummingbirds, parrots) share a striking number of parallels with regards to vocal learning, including the presence of infant babbling, dialects, a need for intact hearing, and in several cases a critical period. Such parallels long hinted that similar parallels might be found in the neural substrates underlying vocal learning. Indeed, the vocal control systems of humans and vocal learning birds have been found to share a number of important features. These include a specialized cortico-striatal-thalamo-cortical loop for vocal acquisition, and a robust direct projection from cortical vocal motor areas (i.e. laryngeal motor cortex - LMC - in humans, analogs of the robust nucleus of the arcopallium - RA - in birds) to brainstem circuits controlling the muscles of the vocal organ (i.e. the larynx in humans, syrinx in birds). By analyzing microarray datasets, Pfenning et al. (2014) found that human LMC and songbird RA also share a set of 55 genes representing convergent molecular specializations, as well as another 78 genes

representing molecular specializations shared by human putamen (Put) and striatal area X in songbirds. In a recent study defining the constitutive transcriptome of the zebra finch song control system, Lovell et al. (2018) reanalyzed the microarray datasets from the Pfenning study, and found a number of discrepancies that warranted further analysis. After removing ~27% of probes deemed non-informative based on poor genomic alignments, correcting or annotating ~10% of previously mis- or un-annotated probes, and redefining p-value cutoffs based on a large collection of *in situ* patterns (ZEBrA), high-confidence sets of shared LMC/RA markers and X/Put markers were defined. *In situ* hybridization verified this more complete analysis of microarray data, confirming >90% of RA markers, but also defining a subset of markers that also label a dorsal intermediate arcopallial domain lateral to RA that has previously been shown to be active during flight. This latter finding suggests that while RA is a highly specialized for song production, some of it's properties may play a broader role in circuits that govern more general motor behaviors. Lastly, a deep-dive into the literature and further bioinformatics analysis provided a better understanding of the biological roles that shared genes and pathways may play in the organization and function of vocal pathways in vocal learning species.

Disclosures: P.V. Lovell: None. **C.V. Mello:** None.

Poster

406. Advances in the Neural Basis of Birdsong

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Program #/Poster #: 406.13/RR13

Topic: F.01. Neuroethology

Support: IOS EDGE: 1645199

Title: NSF EDGE consortium transgenic songbird project: Developing gene manipulation tools and resources for a vocal learning species

Authors: *T. VELHO[1,2], A. KEYTE[3], A. Y. M. SOARES[2], M. T. BIEGLER[4], P. V. LOVELL[5], C. V. MELLO[5], C. LOIS[6], E. D. JARVIS[3]
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Abstract: Studies in zebra finches, a songbird species, have made seminal contributions to our understanding of the behavioral and neural basis of vocal learning, a rare that subserves speech acquisition in humans. However, a deeper understanding of the molecular mechanisms of vocal learning has been hindered by the limited availability of gene manipulation tools, including the ability to generate transgenic birds. Here we provide an update on the activities carried out by

our collaborative consortium funded through an NSF EDGE grant. We have made progress in 3 main lines of research: 1) We have generated mosaic zebra finches that express the genome-editing enzyme Cas9, using our proven method of injecting VSVg-pseudotyped lentiviruses into freshly-laid fertilized eggs. These Cas9 constructs are expressed ubiquitously or are regulated by the recombinase CRE. We are currently screening the resulting mosaics for germline transmission. These Cas9 lines are expected to have broad applicability, including brain gene manipulations through local injections of viral vectors with guide RNA (gRNA) constructs. 2) We have further developed protocols for isolating and culturing zebra finch primordial germ cells (PGCs). Our early stage cultured PGCs are transfectable and are able to incorporate into the embryonic gonads of recipient embryos when injected in the blood stream. Our goal is to create PGC-mosaics that can differentiate and generate genetically modified germ cells that can be transmitted. Being able to isolate and culture PGCs that become germ cells is expected to increase the efficiency and speed of generating zebra finch transgenic lines. Manipulating PGCs *in vitro* would also allow for more targeted gene manipulations in transgenic lines such as knockins and knockouts. 3) We have further developed efficient viral vectors for manipulating genes in zebra finch cells. Direct viral injections in tissues or PGCs of birds from research lines 1) and 2) could then be used to perform intersectional approaches such as with CRE-gRNA (with tissue specific promoters) or Cas9-gRNA vectors. Towards this goal, we are working on developing and testing new viral strains for efficient gene delivery into zebra finch cells, including screening a mutagenized AAV capsid library to identify capsids conferring high infectivity as well as for regional and cell-type specificity in the brain. Overall, we believe this consortium effort will help developed tools for more advanced genetic manipulations in a vocal learning species.

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Poster

406. Advances in the Neural Basis of Birdsong

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Program #/Poster #: 406.14/DP08/RR14

Topic: F.01. Neuroethology

Support: NIH Grant R24 GM120464
NSF Grant 143602
NIH Grant R21 DC014432

Title: Zebra finch Expression Brain Atlas (www.zebrafinchatlas.org): Update and recent analyses

Authors: *C. V. MELLO, P. V. LOVELL
Dept. of Behavioral Neurosci., Oregon Hlth. and Sci. Univ. Sch. of Med., Portland, OR

Abstract: The Zebra finch ExpressionBrain Atlas (ZEBrA; www.zebrafinchatlas.org) is a publicly accessible online resource for investigating the brain distribution of genes of relevance to the development and physiology of functional brain circuits in songbirds. It consists of an *in situ* hybridization database for transcripts expressed in the brain of adult male zebra finches (*T. guttata*), a songbird species and the major model organism for studying vocal learning. ZEBrA's major features and recent updates include: (1) The *In situ* database - an expanding collection of high-resolution (0.46 µm/pixel) digital images presented along with annotated drawings from a reference Histological Atlas. ZEBrA currently houses >2,000 images (>100 GB) corresponding to ~660 brain expressed genes, including markers of all major nuclei that comprise the song system. Notably, in 2018 we have added several dozen image series for genes that are either convergent molecular specializations of cortical vocal motor areas shared between vocal learning birds and humans, or linked to speech and language functions and disorders in humans. (2) A reference Histological Atlas Browser - A set of 18 annotated drawings prepared in registration with Nissl- and Myelin-stained images of sagittal brain sections derived from the Karten/Mitra atlas. (3) Homepage portals that highlight and provide fast access to genes of great interest to songbird biology (e.g. song system markers), human speech and language disorders (e.g. FOXP2 and targets), human neurological diseases (OMIM-based), rodent behavioral and neurological phenotypes (MGI-based), comparative neuroanatomy (e.g. markers from the Allen's mouse brain atlas), and gene functions. Recent uses of ZEBrA include: (1) validation and establishing cut-off criteria for microarray studies of the song system; (2) quantitative analysis of regional gene expression patterns to define molecular signatures of specific avian brain areas; (3) obtaining evidence of heterogeneity of cellular patterns of gene expression as a basis for cellular phenotyping within vocal control nuclei; (4) obtaining molecular data to characterize the internal organization of major avian brain subdivisions (e.g. arcopallium, mesopallium, hippocampus).

Disclosures: C.V. Mello: None. **P.V. Lovell:** None.

Poster

406. Advances in the Neural Basis of Birdsong

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Topic: F.01. Neuroethology

Support: MEXT KAKENHI 17H06063 (YY)
NSERC 402417-2011 (JTS)
FRQ-NT PR-189949 (JTS)

Title: Norepinephrine in auditory processing areas enhances auditory learning and responsiveness in developing songbirds

Authors: *Y. CHEN[1], S. YANAGIHARA[3], Y. YAZAKI-SUGIYAMA[3], J. T. SAKATA[1,2]
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Abstract: Auditory learning serves as the foundation for vocal learning and imitation. Catecholamines such as norepinephrine (NE) have been implicated in various forms of sensory learning but the extent to which they contribute to sensory learning for vocal learning remains largely unknown. Further, little is known about how NE modulates auditory processing during the period of sensory and vocal learning. We investigated how NE impacted the sensory learning of vocal communication signals ('songs') and auditory responses to song in juvenile zebra finches, a species that learns its vocalizations during a sensitive period in development. We previously demonstrated that NE-synthesizing neurons in the locus coeruleus (LC) express more immediate early genes under conditions that promote developmental song learning (Chen et al., 2016), suggesting that NE could facilitate the sensory learning of song. NE-synthesizing neurons in the LC project to parts of the avian auditory cortex that are important for the sensory learning of song, including the caudomedial nidopallium (NCM; Yanagihara and Yazaki-Sugiyama, 2016). To determine the extent to which NE acting within the NCM modulates the sensory learning of song, we infused NE or vehicle into the NCM of juvenile zebra finches as they were briefly tutored with song. Relative to control birds that were infused with vehicle, birds that were infused with NE in the NCM during song tutoring produced songs as adults that were significant more similar to their tutor song. Furthermore, birds given NE during tutoring (but not control birds) showed evidence of rapid song learning (i.e., within days of tutoring). To investigate how NE affects neurophysiological responses to song in developing songbirds, we infused NE into the NCM while recording single unit activity in response to song playbacks. In general, following NE administration, NCM neurons displayed stronger and more temporally precise auditory responses as well as decreased spontaneous firing. Taken together, our data indicate that NE acting within the NCM significantly and rapidly promotes the sensory learning of song and could do so by potentiating auditory responses to song.

Disclosures: Y. Chen: None. **S. Yanagihara:** None. **Y. Yazaki-Sugiyama:** None. **J.T. Sakata:** None.

Poster

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Topic: F.03. Neuroendocrine Processes

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NIH R01 NS082179

Title: Distribution and physiology of dopamine D1 receptors in the songbird secondary auditory cortex

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Abstract: Midbrain dopaminergic projections to the cortex are essential for learning of goal-directed behaviors. In mammals, projections to prefrontal regions govern decision-making, for example, by increasing the drive to seek rewards. Dopaminergic projections to sensory cortices are likely involved in improving and fine-tuning stimulus-response relationships. In humans, dopamine is thought to be central for speech learning and songbirds provide a prominent model for studying auditory learning. In songbirds, a secondary auditory cortical structure, the caudomedial nidopallium (NCM), is thought to play a significant role in the perception of song as well as in the association between songs and behaviorally relevant consequences. Furthermore, NCM is enriched with aromatase (estrogen synthase) and is a site of action of neuromodulators including estradiol and norepinephrine. Despite the abundance of dopamine D1 receptor mRNA in NCM, the presence and distribution of its protein, as well as its physiology has not been explored. Thus, in the present work we analyze the protein distribution and the physiology of dopamine D1 receptors in NCM. First, we validate a primary antibody against D1 receptors in zebra finch telencephalon and use tissue immunofluorescence to show that D1-receptor containing neurons colocalize with the majority of GABAergic neurons and virtually all parvalbumin-GABA positive neurons. Additionally, we observe that more than half of aromatase-positive neurons contain D1 receptors. Third, in whole-cell patch clamp experiments, we observe that the D1 agonist SKF-38393 decreases the amplitude of spontaneous inhibitory post-synaptic currents (under excitatory neurotransmission blockade) and also increases the frequency of spontaneous excitatory NMDA currents (under inhibitory neurotransmission blockade). Our data indicate that D1 receptor protein is abundant in NCM and associated with GABA- and estradiol-producing cells, and that it also exerts effects on both GABAergic and NMDA currents. This suggests that NCM is an important target of dopamine modulation and a site of interaction between neuroestradiol and dopamine, which could be key to NCM's role in auditory learning and memory. Support from NSF IOS 1354906 and NIH R01 NS082179.

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Poster

406. Advances in the Neural Basis of Birdsong

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Topic: F.01. Neuroethology

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Agencia Nacional de Promoción Científica y Tecnológica (Agentina) PICT 2014-1802
University of Buenos Aires (Argentina)

Title: Synchronized cortical activity in auditory perception in canaries

Authors: *S. BOARI, G. B. MINDLIN, A. AMADOR
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Abstract: Songbirds have been the focus of neuroethological research of the principles underlying vocal production, perception and learning. The 'song system' is a network of neural nuclei of a songbird's brain dedicated to these tasks. Telencephalic nucleus HVC is involved in the generation of the motor commands for vocal production and receives highly processed auditory inputs. In asleep or anesthetized adult male zebra finches (*Taeniopygia guttata*), HVC selective single units respond to a playback of the bird's own song (BOS). This elicited auditory response presents a similar firing pattern to the one measured while the bird is singing, suggesting a shared coding mechanism for the motor production and sensory perception of the BOS. For singing zebra finches, a recent study showed that HVC local field potentials (LFP) present defined oscillations in the 25-35 Hz band (Markowitz et al. 2015). While a correlation between LFP features and neuron firing was reported, it remains to be seen whether a link between LFP rhythmicity and song features can be established.
In this work, we studied the auditory responses to BOS at HVC in urethane-anesthetized male adult canaries (*Serinus canaria*). Differently than the highly stereotyped song of zebra finches, canaries present song bouts that are longer and more variable, composed of subunits called phrases. Each phrase is, in turn, composed by the repetition of the same syllable, with repetition rates that range from 2 Hz to 35 Hz. For this species, the BOS was determined by assessing the most probable song bout within the bird's natural repertoire. Acute extracellular neural recordings were conducted at HVC with 32 channel multi-electrode arrays, which provided the possibility to study spatial information together with temporal modulations of the neural activity. We recorded the LFP at different HVC sites and depths. As was reported for singing zebra finches, we have found that the LFPs at HVC present rhythmic oscillations. In this case, we found them to be locked to the syllabic rate of different song phrases, which could provide a new

perspective on the study of the neural coding of auditory processing of song and its link to vocal behavior in songbirds.

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Poster

406. Advances in the Neural Basis of Birdsong

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Program #/Poster #: 406.18/SS4

Topic: F.01. Neuroethology

Title: Premotor control of coordinated vocalizations in zebra finches

Authors: *J. BENICHOV, S. WILCZEK, D. VALLENTIN
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Abstract: Our social lives, and those of many animals, largely rely on the capacity for interactive vocal communication. The male zebra finch, for instance, sings a learned song to court females, who do not sing. Both sexes, however, produce and coordinate acoustically simple short calls. Although the neural principles underlying song production have been studied extensively, the brain mechanisms that enable the coordination of vocal interactions, as in duets or turn-taking, remain unknown. We address this topic in zebra finches as they exchange social calls. To do so, we implement a vocal robot that flexibly engages birds in antiphonal call interactions. Using this paradigm, we have previously shown that birds dynamically control the timing of their calls with reference to a vocal partner. While subcortical brain regions are sufficient for the production of calls, we and others have demonstrated that cortical vocal structures are involved in and necessary for precise call coordination. Here, we begin to identify the neural mechanisms that underlie the coordination of vocal interactions by combining pharmacological perturbation and intracellular recordings in zebra finches during interactions with the vocal robot. Specifically, we focus on the cortical premotor nucleus HVC (proper name) which is known to control the timing of song. Our results suggest that HVC is necessary for the temporal coordination of calls during social interactions. We also found that pharmacological manipulation of HVC affects call structure. Furthermore, we demonstrate that individual cells within HVC can play multiple roles: HVC premotor neurons generate learned song and long calls, and also initiate non-learned social calls. These findings support the notion that a highly specialized cortical vocal motor pathway supports developmental vocal motor learning as well as dynamic vocal regulation for social interactions.

Disclosures: J. Benichov: None. **S. Wilczek:** None. **D. Vallentin:** None.

Poster

406. Advances in the Neural Basis of Birdsong

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 406.19/SS5

Topic: F.01. Neuroethology

Support: NSF IOS 0917918

Title: Roles of sensory and motor activity in coordinating a cooperative behavior between female and male wrens

Authors: *M. J. COLEMAN[1], N. F. DAY[2], P. RIVERA PARRA[3], E. S. FORTUNE[4]
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Abstract: Cooperation between animals requires that individuals integrate sensory information produced by partners to modulate their own behavioral output. We investigated how sensory cues generated by each partner modifies motor programs in the brain to achieve tightly coordinated cooperative performances. Female and male plain-tailed wrens (*Pheugopedius euophrys*) alternate vocal output to produce duet songs that sound as if a single bird is singing. These duets are well-suited for studying the mechanisms for coordination of behavior because duetting can rely on a single sensory modality (audition), use sex-specific syllable types, and involve the rapid (on the order of 10s to 100s of milliseconds) interchange of information between females and males. This rapid exchange requires self-generated 'autogenous' auditory feedback, and auditory cues from the partner, known as 'heterogenous' feedback. To understand the roles of both autogenous and heterogenous feedback we recorded chronically from HVC, a sensorimotor area of the songbird, from duetting wrens. Subsequently, we anesthetized both birds with urethane and measured HVC responses to playbacks of their duets. Unexpectedly, heterogenous cues did not generate changes in HVC activity in awake birds. In males, activity during singing occurred during autogenous syllable production, but shifted to responding to playback of female syllables when under urethane anesthesia. In contrast, female activity also occurred during autogenous syllables in awake birds, but HVC neurons responded equally well to both autogenous and heterogenous syllables. These data demonstrate that female and male wrens use different neural mechanisms for coordinating the shared duet performance. Further, these data suggest a role for HVC in the coordination of the duet performance.

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Poster

406. Advances in the Neural Basis of Birdsong

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Topic: F.01. Neuroethology

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NSF - IGERT Traineeship
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Title: Mating posture and female choice in songbirds

Authors: *A. PERKES[1], C. MESSIER[1], M. WILD[1], B. PFROMMER[2], M. F. SCHMIDT[1]
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Abstract: Female cowbirds produce a copulation solicitation display (CSD) in response to male courtship, both inviting and facilitating copulation. This behavior can be generated with song playback under isolation, where the frequency with which females produce CSD reveals the relative potency of male song. The highly selective nature of the behavior makes this species a valuable model for studying sexual preference, but the mechanisms driving selectivity are still poorly understood. We have previously shown (Maguire et al. 2013) that targeted lesions to the forebrain nucleus HVC cause a loss of CSD selectivity. While this suggests a testable model for modulating sexual preference, no precise method exists for quantifying or comparing postures, and the connectivity of HVC is poorly characterized in the female songbird.
Our findings reveal that the overall connectivity pattern of HVC is identical in females to that observed in males, even though females do not sing. To quantify CSD, we use an array of cameras to record birds with high temporal and spatial precision, combined with computer vision approaches to reconstruct posture. Given a quantitative model of bird pose, we can observe intrinsic variability in posture and whether this variability correlates with song preference. We can then observe the neural correlates of the CSD behavior, as well as measuring the impacts of lesions to downstream targets of HVC. We hypothesize that this circuit serves the broader function of controlling courtship behavior and selectivity rather than driving song learning and production alone.

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Poster

406. Advances in the Neural Basis of Birdsong

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Topic: F.01. Neuroethology

Support: NSF IOS CAREER 1453084

Title: From perception to action: Sensory cortices and motor pathways underlying female mate choice

Authors: *J. F. PRATHER[1], K. S. LAWLEY[1], J. L. DUNNING[2]
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Abstract: Females of many species use male courtship displays as a proxy of male fitness to inform decisions of mate choice. Female mate choice has been studied extensively in songbirds in which females identify males based on their songs, evaluate the quality of those songs, and use that information to guide their mate selection. Song plays a central role in that choice, as female songbirds will exhibit copulatory behaviors (i.e. copulation solicitation displays (CSDs) and calls) in response to songs played through a speaker, even when no male is physically present. Studies of female response to song have implicated auditory cortical regions such as the caudal mesopallium (CM) and the caudal nidopallium (NC) in perception of song quality and the associated influence on production of copulatory behaviors. Here we are employing a combination of excitotoxic lesioning, anterograde and retrograde pathway tracing, and optogenetic stimulation to investigate: 1) the role of those cortical areas in song evaluation and mate choice, 2) the connectivity of those areas with other cortical sites and motor sites that underlie production of courtship behaviors, and 3) the role of specific pathways in specific aspects of song evaluation and mate selection. Our preliminary results from lesion studies demonstrate roles for each of CM and NC in female evaluation of song quality. In ongoing experiments, those results are confirmed and extended to specific pathways using optogenetic stimulation. Furthermore, our pathway tracing data reveal projections from each of those cortical sites to areas implicated in behavioral motivation and production of courtship behaviors, demonstrating possible pathways through which sensory perception may influence motor activation. Future experiments will seek to disambiguate the degree to which activity in specific pathways contributes to sensory perception versus motor activation, and thus the degree to which activity in those brain regions contributes to specific aspects of song evaluation and mate selection.

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Poster

406. Advances in the Neural Basis of Birdsong

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Program #/Poster #: 406.22/SS8

Topic: F.01. Neuroethology

Title: Monitoring of social interactions in western jackdaws by a 3D tracking system

Authors: *A. L. VYSSOTSKI[1], S. J. VAN HASSELT[2], G.-J. MEKENKAMP[2], A. N. ABRAMCHUK[3], N. C. RATTENBORG[4], P. MEERLO[2]
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Abstract: Corvids exhibit some of the largest relative avian brain sizes, as well as the most flexible cognition in the animal kingdom. The most striking example of outstanding cognitive abilities of corvids is tool fabrication by New Caledonian crows. Other representatives of this family, ravens and jackdaws are famous for their abilities of vocal imitation, an ability to copy sounds of other birds and even some human words. Crows have fixed dominance ranking helping to decrease stress of group leaving, and jackdaws are known for food sharing, a behavior when one individual gives food to another and can get food reciprocally later. We are interested in social interactions laying the bases of dominance formation, as well as in social interactions associated with solving creative tasks, such as adaptation to novel environments.
Social interactions in animals are mediated by a large variety of signals including body movements and vocalizations. In many species, the exchange of signals is often too fast for direct observation and interpretation by human experimenters. While posthoc analysis of videos can partly overcome this problem, this approach can be time-consuming. We are therefore developing a 3D tracking system that allows for automated and detailed data collection of behavior and brain activity of socially interacting animals.
To study social interactions in jackdaws we equipped birds with small data loggers (Neurologger 3, Evolocus LLC, USA). Each logger, attached to an animal head, recorded brain activity from 28 epidurally implanted electrodes (15.625 kHz), 3D animal position (15 Hz), and head orientation (625 Hz, by 3D magnetometer, 3D accelerometer and 3D gyroscope). Vocalization of animals was recorded by a built in microphone (125 kHz, similarly to Nature Methods, 2014, doi:10.1038/nmeth.3114). Position measurement was based on detection of time of intersection of infrared (IR) planar laser beams with the IR sensor on the logger. Planar laser beams scanning the environment periodically were emitted by four sources in the upper corners of the aviary. This method of tracking achieves sub-centimeter accuracy necessary for determination of relative positions of birds.
We monitored social interactions during habituation to modified environment in two stable

groups consisting of 4 males and 4 females respectively. Then, we observed social interactions initiated by introduction of unfamiliar members to stable social group. Males and females were unfamiliar to each other as they were always kept separately before the experiment. Thanks to advanced 3D tracking we detected complicated dynamics of social interactions and classified interesting behavioral patterns.

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Poster

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Topic: F.01. Neuroethology

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Title: Immediate early gene response to gargle calls of intact and HVC-lesioned black-capped chickadees (Poecile atricapillus)

Authors: *S. K. MISCHLER[1], R. A. FERNÁNDEZ[2], S. A. MACDOUGALL-SHACKLETON[3], C. B. STURDY[1]
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Abstract: Bird calls play a crucial role in social interactions among flock members, whether it be by communicating information about movements, food sources, or an impending predator. Similar to songs, which are used for mate attraction and territory defense, some songbird calls require imitative vocal learning in order to produce them. Songbirds possess a series of discrete nuclei, together known as the song-control system, that are critical for learning and producing both song and learned calls. This circuit includes the brain region HVC (proper name), which when lesioned has an impact on learned call production. Black-capped chickadees are songbirds that produce a variety of highly complex calls used in a variety of contexts, such as the *gargle* call which is an aggressive vocalization used primarily in male-male agonistic encounters. Lesioning HVC in male chickadees alters the bioacoustic properties of the *gargle* call, rendering it acoustically simpler overall. In this study, we measured immediate-early gene (IEG) immunoreactivity to assess whether the auditory forebrain, specifically the caudomedial mesopallium (CMM) and the caudomedial nidopallium (NCM) regions, were similarly active when female birds heard either intact or HVC-lesioned male produced *gargle* calls. Subjects

were either exposed to recordings of intact male *gargle* calls, *gargle* calls produced from HVC-lesioned male chickadees, or pink-noise within the same frequency ranges as these calls. Following playback, brains were processed using immunohistochemistry to visualize IEG protein in the auditory forebrain (CMM and NCM). Chickadees that heard either intact or HVC-lesioned *gargle* calls did not show significantly different levels of IEG activation in CMM or NCM; however, both of these calls elicited more IEG activation than the pink-noise condition. The post-lesion *gargle* calls differed from intact ones by having increased end frequency, decreased peak frequency, and decreased branching in complex notes. Although these types of *gargle* calls were bioacoustically different it appears that they are processed similarly by the female chickadees according to IEG response. Since male chickadees typically produce *gargle* calls and produce them in male-male agonistic interactions, it is possible that the females are not attuned to differences in these calls, thus perceiving them similarly. It is also possible that because the *gargle* call varies greatly across individuals, both in note composition and complexity that differences between HVC-lesioned *gargle* calls and intact *gargle* calls are within the normal range of acoustic variation of *gargle* calls.

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Poster

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Title: Calcium imaging in canary (serinus canaria) HVC reveals latent states supporting behavioral sequencing with long range history dependence

Authors: *Y. COHEN[1], J. SHEN[1], D. SEMU[1], D. P. LEMAN[1], W. A. LIBERTI, III[2], N. PERKINS[1], T. J. GARDNER[1]
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Abstract: Orchestrating sequential movements in real time is required in many tasks. In skills like speech or dance, this control follows syntactic rules with long range dependencies - behavioral transitions that depend on what actions were taken multiple time steps in the past. Songbirds have a learned and naturally recurring behavior, song, whose temporal structure is largely governed by the premotor nucleus HVC (Hahnloser 2002, Long 2008, Wang 2008, Nottebohm 1976). Canary song repertoires are defined by repeated syllables, known as phrases,

whose syntax is controlled separately from the syllables' identity (Gardner 2005) and which exhibit long range order (Markowitz 2013). Specifically, certain phrase transitions have history dependence, originating 2-3 phrases upstream. Accordingly, the neural underpinning of the phrase sequence must either rely on a complex memorized repertoire or maintain historic context to influence ongoing transitions. To discriminate such mechanisms, we expressed the calcium indicator GCaMP6f in projection neurons and used head-mounted fluorescence microscopes to record from HVC in freely behaving canaries.
We find that song history is reflected in identified Ca^{2+} ROI signals (purported single neurons) up to 4 phrases apart — a temporal separation of up to 3 seconds and 40 individual syllables. Examining correlations to past phrase types we find that many ROIs exhibit mixed selectivity — suggesting that the network dynamics does not follow a memorized, but instead reflects historic context relevant to flexible transitions. Moreover, we find that signals, reflecting sequence history information are more frequently found during phrase transitions that are history dependent compared to history insensitive ones - suggesting that the necessary information is indeed carried by hidden network states.
Beyond phrase-restricted signals, we find ROIs with signals that last several seconds and span 3-4 phrases. Such Ca^{2+} changes cannot be explained by syllable type or transition specific HVC projection neuron bursting (Fujimoto 2011). These signals are often not modulated by syllable or phrase boundaries and appear mostly during highly stereotyped phrase sequences. These properties suggest distinct network dynamics during stereotyped and variable phrase sequences. That long order syntax generation is reflected by hidden network states and signals with qualitatively different time scales suggest that behavioral sequencing may be specified by ongoing network dynamics. Investigating the underlying mechanisms may reveal neural substrates that also exist in other motor and cognitive functions such as inference and decision making.

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Poster

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Topic: F.01. Neuroethology

Title: Combining light sheet and expansion microscopy for large scale, high resolution projectomics of the zebra finch brain

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Abstract: Birdsong is a neuroethological model of speech learning and language acquisition (Brainard & Doupe, 2013). A key premotor area of the songbird brain, HVC, plays a major role in song learning and takes part in both song perception and song production (Nottebohm et al., 1990; Brainard & Doupe 2002). Mapping the wide network of afferent and efferent connections of HVC neurons presents an important step towards understanding the neural mechanisms underlying song production and learning. A major challenge for mapping neural circuits is to achieve sufficient optical resolution for identifying morphological fine structures while preserving the topographic information of neural origin. Recent advances in fluorescent light microscopy (LM) imaging allows for imaging beyond Abbe's diffraction limit (Hell, 2007), sufficient to reliably quantify dendritic spines and fluorescently labeled synaptic markers. However, trade offs among acquisition times, resolution, and photo damage hinder investigations of large sample volumes using traditional light microscopy.
To probe for new avenues into circuit-level investigations of the songbird brain, we use single plane illumination microscopy (SPIM) in combination with expansion microscopy (ExM). SPIM improves on both imaging speed and reduced bleaching by virtue of simultaneous excitation and acquisition of entire cross sectional planes of large tissue volumes. However, the quantification of morphological fine structures remains challenging, because the physical characteristics of the excitation light sheet limits the resolution to the micrometer range. Expansion microscopy (ExM) improves on imaging resolution thanks to physical magnification of the brain prior to imaging (Tillberg et al., 2016). Thus, the combination of ExM and SPIM (ExSPIM) enables us to look at intact long range projections in the zebra finch brain while preserving morphological fine structures on the synaptic level.

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Poster

406. Advances in the Neural Basis of Birdsong

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Title: Viral methods for reversible knockdown of speech-related genes in the songbird

Authors: *L. XIAO[1], M. CAO[3], F. GARCIA OSCOS[3], M. CO[2], G. KONOPKA[3], T. F. ROBERTS[3]
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Abstract: Songbirds are the preeminent animal model for studying neural circuit mechanisms for vocal communication and vocal learning. Songbirds learn their songs by imitating adult birds during a developmental critical period, a rare behavioral feat with strong parallels to speech learning. Moreover, knocking down genes, like FoxP2, or disrupting neuronal circuits, like the basal ganglia, that are known to be involved in speech, disrupts birdsong in ways that largely phenocopy speech disruptions in people, illustrating the strong behavioral, circuit and genetic parallels between song and speech learning. However, the translational potential for research in the songbird field has long been hampered by a lack of sophisticated genetic tools. We have developed a new viral method for the reversible KD of genes in songbirds using Cre-switch site-specific recombinase vectors for expression of shRNAs. Cre-switch vectors contain protein coding sequences inserted with inverted orientations relative to each other, downstream of a single promoter. Cre-mediated recombination flips the orientation of the protein coding sequences, switching the protein expressed. We generated shRNAs against FoxP2 downstream of a fluorescent protein in our Cre-switch AAV construct, such that Cre-mediated recombination will turn off expression of the shRNA and change the fluorescent protein being expressed from mCherry to TagBFP. In vitro and in vivo work demonstrates significant knockdown of FoxP2 using this construct and that expression of Cre reliably restores FoxP2 expression levels. Surprisingly, we found that AAV mediated knockdown of FoxP2 in the basal ganglia (Area X) of adult zebra finches caused severe vocal deficits, including changes in syntax, repetition (similar to ‘‘stuttering’’), deletion and creation of individual syllables or strings of syllables. However, these abnormal vocalizations are rescued following restoration of FoxP2 levels in Area X in adult birds. These results suggest a greater role for FoxP2 in adult song maintenance than previously appreciated. This novel viral approach opens the door for dissecting developmental contributions of FoxP2 and examining additional genes implicated in speech development using reversible genetic knockdown techniques.

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Poster

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Title: A neural circuit for renewed motor exploration in birdsong

Authors: *D. ALAM[1], T. F. ROBERTS[2]
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Abstract: Learning of skilled motor behaviors involves consolidation of motor patterns that result in better performance outcomes. This process of consolidation is thought to depend critically on sleep. However, the synaptic circuits contributing to sleep-based consolidation of skilled motor behaviors are still poorly defined. Birdsong is learned during a developmental critical period and provides an experimentally tractable model for examining the mechanisms underlying sleep-based consolidation. Previous studies indicate that birds achieve daily improvements in their song performance during this critical period and that these improvements are consolidated during sleep. This consolidation is thought to permit renewed vocal exploration each morning, which in turn is thought to fuel further improvements in song. The brain regions involved in this sleep-based consolidation and renewed vocal exploration are not known. Here we test the role the cortical premotor song nucleus RA in sleep based consolidation renewed motor exploration. RA is necessary for production of learned song and receives afferents from two functionally distinct premotor cortical circuits, HVC and LMAN. The premotor region HVC is vital for production of learned aspects of song while the premotor region LMAN is necessary for song plasticity. We show that reversible pharmacological silencing of RA during the night is sufficient to disrupt increased vocal exploration in the morning. Nightly silencing of RA does not disrupt vocal practice or reduce normal vocal variability, but does reduce the transient increases in vocal exploration seen during this stage of development. To examine if increased vocal exploration is important to song learning, we tracked changes in similarity to the tutor song over development. We find that nightly silencing of RA does not disrupt consolidation of the previous day's improvements in song, but significantly reduces within day improvements in song similarity. These findings indicate that RA is at least one region where activity during sleep functions to allow renewed plasticity of motor patterns which drive learned improvements in song performance, and supports the idea that activity in premotor cortical circuits during sleep is important for motor flexibility and learning of motor skills.

Disclosures: D. Alam: None. **T.F. Roberts:** None.

Poster

406. Advances in the Neural Basis of Birdsong

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Title: Reafferent thalamo-cortical pathway connects motor and auditory circuits important for song

Authors: *M. Z. IKEDA[1], T. F. ROBERTS[2]
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Abstract: Circuits relaying a copy of descending vocal motor commands back to the auditory system are thought to facilitate vocal learning by allowing comparison of vocal motor goals with performance outcomes. In songbirds, a descending projection from the cortical song nucleus RA controls production of song. RA receives excitatory projections from two vocal-motor cortical regions, HVC and lMAN. HVC is necessary for producing the learned components of song and lMAN, the output structure of a basal ganglia circuit, is necessary for vocal plasticity. A circuit connecting the premotor song nucleus HVC with the auditory region Avalanche (Av) has been recently identified. Consistent with longstanding models, genetic lesions show that this circuit is important for normal song learning and for certain forms of adult vocal plasticity. However, since HVC provides only one arm of the vocal-motor input to RA, the projection from HVC to Avalanche is likely to provide the auditory system with only an incomplete picture of 'vocal motor goals'. Here we ask if circuits emanating from RA's descending projections provide a unique feedback circuit back into the auditory system. A subset of RA neurons that provide descending motor commands for song also send collaterals to the ipsilateral thalamic nucleus DMP (dorsal medial thalamus (DMP)), which in turn projects bilaterally back to the cortex, to the medial magnocellular nucleus of the anterior nidopallium (mMAN). Using dual tracer injections into premotor nuclei and the auditory system we find that the auditory region Avalanche receives a direct projection from mMAN. This indicates that Avalanche receives input from two separate vocal motor sources, a direct projection from the song nucleus HVC and a relayed projection from RA. Future studies involve testing the specific roles of mMAN-Av neurons and mMAN-HVC neurons in varying aspects of song learning and vocal plasticity.

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Poster

406. Advances in the Neural Basis of Birdsong

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Topic: F.01. Neuroethology

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Title: Inception of vocal memories for song syllable timing

Authors: *W. ZHAO[1], F. GARCIA OSCOS[2], T. F. ROBERTS[2]
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Abstract: Songbirds, like humans, learn their vocalizations by imitating their parents or other adults. Juvenile zebra finches learn their courtship song by first memorizing the song of their father and then use this memory to guide iterative changes in their own song. However, the networks and synapses in the brain involved in encoding a memory of the fathers' song are still poorly defined. Previous studies suggest that the premotor nucleus HVC (proper name) is necessary for production of song and for encoding a memory of the tutor song. Moreover, it is thought that tutor song information is relayed to HVC from the cortical nucleus NIf (Nucleus Interface). Using dual tracer injections we confirmed that NIf connects to HVC via two, largely non-overlapping pathways: a primary pathway projecting directly to HVC and a secondary pathway projecting to HVC via another auditory forebrain structure termed Avalanche (Av). To investigate NIf's role in tutor song learning, we manipulated activity in the direct NIf-HVC pathway by injecting an AAV expressing axon-targeted Channelrhodopsin 2 in NIf of juvenile birds prior to tutoring. We then conducted closed-loop experiments in which the optogenetic manipulation of NIf axon terminals in HVC was yoked to the tutor's singing behavior. We found that tutoring-contingent optogenetic manipulation of the NIf-HVC pathway blocks tutor song imitation, indicating that the NIf-HVC pathway is necessary for encoding of tutor song memories. To test the sufficiency of this pathway in tutor song learning we optogenetically manipulated NIf axon terminals in HVC of juvenile birds that were never tutored. Even in the absence of song tutoring, optogenetic activation of the NIf axons in HVC of juvenile birds is sufficient to direct learning of their adult songs. Long light pulses (300ms) or short repetitive light pulses (50ms) in tutor-naïve juveniles resulted in birds singing songs containing either abnormally long syllables, or short trilled notes, respectively. This indicates that NIf inputs to HVC provide temporal information about a 'song model' that is sufficient to guide vocal imitation. Together, these results indicate that the direct NIf to HVC pathway is necessary for learning from a song tutor and that this circuit is also sufficient to guide learning of song syllable timing.

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Poster

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Title: Precise neural sequences for birdsong emerge from functionally distinct networks of premotor neurons

Authors: *V. K. DALIPARTHI[1], B. G. COOPER[2], S. J. SOBER[3], T. F. ROBERTS[4]
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Abstract: Precise neural sequences underlie the voluntary production of well-learned skilled behaviors. A key question is how these neural sequences are initiated and brought to a close to begin and end behavioral performances, and how these transitions relate to changes in descending motor commands and movements. We used cell-type specific calcium imaging from populations of premotor neurons, and recordings from the respiratory system and from cortical output neurons that innervate brainstem vocal and respiratory motor nuclei in songbirds to examine the neural correlates of song initiation and cessation. Premotor projection neurons in the cortical song nucleus HVC (HVC-RA neurons) are necessary for the production of learned song and exhibit precise sequential activity during singing. Imaging from populations of HVC-RA neurons reveals distinct transitions in activity patterns as birds prepare to sing and following song cessation. We characterize populations of HVC-RA neurons that are active immediately preceding and or following song production, but not during singing, or at other times. These neurons predict impending song and also mark silent periods between song bouts. We also identify populations of neurons that increase their activity before song and transition into precisely sequenced activity during singing. Our results suggest that precise neural sequences emerge from transitions among anatomically intermingled, but functionally distinct groups of cortical premotor neurons. Recordings from cortical output neurons in the song nucleus RA and from the respiratory system identify changes in activity that are temporally coincident with peri-song activity observed in HVC, suggesting that peri-song activity results in descending motor commands that help coordinate respiratory patterns as birds prepare to sing and as they recover

from singing-related respiratory exertion. We suggest that transitions between functionally identified groups of HVC-RA neurons may set network dynamics important for initiation and cessation of precise neural sequences and the coordination of respiratory patterns necessary for vocal production. Together, these results shed light on the neural origins of precise neural sequences and how network transitions relate to the initiation and termination of voluntarily produced skilled behaviors.

Disclosures: V.K. Daliparthi: None. **B.G. Cooper:** None. **S.J. Sober:** None. **T.F. Roberts:** None.

Poster

407. Social Communication in Non-Avian Species

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 407.01/TT3

Topic: F.01. Neuroethology

Support: NIH Grant R33MH104188

Title: Forebrain control of behaviorally-driven social orienting in zebrafish

Authors: *S. J. STEDNITZ[1], E. MCDERMOTT[2], D. NCUBE[2], P. E. WASHBOURNE[3]
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Abstract: Deficits in social engagement are diagnostic of multiple neurodevelopmental disorders, including autism and schizophrenia. Genetically tractable animal models like zebrafish (*Danio rerio*) could provide valuable insight into developmental factors underlying these social impairments, but this approach is predicated on the ability to accurately and reliably quantify subtle behavioral changes. Similarly, characterizing local molecular and morphological phenotypes requires knowledge of the neuroanatomical correlates of social behavior. We leveraged behavioral and genetic tools in zebrafish to both refine our understanding of social behavior and identify brain regions important for driving it. We characterized visual social interactions between pairs of adult zebrafish, and discovered that they perform a stereotyped orienting behavior that reflects social attention. Furthermore, in pairs of fish, the orienting behavior of one individual is the primary factor driving the same behavior in the other individual. We used manual and genetic lesions to investigate the forebrain contribution to this behavior and identified a population of neurons in the ventral telencephalon whose ablation suppresses social interactions, while sparing other locomotor and visual behaviors. These neurons are cholinergic and express the gene encoding the transcription factor Lhx8a, which is required for development of cholinergic neurons in the mouse forebrain. The neuronal population identified in zebrafish lies in a region homologous to mammalian forebrain regions implicated in social behavior such

as the lateral septum. Our data suggest that an evolutionarily conserved population of neurons controls social orienting in zebrafish.

Disclosures: S.J. Stednitz: None. **E. McDermott:** None. **D. Ncube:** None. **P.E. Washbourne:** None.

Poster

407. Social Communication in Non-Avian Species

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Program #/Poster #: 407.02/TT4

Topic: F.01. Neuroethology

Support: NIH Grant 1R15NS091977-01

Title: Understanding the cellular and genetic bases of sexually dimorphic vocal behaviors in the African clawed frog

Authors: *T. STEELE[1], C. J. WILSON[1], C. L. BARKAN[2], E. ZORNIK[2]
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Abstract: Sexually dimorphic circuits provide a framework to study the role of hormones in establishing behavioral diversity. The vocal behaviors of the African clawed frog, *Xenopus laevis*, offer a unique opportunity to study sexually dimorphic behavior. The mating vocalizations of male and female *X. laevis* are dimorphic both in rate and pattern: females produce monophasic calls with slow pulse frequencies, while male advertisement calls are biphasic with faster pulse frequencies. The circuit responsible for these vocalizations is a central pattern generator consisting of androgen sensitive premotor and motor nuclei. Cells in the premotor nucleus of males exhibit activity closely correlated with song production. Androgen treated female *X. laevis* develop the capacity for male-like call production. We utilized comparative electrophysiological and transcriptomic approaches to characterize differences in function and gene expression in the premotor nuclei of males, females, and testosterone-treated females (T-females). We identified cells in the premotor nuclei of T-female *X. laevis* with activity during song resembling that of male premotor vocal cells. These cells increased in size over the course of testosterone treatment, reaching male-like sizes by eight weeks; they also possessed the hyperpolarization activated cation current, I_H, as well as NMDA receptor-dependent oscillations, both of which are characteristic of male premotor cells. RNA sequencing of the premotor nuclei of males, females, and T-females identified 10211 genes of interest, of which 821 were significantly differentially expressed (padj < 0.05) between groups. Gene ontology analysis identified the Wnt pathway and signal transduction as categories of significant enrichment. Within the signal transduction category, genes upregulated in T-females and males compared to females included: tweety family member 3 (a Ca^{2+}-activated large conductance Cl^-

channel); GTPases implicated in cell morphology changes; and prostaglandin E(2)-9 reductase (which may influence sexually dimorphic responses to prostaglandins). The Wnt pathway is downregulated in T-females compared to males and females; however, the function of this pathway in the regulation of vocal behavior is unknown. Our findings suggest that changes to cell morphology, intrinsic currents, and intracellular signalling are all involved in the transition between the production of male and female vocal behavior in *X. laevis.*

Disclosures: T. Steele: None. **C.J. Wilson:** None. **C.L. Barkan:** None. **E. Zornik:** None.

Poster

407. Social Communication in Non-Avian Species

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Program #/Poster #: 407.03/TT5

Topic: F.01. Neuroethology

Support: NSF Grant IOS 1557945
University of Utah USTAR

Title: Speech-related gene FOXP2 expression in the central vocal pathways of the African clawed frog

Authors: *A. YAMAGUCHI, D. J. WOLLER, K. MEREDITH, A. NGUYEN
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Abstract: Forkhead box protein P2 (FOXP2) is a transcription factor known to be critical for the development of speech and language in humans; a mutation in the FOXP2 gene results in devastating linguistic impairments. Orthologs of FOXP2 are found across vertebrate species including birds, crocodiles, and fish. FOXP2 has a highly conserved amino acid sequence as well as conserved expression patterns in the brain of vertebrate species. FOXP2 disruption in mice and songbirds results in loss of pup calls and incomplete imitation of adult songs, respectively, suggesting the involvement of the gene in vocal communication. Here, we asked if FOXP2 is expressed in the central vocal pathways of African clawed frogs, *Xenopus laevis*. Male and female *X. laevis* generate sex-specific vocalizations to coordinate reproduction during the breeding season. The vocalizations of *X. laevis* are generated by central pattern generators (CPGs) contained in the brainstem that consist of the dorsal tegmental area of medulla (DTAM, homologue of parabrachial nucleus in mammals) and the laryngeal motor nucleus (n.IX-X, homologue of nucleus ambiguus in mammals). We found FOXP2 immunopositive neurons in a variety of brain regions including the telencephalon, optic tectum, cerebellum, and brainstem of *X. laevis*. In particular, we found dense labeling of FOXP2-positive neurons in the DTAM in both males and females. Given that FOXP2 is known to be expressed in the parabrachial nucleus of mammals, our results represent a conserved expression pattern of the FOXP2 gene. There

were no sex differences in the expression patterns of FOXP2 immunopositive neurons. Further immunohistochemical analyses showed that FOXP2-positive neurons in the DTAM do not express GABA or parvalbumin, but may express cholinacetyltransferase. The discovery of FOXP2 expression in the *X. laevis* DTAM suggests a contribution of this gene to vocal communication in amphibians.

Disclosures: A. Yamaguchi: None. **D.J. Woller:** None. **K. Meredith:** None. **A. Nguyen:** None.

Poster

407. Social Communication in Non-Avian Species

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 407.04/TT6

Topic: F.01. Neuroethology

Title: Dietary tuning of begging behavior in a social tadpole

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Abstract: Adequate nutrition during infancy is required for the development of healthy children and lifelong wellbeing in adults. Infants must integrate their nutrition levels with the decision to spend energy reserves communicating need to parents. Although diet is an important factor in infant behavior, little is known about the underlying neural mechanisms driving this social communication. We examined the neural basis of infant communication of nutritional need in the Mimetic poison frog (*Ranitomeya imitator*). In order to receive food from their mothers, tadpoles perform a begging display characterized by vigorously vibrating back and forth. This begging behavior is an honest indicator of need because it is a costly display for the tadpole and is more likely to occur when nutritional state is poor. We first examined the neural basis of begging behavior by quantifying neural activation (via a phosphorylated ribosome marker) throughout the brain and found several hypothalamic and forebrain regions that are more active in begging tadpoles compared to non-begging controls. We then quantified gene expression specifically in active neurons using ribosome capture and found that neurons active during begging behavior are enriched for arginine vasopressin and orexin pathways, suggesting circuitry involved in social behavior and nutritional state are important. Moreover, we also found that neurons active during begging behavior express *forkhead box protein 2* (*foxp2*), which is important for communication in humans. Current experiments involve disrupting the function of these pathways and testing the effect on tadpole begging behavior. Overall, this work suggests that social- and nutrition-related neuronal pathways interact to promote begging behavior in neonates.

Disclosures: L.A. O'Connell: None. **S.V. Edwards:** None. **K. Summers:** None.

Poster

407. Social Communication in Non-Avian Species

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Program #/Poster #: 407.05/TT7

Topic: F.01. Neuroethology

Support: GSU Brains & Behavior Program
Center for Behavioral Neuroscience

Title: Effects of acquisition and maintenance of social status on cytochrome oxidase activity in the preoptic area and amygdala in green anole lizards

Authors: D. SHUKLA, L. DUMANOWSKY, J. KIM, G. M. GUTZMAN, J. HONG, *W. WILCZYNSKI
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Abstract: Male green anole lizards form dominant-subordinate dyads when paired together that remain stable over time. Acquisition and maintenance of social status results in a change in a multitude of behaviors in anoles and many other species. Previous studies in our lab have consistently shown a decline in aggression and courtship behaviors in subordinate anoles after cohabitation with a dominant animal for 1 week. Such a change in territorial behaviors suggests a change in the activity of or relationship among the nodes in the social behavior network (SBN). The social behavior network is an evolutionary conserved set of reciprocally connected forebrain nuclei that have been implicated in a number of social behaviors including aggression and appetitive sexual behaviors. We investigated changes in the amygdala and preoptic area by quantifying changes in cytochrome oxidase activity in these nuclei. Cytochrome oxidase is a rate-limiting enzyme in the electron transport chain in the mitochondria and cytochrome oxidase activity reflects long-term changes in metabolic activity of neurons due to changes in the amount of excitation and inhibition received by the neurons. Individually housed male anoles were paired with size-matched opponents to form subordinate-dominant dyads that were housed together for 10 days. At the end of the cohabitation period, brains were flash frozen, cryostat sectioned, and cytochrome oxidase histochemistry was carried out using 3,3'-diaminobenzidine as the chromogen in the reaction. The sections were imaged at 40x magnification and the images were analyzed to obtain optical density values using Image J that reflected changes in cytochrome oxidase activity. We observed a difference in optical density values in the preoptic area with dominants possessing higher O.D values relative to subordinates (n=6 pairs, paired t test: t= 2.652, p= 0.045) but not in the amygdala (n=6 pairs, paired t test: t= 1.453, p= 0.211). The higher activity in the preoptic area in dominants is consistent with its role in aggression and sexual behavior and the decline in territorial behaviors in subordinates. Furthermore, the correlation between activity in the amygdala and preoptic area in the subordinates (r= 0.258) was

stronger relative to the dominant animals where it was negligible (r= 0.0346) suggesting a change in functional connectivity in addition to changes in mean activity levels.

Disclosures: D. Shukla: None. **L. Dumanowsky:** None. **J. Kim:** None. **G.M. Gutzman:** None. **J. Hong:** None. **W. Wilczynski:** None.

Poster

407. Social Communication in Non-Avian Species

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Program #/Poster #: 407.06/TT8

Topic: F.01. Neuroethology

Support: BrainHub Postdoctoral Fellowship

Title: Epigenomic dissection of regulatory elements underlying the evolution of learned vocal behavior

Authors: *M. WIRTHLIN[1], L. CANTIN[2], S. ANNALDASULA[1], T. PARK[1], E. D. JARVIS[2], A. R. PFENNING[1]
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Abstract: Complex behaviors, such as vocal learning (the ability to actively modify vocal production in response to auditory feedback, as in human speech), require tight organization of gene activity across a vast array of interconnected cell types and tissues. In the brain regions that control the production of learned vocalizations, we previously identified convergent changes in gene expression that were unique to multiple vocal learning species, and not present in their vocal non-learning relatives. Given that convergent evolution of behavior is associated with convergent specializations in gene expression, we sought to discover whether this convergence also exists at the level of gene regulation, specifically within the domain of epigenetic modification of enhancer sequences that control gene expression. To study enhancer regions across large evolutionary distances, we built and validated a specialized pipeline. First, we used multiple sequence alignments to map a set of ~150,000 known brain enhancers to the genomes of 100 different vertebrate species. In the orthologous enhancers of macaque, mouse, and zebra finch, we estimated enhancer activity using ChIP-Seq for an associated chromatin modification (H3K27ac) in two brain regions, the cortex and the striatum. Despite the extensive nucleotide turnover occurring over the >300 million years of evolutionary distance between birds and mammals, we found conservation of cortical vs. striatal enhancer specificity. Using this framework, we next identified enhancers found near critical genes associated with specialized expression in vocal learning songbirds and humans, that also show specialized enhancer activity in speech motor cortex of humans. Remarkably, investigations of this set revealed that the

change in enhancer activity correlated with the specialized transcriptional changes in brain gene expression in vocal learners. In sum, our work has identified conserved enhancers that control convergently specialized genes, supporting enhancer evolution as a prime candidate for driving the evolution of complex behavior.

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Poster

407. Social Communication in Non-Avian Species

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Program #/Poster #: 407.07/TT9

Topic: F.01. Neuroethology

Support: NSF 1557846

Title: Neural adaptations for coding social communication in the presence of social noise

Authors: *K. M. ALLEN[1], G. T. SMITH[2], G. MARSAT[1]
[1]Biol., West Virginia Univ., Morgantown, WV; [2]Indiana Univ., Bloomington, IN

Abstract: Studying the production and reception of social communication signals represents a unique opportunity to examine how sensory systems evolve and specialize. Selective pressures can act on both the production and reception of the signal within the same organism, leading to marked sender-receiver matching. Behavioral context can also influence the shape of the signal to be perceived and social species might have to evaluate a single signal from a complex sensory stream. Using three species of weakly electric fish, this study demonstrates changes within the electrosensory system correlated to changes in divergent communication strategies. The fish *Aperotonotus leptorhynchus*, *Apteronotus albifrons*, and *Adontosternarchus devenanzii* have large differences in social behaviors such as group size, communication frequency, and signal structure. By studying the social behavior of these three species, we show that communication signal production and use varies between these three species. Recordings from the primary electrosensory area of the brain show differences between species in frequency tuning and feature coding. These adaptations correlate with enhanced ability to detect social signals and discriminate signal features, particularly in conditions simulating noisy group interactions. In both behavior and physiology, species that are more socially gregarious exhibit distinct adaptations that support the detection and discrimination of social signals in large and complex social groups.

Disclosures: K.M. Allen: None. **G.T. Smith:** None. **G. Marsat:** None.

Poster

407. Social Communication in Non-Avian Species

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Program #/Poster #: 407.08/TT10

Topic: F.01. Neuroethology

Support: DP1 MH103908
R01 NS099288

Title: A midbrain-to-hindbrain circuit for the control of vocalization

Authors: *K. A. TSCHIDA, V. MICHAEL, J. TAKATO, S. ZHAO, B.-X. HAN, R. MOONEY, F. WANG
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Abstract: Vocalizations are an essential medium for communication and courtship in numerous mammalian species ranging from mice to humans. In mammals, the midbrain periaqueductal gray (PAG) serves as an obligatory node in a vocalization-related network that spans the forebrain and brainstem, as bilateral lesions of the PAG result in mutism. Despite the PAG's importance for vocal production, the identity, function, and connectivity of PAG neurons involved in vocalization has remained elusive, in part because the PAG is a functionally and anatomically heterogeneous structure that serves myriad roles including nociception, defensive behaviors, and autonomic regulation. To this end, we previously used a viral genetic strategy to identify and characterize PAG neurons whose activity is necessary and sufficient for the production of ultrasonic vocalizations (USVs) in the mouse. While this work established the identity of the PAG neurons selectively required to gate the production of USVs (i.e, PAG-USV neurons), an important remaining issue is to determine how descending projections from PAG-USV neurons recruit brainstem premotor circuits to shape the spectral and temporal acoustic features of vocalization.

In the current study, we test the hypothesis that PAG inputs to the nucleus retroambiguus (RAm), a vocal/respiratory premotor region of the caudal brainstem, are a key site whereby descending vocal motor commands recruit premotor circuits to shape vocal output. We have observed that PAG-USV neurons send a dense axonal projection to RAm, and that RAm neurons upregulate the immediate early gene Fos following the production of USVs, suggesting that PAG inputs to RAm may be important for vocal control. We are currently using optogenetic methods to activate and silence PAG inputs to RAm to test the role of this projection in vocal control. We are also monitoring the activity of PAG-USV and RAm neurons to understand how they are recruited during both vocalization and by different patterns of respiration. Taken together, these experiments will shed light on how midbrain neurons essential to USV production recruit premotor circuits to ultimately control the complex acoustic features of vocalizations.

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Poster

407. Social Communication in Non-Avian Species

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Program #/Poster #: 407.09/TT11

Topic: F.01. Neuroethology

Support: NIH New Innovator Award
Sloan Fellowship
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McKnight Foundation
The Pew Fellowship

Title: A 'Go-signal' for vocal production in the brain of a developing vocal learning mammal

Authors: *M. C. ROSE[1], M. M. YARTSEV[2]
[1]Helen Wills Neurosci. Inst., [2]Bioengineering, Univ. of California Berkeley, Berkeley, CA

Abstract: Infants and children are expert vocal learners; however, the neural mechanisms supporting vocal learning in mammals have never been studied before due to the remarkable sparsity of vocal learning capabilities across mammalian species. To address this knowledge gap we study vocal learning in one of the only known non-human vocal learning mammals: bats, specifically, the Egyptian fruit bat (*Rousettus aegyptiacus*). We chose bats not only because of their mammalian brain organization, but also for their impressive social vocal repertoire and learning capabilities. Previous neurophysiological investigations in bats have focused primarily on the perception of acoustic signals and not on the production of learned vocalizations. Importantly, no study to date has examined the detailed neural computations which might support vocal production in a developing mammal of any species at cellular resolution. To bridge this gap, we have made first efforts toward studying the mechanisms that support vocal production and learning in the developing mammalian brain during free, naturalistic behavior. We used lightweight wireless electrophysiology to study the activity in a frontal motor cortical area (FMA) of juvenile bats engaged in natural social interactions. We observed a substantial fraction of single neurons exhibiting responses tightly aligned to the production of learned vocalizations, but not during auditory playback. Consistent with the single neuron results, we observed a strong modulation of high-frequency local field potential power aligned to vocal production. FMA responses were independent of both the temporal ordering, acoustic structure, and rhythmic structure of the produced vocalizations, but were tightly locked to the onset of learned vocalizations. Furthermore, only a relatively small number of the same FMA neurons modulated their activity during the production of echolocation calls - a major component of the

bat acoustic production system that is believed to be innate. These results suggest a potential encoding of a "go-signal" for the production of learned vocalizations. With ongoing experiments, we will compare these results to neural activity in the FMA of fully developed adult bats to determine the effect of vocal learning on neural activity in this region. Combined, we present here the first evidence of cellular-resolution neural activity related to the production of learned vocalizations in the developing mammalian brain.

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Poster

407. Social Communication in Non-Avian Species

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Topic: F.01. Neuroethology

Support: DP1 MH103908
R01 NS099288

Title: A hypothalamic-to-midbrain circuit for the control of vocalization

Authors: *V. C. MICHAEL[1], K. A. TSCHIDA[2], S. ZHAO[1], F. WANG[3], R. D. MOONEY[4]
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Abstract: Vocal communication is a key social behavior by which humans and other mammals form and maintain social bonds, convey information about social status and mating fitness, and engage in cooperative behavior. Successful vocal communication is essential for social affiliation and impairments in vocal communication typify neuropsychiatric disorders. Mice produce complex ultrasonic vocalizations (USVs) in a variety of social contexts, allowing researchers to study the circuits underlying vocal control using genetic tools. The midbrain periaqueductal gray (PAG) is a critical node in the vocal motor circuit whose activity is obligatory for vocalizations. Studying the identity and connectivity of PAG neurons that control vocal production is challenging as these neurons reside amidst a heterogenous population of neurons controlling defensive behaviors, nociception, and respiratory modulation. In previous studies, we used a viral genetic method (CANE, Capturing Activated Neuronal Ensembles) to identify and tag a subpopulation of PAG neurons whose activity are necessary and sufficient to produce USVs (PAG-USV neurons). An important remaining question is how PAG-USV neurons integrate upstream information to appropriately gate vocal output. Engaging in appropriate vocal behavior requires the active integration of dynamic sensory cues, as well as state-dependent internal signals that relate to the individual's motivational drive and social experience. We sought to understand how inputs to PAG-USV neurons convey information about social context to drive or

shape USV production.
We used CANE-based trans-synaptic rabies tracing from PAG-USV neurons to identify their afferents to dissect the larger circuit in which these neurons reside. We found that PAG-USV neurons receive input from a variety of cortical and subcortical regions implicated in social behavior, supporting the idea that PAG-USV neurons integrate information about social context to gate vocal output. Amongst these various inputs, we focused on the identity and function of neurons in the preoptic area (POA) of the hypothalamus that project to PAG-USV neurons. We used genetic tools to selectively activate subpopulations of POA neurons that project to the PAG in order to identify the projection cell types that drive vocalization. We then monitored the activity of PAG-projecting POA neurons during social interaction assays to test when these neurons are active during social encounters and what information they convey to PAG-USV neurons. Overall, these experiments further our understanding of how vocal communication is embedded in and recruited by networks that control social behavior.

Disclosures: V.C. Michael: None. **K.A. Tschida:** None. **S. Zhao:** None. **F. Wang:** None. **R.D. Mooney:** None.

Poster

407. Social Communication in Non-Avian Species

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Topic: F.01. Neuroethology

Support: P50 MH106428
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T32 DA007278

Title: DeepSqueak: A deep learning based system for quantification of ultrasonic vocalizations

Authors: *R. MARX, K. R. COFFEY, J. F. NEUMAIER
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Abstract: Rodents engage in social communication through a rich repertoire of ultrasonic vocalizations (USVs). Recording and analysis of USVs has broad utility during diverse behavioral tests and can be performed non-invasively in almost any rodent behavioral model to provide rich insights into the emotional state and motor function of the test animal. Despite strong evidence that USVs serve an array of communicative functions, technical and financial limitations have been barriers for most laboratories to adopt vocalization analysis. Recently, deep learning has revolutionized the field of speech recognition and computer vision, allowing computers to see, listen and speak with near-human accuracy. Such systems are constructed from biomimetic, 'deep', artificial neural networks. Here we present DeepSqueak, an open source USV

detection and analysis software suite designed around cutting edge regional convolutional neural network architecture (Faster-RCNN), capable of performing human-quality USV detection and classification automatically, rapidly and reliably. DeepSqueak was engineered to allow non-experts easy entry into USV detection and analysis yet is flexible and adaptable. DeepSqueak has a user friendly graphical user interface, is freely available, flexible, feature packed, and systematically addresses the limitations of other available software. This package allows USV recording and analysis to be added inexpensively to existing rodent behavioral procedures, revealing a wide range of innate responses that provide another dimension of insights into behavior, especially when combined with conventional outcome measures.

Disclosures: R. Marx: A. Employment/Salary (full or part-time):; University of Washington. **K.R. Coffey:** None. **J.F. Neumaier:** None.

Poster

407. Social Communication in Non-Avian Species

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Topic: F.01. Neuroethology

Support: DAAD
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Searle Scholar
Pew Scholar

Title: Long-term and persistent vocal plasticity in adult bats

Authors: *M. M. YARTSEV[1], D. GENZEL[2]
[1]Bioengineering, Univ. of California Berkeley, Berkeley, CA; [2]Helen Wills Inst. of Neurosci. and Dept. of Bioengineering, Univ. of California, Berkeley, CA

Abstract: Most bat species exhibit a diverse and complex vocabulary of social communication calls with which they can facilitate intraspecific information exchange. Evidence is accumulating that a vocal learning mechanism is underlying this capability resulting in a high-degree of vocal plasticity. As many bats live in socially tight-knit and acoustically noisy communities and live up to a relatively high age they most likely require long-term vocal plasticity to exist not only in adolescence but in adulthood as well. The goal of this project was therefore to investigate for the existence of persistent vocal plasticity in different groups of adult Egyptian fruit bats (*Rousettus aegyptiacus*) in response to long-term noise exposure. When exposed long-term to acoustic background noise, the groups independently adapted their vocalizationsto minimize interference with the noise.Different spectral shapes of the background noise targeted different frequency bands of the bats' calls and influenced how the bats adapted their call parameters. Next, we

examined the hypothesis that changes in call parameters emerging from the noise exposure would persist even after cessation of the noise. Our results indicate that not only do these bats modify distinct parameters of their vocalizations in response to the presented noise but that these changes persist for multiple weeks after cessation - suggestive of vocal plasticity. Further analysis shows that this change is not driven by a switch of call type or influenced by group constellation - supporting a global adaptation of the produced sounds. The obtained results demonstrate persistent vocal adaptation in an adult bat and extensive vocal plasticity abilities of this bat species. As a conclusion, bats could potentially play an important role acting as a model system for vocal production plasticity and learning.

Disclosures: M.M. Yartsev: None. **D. Genzel:** None.

Poster

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Pew Charitable Trust

Title: Mapping vocal production circuits in a vocal learning mammal - the bat

Authors: *T. A. SCHMID[1], A. A. NEVUE[3], P. V. LOVELL[4], M. B. JI[5], V. A. SHVAREVA[5], C. V. PORTFORS[6], C. V. MELLO[4], M. M. YARTSEV[2]
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Abstract: The production of learned vocalizations is one of the most complex motor skills that humans effortlessly perform, requiring the coordinated control of numerous respiratory, orofacial, and phonatory muscles. Among the latter, the cricothyroid muscle in the larynx is solely responsible for controlling an increase in pitch during phonation. Though many animals vocalize, the ability to produce learned vocalizations has only been found in a few animal groups, including some birds, cetaceans, and bats. Notably, humans and songbirds share several features related to vocal learning, including the presence of specialized corticobulbar projections

onto brainstem motor circuits that control a vocal organ, and shared molecular specializations in cortical vocal-motor areas. Yet, the absence of a tractable mammalian model system for probing vocal learning behavior has limited our ability to explore the neural basis of this trait in mammals. Recent evidence has shown that Egyptian fruit bats (*Rousettus aegyptiacus*) are capable of learning their social communication calls, thus suggesting that this species may be a suitable model for mammalian vocal learning studies. Here, we use molecular profiling and neuronal tracing techniques to investigate whether bats possess a corticobulbar projection that may facilitate the fine motor control needed for the acquisition and production of learned vocalizations. Specifically, we first performed *in situ* hybridizations for convergent markers of cortical vocal-motor neurons shared between songbirds and humans (e.g. GPM6A, PVALB, GAP43, SLIT1) in order to identify brain regions that may correspond to a laryngeal motor cortex (LMC) in two species of bats, Seba's short-tailed bats (*Carollia perspicillata*) and Egyptian fruit bats. We also injected anterograde tracers into putative LMC simultaneously with retrograde tracers into the cricothyroid muscle of Egyptian fruit bats. The *in situ* data provide evidence of molecular cortical specializations reminiscent of LMC. We also show that cortical projections from putative LMC may directly synapse onto nucleus ambiguus motoneurons that innervate the cricothyroid muscle. We are working towards verifying these findings in both species and testing them functionally to further establish bats as a mammalian model for vocal learning.

Disclosures: T.A. Schmid: None. **A.A. Nevue:** None. **P.V. Lovell:** None. **M.B. Ji:** None. **V.A. Shvareva:** None. **C.V. Portfors:** None. **C.V. Mello:** None. **M.M. Yartsev:** None.

Poster

407. Social Communication in Non-Avian Species

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 407.14/TT16

Topic: F.01. Neuroethology

Title: Investigating the role that social status plays in vocal courtship behavior using a sound source localization system

Authors: *R. S. CLEIN[1], D. T. SANGIAMO[3], J. P. NEUNUEBEL[2]
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Abstract: Across the animal kingdom, social status profoundly affects access to the resources necessary for survival and reproductive success (Chase, 1982). Hierarchical rank is determined by the outcomes of agonistic behaviors and stabilizes over time (Williamson et al., 2017). In established, stable dominance hierarchies, vocal courtship displays are more prevalent in animals with a higher social status (Wang et al., 2011). However, as an animal's rank in a hierarchy

emerges, it is unknown how male agonistic social interactions impact their courtship displays. To answer this question, we employed a sound-source localization system (Warren et al., 2018) to track ultrasonic courtship vocalizations and the social behavior of individual mice (B6.CAST-Cdh23Ahl+/Kjn, 13-21 weeks old) during 5-hours of unrestrained group interaction between 2 males and 2 females after a minimum of two weeks of isolation (n = 11 groups). Using a machine-based learning program (Kabra et al., 2013), male courtship and agonistic behaviors were extracted (courtship: chasing female, 368.6 ± 32.1 (average ± SEM); agonistic: chasing and fleeing, 311.2 ± 51.9). A step function was calculated for every recording based on instances males performed agonistic behaviors and was normalized by the total number of aggressive behaviors. Each function provided a metric for quantifying the magnitude of the overall difference in social status. Large differences in the number of aggressive behaviors performed by each mouse indicated bigger disparities in status. Moreover, each function allowed us to define discrete periods of time where one male was engaging in all of the agonistic behaviors (aggressive state), or in all of the submissive behaviors (submissive state). Using the functions, we quantified the number of times that the identity of the male performing agonistic behaviors changed (state changes). Across experiments, the number of state changes (14-125) and the overall differences in status (absolute values: 0.02-0.97) was variable. When examining the courtship behavior of males in aggressive and submissive states, we found that the number of times a male interacted with a female was negatively correlated to the proportion of courtship chases with vocalizations (aggressive state: $r = -0.46$, $p < 0.002$; submissive state: $r = -0.31$, $p < 0.05$). The relationship was significantly influenced by the overall difference in social status ($r = -0.61$, $p < 10^{-5}$). These results suggest that establishing social rank is a highly dynamic process, and both the behavioral state of an animal and the overall difference in status impact courtship.

Disclosures: R.S. Clein: None. **D.T. Sangiamo:** None. **J.P. Neunuebel:** None.

Poster

407. Social Communication in Non-Avian Species

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 407.15/TT17

Topic: F.01. Neuroethology

Title: Using a sound source localization system to quantify autism-like deficits in mice during naturalistic group interaction

Authors: *M. R. WARREN[1], J. P. NEUNUEBEL[2]
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Abstract: Autism is a behaviorally classified disorder characterized in part by deficits in social interaction and communication. As numerous genetic perturbations are associated with autism, multiple mouse models have been genetically engineered to target candidate genes for autism. To

subsequently evaluate behavioral deficits similar to those observed in humans diagnosed with autism, numerous assays have been developed to measure discrete facets of autism-like behavior in these mouse models (Moy et al., 2007). Quantifying autism-like behavioral phenotypes during naturalistic interactions between mice, however, has been difficult. To overcome this challenge, we used a novel microphone array system (Warren et al., 2018) and two established mouse models of autism (Cacna1c (Bader et al. 2012) and Shank3b (Peca et al. 2011)) to examine the vocal and behavioral repertoires of individual adult animals (8-12 weeks old) during unrestrained group interactions. Groups of four mice, two males and two females, all of the same genotype (Cacna1c groups: wild-type, n = 7, heterozygous TS2-neocassette knock-in, n = 6; Shank3b groups: wildtype, n = 7, homozygous knockout, n = 6), were allowed to freely interact over a 5-hour period. For Cacna1c model mice, the number of social interactions between heterozygous males was similar to the number between wildtype males ($t_{11} = 1.11$, $p > 0.2$). Further, the number of vocal signals each male emitted was comparable regardless of genotype ($t_{24} = 1.28$, $p > 0.2$). For the Shank3b model, however, homozygous males interacted with each other less frequently than wildtype males ($t_{11} = 3.72$, $p < 0.01$). Moreover, the Shank3b knockout mice emitted significantly fewer vocal signals than their wildtype counterparts ($t_{24} = 3.59$, $p < 0.002$). Together, these data indicate that assessing behavior in a more naturalistic setting for longer periods of time may be a more sensitive measure of autism-like behavior in mice. Strikingly, however, we found that the relationship between vocal and behavioral repertoire remained constant regardless of genotype. By using an automatic behavioral classification program (Kabra et al., 2013) to extract discrete instances of behavior, and a novel vocal classification program to group similarly shaped vocal signals, we found that regardless of genotype, male mice emit discrete types of vocalizations depending upon their role in a social encounter. These results suggest that autism-like behavior quantified via typical measures may not be indicative of behavior in more naturalistic settings. Additionally, vocal emission appears to be linked to behavioral repertoire, regardless of genotype.

Disclosures: M.R. Warren: None. **J.P. Neunuebel:** None.

Poster

407. Social Communication in Non-Avian Species

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 407.16/TT18

Topic: F.01. Neuroethology

Support: DFG EXC 307

Title: Acoustic characteristics of vocal motor units in phee vocalizations of marmoset monkeys

Authors: *C. RISUENO SEGOVIA[1], S. R. HAGE[2]
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Abstract: The common marmoset, *Callithrix jacchus*, is endowed with a flexible vocal apparatus leading to an extensive vocal repertoire. The species-specific contact call, the phee call, is normally uttered as a simple call with one or multiple consecutive syllables. Previously, we reported the occurrence of periodically segmented phees indicating that vocalizations do not consist of one discrete call pattern but are built of many sequentially uttered units. Interestingly, the duration of these units matches the duration of other call types suggesting a general principle of vocal pattern generation. In the present study, we aim to characterize the acoustic features of segmented phee vocalizations. Our first results indicate a flexible yet constrained complex vocal pattern generating mechanism.

Disclosures: C. Risueno Segovia: None. **S.R. Hage:** None.

Poster

407. Social Communication in Non-Avian Species

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 407.17/TT19

Topic: F.01. Neuroethology

Title: Does Limiting parental interaction interrupt or regress vocal development in marmoset monkeys?

Authors: *Y. GÜLTEKIN[1], D. G. HILDEBRAND[2], S. R. HAGE[3]
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Abstract: Vocalizations of human infants change dramatically across the first year by becoming increasingly mature and speech-like, a process that is driven by learning from caretakers. In contrast, vocalizations of non-human primates are mainly innate and traditionally thought to change during development purely due to maturation. Like humans, marmosets exhibit infant-specific vocal behaviour including distinct infant call types that become increasingly mature during the first postnatal months until the proper adult vocal behaviour and repertoire is maintained. Although non-human primate vocalizations are largely innate, recent studies revealed vocal developmental processes in marmoset monkeys are influenced by parental feedback. However, the extent of this influence remains unclear. In particular, it is not yet known whether parental feedback in marmosets is an obligate requirement for proper vocal development as in humans, or whether it simply accelerates vocal development without a detrimental effect if

absent. To address this question, we tracked the vocal development of subadult marmosets with different parental experiences at different postnatal stages in a comparative study. We first studied two sets of offspring from the same parents: one set was normally raised, while the other was separated from the parents after the third postnatal month. Using quantitative measures to compare distinct call parameters and vocal sequence structure of the litters, we showed that parental feedback is necessary for normal vocal development in marmosets. While normally raised monkeys exclusively produced adult calls at the age of seven months, monkeys that had limited parental feedback still produced infant calls at the age of 13 months. In addition, limited parental feedback monkeys still exhibited the infant-specific babbling behaviour. To determine the extent to which their vocal development matured, we now investigate whether the limited parental interaction monkeys got stuck at or even regressed from the level of vocal development at separation by comparing their vocal behaviour with vocal output from normally raised monkeys at multiple developmental stages. Our aim is to thoroughly understand the impact of losing direct parental interaction on the vocal behaviour of marmosets in long term. Overall, our findings suggest a significant role for social feedback on primate vocal development that can be modified by postnatal experiences and show that marmoset monkeys are a compelling model system for human vocal development.

Disclosures: Y. Gültekin: None. **D.G. Hildebrand:** None. **S.R. Hage:** None.

Poster

407. Social Communication in Non-Avian Species

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 407.18/TT20

Topic: F.01. Neuroethology

Support: Stanley Center

Title: Neurobehavioral and vocal development in marmoset infants

Authors: *R. LANDMAN[1], O. MEISNER[2], S. PARMAR[2], R. DESIMONE[4], G. FENG[3]
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Abstract: The prospect of transgenic models in the Common marmoset (Callithrix jacchus) monkey creates a need for baseline data on development in wild type animals. To create a baseline for neurological, social and behavioral functioning in our colony, we used the Primate Postnatal Neurobehavioral Assessment Scale for marmoset monkeys (PPNAS-M) (Brain et al, 2015). This scale is based on a similar scale for Rhesus monkeys and the human infant scales Brazelton Newborn Behavioral Assessment Scale and Bayley Scales of Infant Development. The 41-item screening consists of 5 testing categories: visual orienting, auditory and spatial orienting,

motor responses, righting and body strength, and temperament tests. Marmoset infants were tested on the scale at age 5, 10, 15, 20, 30, 45 and 60 days of age. In addition, the infants' vocalizations were recorded for a 10 minute period while an audio recording of calls of an unfamiliar adult marmoset was played. On the PPNAS-M, our results are similar to the original publication. For most categories, the biggest development took place between 10 and 30 days of age. The general curve is similar between the different categories. To examine changes in vocalization in response to other animals, we evaluated the rate of vocalizations uttered by the infants just after recorded calls. Starting at 45 days of age, we noted an approximately 5 sec inhibition of calling by the infant following recorded calls. There was a weak correlation between scores on the PPNAS-M and inhibition of vocalization. The results confirm the usefulness of the PPNAS-M as a measure for development in the first month. Our vocal interaction test could be an indicator of vocal communication abilities starting at 45 days of age.

Disclosures: R. Landman: None. **O. Meisner:** None. **S. Parmar:** None. **R. Desimone:** None. **G. Feng:** None.

Poster

407. Social Communication in Non-Avian Species

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 407.19/TT21

Topic: F.01. Neuroethology

Support: DFG EXC 307
DFG HA5400/3-1

Title: Operant conditioning of vocal behavior in marmoset monkeys

Authors: *T. POMBERGER[1,2,3], C. RISUENO-SEGOVIA[3,2], D. DOHMEN[3,2], Y. B. GULTEKIN[3,2], S. R. HAGE[3]
[1]Werner Reichardt Ctr. For Integrative Neuroscien, Tübingen, Germany; [2]Grad. Sch. of Neural & Behavioural Sci. - Intl. Max Planck Res. School, Univ. of Tübingen, Tübingen, Germany; [3]Werner Reichardt Ctr. for Integrative Neuroscience, Univ. of Tübingen, Tübingen, Germany

Abstract: Current studies have revealed distinct learning mechanisms during vocal development and observed rapid vocal flexibility in vocalizations of marmoset monkeys. However, it is still a matter of debate whether they are able to achieve cognitive control over their vocalizations and, therefore, to decouple them from accompanied levels of arousal or specific events. We show that marmoset monkeys (*Callithrix jacchus*) can be trained to elicit vocalizations on command in response to arbitrary visual cues. Furthermore, we show that these animals can learn to produce distinct call types in response to different visual cues on a trial-by-trial basis. These findings indicate that marmoset monkeys are able to volitionally initiate their vocal production and,

therefore, are able to instrumentalize their vocal behavior to perform a behavioral task successfully. This conditioning approach will pave the way for further electrophysiological investigations of vocal motor control in marmoset monkeys, a highly loquacious monkey species, in a highly controlled experimental approach.

Disclosures: T. Pomberger: None. **C. Risueno-Segovia:** None. **D. Dohmen:** None. **Y.B. Gultekin:** None. **S.R. Hage:** None.

Poster

407. Social Communication in Non-Avian Species

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 407.20/TT22

Topic: F.01. Neuroethology

Support: NIH Grant R01NS054898
NSF Graduate Research Fellowship

Title: The anterior cingulate cortex as a nexus for vocal communication and energy allocation in marmoset monkeys

Authors: *D. A. LIAO[1], Y. S. ZHANG[1], D. Y. TAKAHASHI[1], A. EL HADY[1,2], A. A. GHAZANFAR[1]
[1]Princeton Neurosci. Inst., Princeton, NJ; [2]Howard Hughes Med. Inst., Chevy Chase, MD

Abstract: We recently showed that arousal levels are linked, but not inextricably, to vocal production (Liao et al., PNAS, 2018). In contexts where marmosets are interacting, the production of different call types is determined by both arousal levels and extrinsic factors such as the timing of a conspecific's vocalization. These data suggested that primates use their vocalizations strategically in that the changes in vocal output (i.e., different call types) as a function of context seem to reflect the tradeoffs between the drive to perpetuate vocal contact and conserving energy usage. For example, the production of loud, long, and tonal contact calls when conspecifics are out of sight is energetically demanding, eliciting a high metabolic cost. Increases in arousal could serve to allocate metabolic energy in order to prepare the body for this adaptive action.
Given how intrinsic states like arousal and external factors such as the vocalizations of conspecifics are intertwined, we investigated the neural circuits that might coordinate both processes. The anterior cingulate cortex (ACC) has long been implicated in modulating arousal, self-monitoring and vocal production . However, the ACC is broad and heterogeneous, with multiple functional subdivisions. Thus, we are using functional ultrasound imaging (fUSi) combined with heart rate measures via electromyography to capture the network of medial brain areas engaged when marmosets participate in vocal communication. Analyses of fUSi signals,

vocal playbacks, and heart rates identified a region spanning segments of pre- and sub-genual ACC responsive to call perception. Further analyses will investigate the relationship between the heart rate and the fUSi signal, and how together they might be modulated by social context. Interrogation of the interplay between intrinsic and extrinsic factors and their effects on neural activity will provide insights into the mechanisms governing vocal behavior.

Disclosures: D.A. Liao: None. **Y.S. Zhang:** None. **D.Y. Takahashi:** None. **A. El Hady:** None. **A.A. Ghazanfar:** None.

Poster

407. Social Communication in Non-Avian Species

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 407.21/TT23

Topic: F.01. Neuroethology

Support: NIH R01NS054898
PNI Innovation Fund

Title: Dynamic functional ultrasound imaging of socio-vocal network in marmoset monkeys

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[1]Psychology, [2]Princeton Neurosci. Inst., Princeton, NJ; [3]Howard Hughes Med. Inst., Chevy Chase, MD; [4]Neuro-Electronics Res. Flanders, Leuven, Belgium

Abstract: Vocal communication is the quintessential form of social interaction. Humans and other animals coordinate their social behaviors by producing and perceiving distinct vocalizations. Brain networks related to vocal communication include areas at the intersection of social behavior and vocal production-perception networks. Recent studies of primate vocal communication focused on lateral cortical regions, despite the fact that medial cortical and subcortical areas constitute the main vocal production and social behavior network (SBN). Hence, we aim to describe the brain-wide network underlying social communication focusing on the role played by medial cortical and subcortical areas. We use as our model the marmoset monkey, a highly vocal New World species. To image large-scale neural activity, we use functional ultrasound imaging which has a large spatial coverage and high spatio-temporal resolution. Furthermore, we built a stochastic dynamical systems model of vocal behavior that interacts with the marmoset in a closed-loop to fully control the vocal interaction and make quantitative predictions about brain dynamics during communication. We first show the existence of a medial brain system at the intersection of vocal production-perception and SBN; we call it the *socio-vocal network* (SVN). These areas differentially respond to affiliative vocalizations—contact, trillphee, and trill calls—produced in different contexts, exhibiting the

highest and quickest response to contact calls. Given that the contact calls reflect the highest arousal state of the vocalizing animal, this is consistent with the hypothesis that SVN is related to the monitoring of others motivational state through vocalization. Second, through a closed-loop interaction between the computational model and a marmoset, together with large-scale functional imaging, we found that the marmoset anterior cingulate cortex (which is part of SVN) and the model's "SVN" are entrained. These results demonstrate what the SVN encompasses and its roles in vocal communication.

Disclosures: D.Y. Takahashi: None. **A. El Hady:** None. **G. Montaldo:** None. **A. Urban:** F. Consulting Fees (e.g., advisory boards); Alan Urban Technology & Consulting. **A.A. Ghazanfar:** None.

Poster

407. Social Communication in Non-Avian Species

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 407.22/TT24

Topic: F.01. Neuroethology

Support: NIH Grant R01NS054898
NIH Grant F31NS089303
NIH Grant U19NS104648
NSF Graduate Research Fellowship

Title: Neurovascular anatomy of the marmoset brain: Links to the default mode network

Authors: *Y. S. ZHANG[1], T. J. PISANO[1,2], D. Y. TAKAHASHI[1], A. EL HADY[1,3], D. A. LIAO[1], S. S.-H. WANG[1], A. A. GHAZANFAR[1]
[1]Princeton Neurosci. Inst., Princeton, NJ; [2]Rutgers-Robert Wood Johnson Med. Sch., New Brunswick, NJ; [3]Howard Hughes Med. Inst., Chevy Chase, MD

Abstract: Dynamically correlated brain activity during resting state consistently reveals a large-scale network of functionally-linked areas known as the "default mode network". One hypothesis is that—in addition to neural connectivity—the structure of the default network is determined by the vascular architecture of the brain. This architecture represents the physical constraints of energy transport necessary for neural activity. In this study, we present our progress towards building the connection between the stationary vascular structure and the dynamics of blood flow in the primate brain. We combined the high-throughput technique of brain clearing (iDISCO) and high-contrast vascular staining with light-sheet microscopy to reconstruct the whole brain vasculature. Using an automated image processing pipeline, we quantitatively analyzed the vessel morphology and distribution, down to the level of microvessels. To link structure to function, we used functional ultrasound imaging which has a high spatiotemporal resolution to

image changes in cerebral blood volume in the medial sagittal section of the brain during the resting state. We found that temporally-correlated activity forms distinct clusters in cortical and subcortical areas as a function of both the brain area and the size of the blood vessels. Using current models of hemo-neural coupling, our results could provide a new approach to predict large-scale neural dynamics using neurovascular anatomical data.

Disclosures: Y.S. Zhang: None. **T.J. Pisano:** None. **D.Y. Takahashi:** None. **A. El Hady:** None. **D.A. Liao:** None. **S.S. Wang:** None. **A.A. Ghazanfar:** None.

Poster

407. Social Communication in Non-Avian Species

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 407.23/UU1

Topic: E.04. Voluntary Movements

Title: CRISPR/Cas9-based genome editing of the FoxP locus in *Drosophila*

Authors: *A. E. ERIKSSON, O. PALAZZO, B. BREMBS
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Abstract: In humans, variants of the Forkhead Box P2 (FOXP2) gene can cause severe speech and language disorders (verbal dyspraxia). The FoxP2 gene sequence is highly conserved in vertebrates and orthologs of the FoxP gene family exist in all bilaterians. Paralleling the interpretation of the verbal dyspraxia in FOXP2 patients as a motor deficit, FOXP1 patients show additional motor deficits, e.g. delayed development of walking. The Drosophila melanogaster ortholog, dFoxP, provides an opportunity to study the evolutionary conserved function of this gene family in operant self-learning, a type of motor learning. Operant self-learning of yaw torque (attempted rotations around the vertical body axis) in tethered flies is conceptually analogous to vocal learning: Vocal learning by imitation is a form of motor learning that proceeds slowly from “babbling” in humans and “subsong” in birds, towards speech and language in humans and crystallized song in birds. Analogously, flies tethered at the torque meter first initiate erratic exploratory actions followed by a reduction in behavioral variability as a result of sensory feedback. In vocal learning, the feedback shaping the motor actions is auditory, in flies we use heat to indicate motor errors. The analogy between fly operant self-learning and vocal learning is more than superficial: In humans and song birds, alterations of the FOXP2 gene cause a deficit in vocal learning. Also in flies, alterations of the dFoxP gene cause a deficit in operant self-learning, but leaving other forms of learning intact. In this work, we generated three transgenic Drosophila lines using the CRISPR/Cas9 technology: a conditional knockout of the dFoxP gene in a spatio-temporally controlled manner, a null-mutant knockout, and a knock-in of the Gal4 reporter gene sequence directly into the FoxP gene. Due to this latter knock-in reporter, we were able to study FoxP gene expression at the cellular level and compare

it with previously existing fly lines where FoxP had been tagged. These lines also enabled us to specifically study the expression of the IsoB isoform, which was previously associated with operant self-learning. Gene expression profiles were generated at the embryonal, larval, pupal and adult stage. In addition to the expression pattern, we also performed a behavioral characterization of the FoxP manipulated lines with regards to their performance in experiments testing spontaneous behavior, operant learning and decision making.

Disclosures: A.E. Eriksson: None. **O. Palazzo:** None. **B. Brembs:** None.

Poster

408. Stress and the Brain: Neuroimmunology

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 408.01/UU2

Topic: F.04. Stress and the Brain

Support: NIH Grant MH084970
NIH Grant MH109484

Title: Role of the anti-inflammatory cytokine IL-10 on anxiety and sickness-like behaviors in adult male rats

Authors: *S. MUNSHI[1,2], V. PARRILLI[2], J. A. ROSENKRANZ[2,3]
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Abstract: Anti-inflammatory cytokines are known to counteract damage caused by inflammatory processes and are beneficial in limiting the severity of inflammation. A growing body of evidence suggests that pro-inflammatory cytokines produce manifestations of sickness-like behaviors seen during inflammation, such as malaise and lethargy, and contribute to effects of inflammation on mood. Very little is known about the role of anti-inflammatory cytokines in this regard. Interleukin (IL)-10 is a cytokine whose anti-inflammatory effects have been studied extensively. However, its effects on sickness behavior and mood changes caused by inflammation have not been extensively explored. This aspect is commonly tested in rodents by measuring changes in behavioral activity levels that reflect lethargy or malaise and anxiety-like behaviors that represent changes in affective state. The first step towards understanding if IL-10 exerts effects opposite of pro-inflammatory cytokines is to determine if IL-10 itself produces a change in behavioral activity level and anxiety-like behavior. In the present study, we tested the effects of peripherally injected IL-10 on these behaviors of adult male Sprague Dawley rats. Rats were injected with a single dose of IL-10 (1 μg, i.p.) and its acute effect on open field exploration, social interaction and elevated plus maze tests were measured at 30 min post-treatment. IL-10 caused anxiety-like behavior as evidenced by reduced central area exploration

in the open field and reduced open arm exploration in elevated plus maze, but without any effect on locomotor activity. While the prototypical pro-inflammatory cytokine IL-1β (1 μg, i.p.) caused a decrease of locomotor activity, indicative of lethargy/malaise, rats co-treated with both IL-10 and IL-1β showed locomotor activity, open field, social interaction and elevated plus maze behaviors very similar to that of the control groups. This suggests that IL-10 is capable of mitigating the sickness-like effects caused by IL-1β. This provides a significant insight into the therapeutic potential of IL-10 in the management of depression.

Disclosures: S. Munshi: None. **V. Parrilli:** None. **J.A. Rosenkranz:** None.

Poster

408. Stress and the Brain: Neuroimmunology

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 408.02/UU3

Topic: F.04. Stress and the Brain

Support: NIH Grant R01MH108523
NiH Grant F32AG048672
NIH Grant R21MH116263

Title: Immunization with mycobacterium vaccae induces an anti-inflammatory milieu in the CNS: Attenuation of stress-induced microglial priming, alarmins and anxiety-like behavior

Authors: *M. G. FRANK[1], L. K. FONKEN[2], S. D. DOLZANI[3], J. L. ANNIS[3], P. H. SIEBLER[3], D. SCHMIDT[3], L. R. WATKINS[3], S. F. MAIER[3], C. A. LOWRY[3,4,5,6]
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Abstract: Exposure to stressors induces anxiety- and depressive-like behaviors, which are mediated, in part, by neuroinflammatory processes. Recent findings demonstrate that treatment with the immunoregulatory and anti-inflammatory bacterium, *Mycobacterium vaccae* (*M. vaccae*), attenuates stress-induced exaggeration of peripheral inflammation and stress-induced anxiety-like behavioral responses. However, the effects of *M. vaccae* on neuroimmune processes have largely been unexplored. In the present study, we examined the effect of the *M. vaccae* NCTC11659 on neuroimmune regulation, stress-induced neuroinflammatory processes and anxiety-like behavior. Adult male rats were immunized 3x with a heat-killed preparation of *M. vaccae* (0.1 mg, s.c.) or vehicle. *M. vaccae* induced an anti-inflammatory immunophenotype in hippocampus (increased interleukin (Il)4, Cd200r1, and Mrc1 mRNA expression) and increased IL4 protein 8 d after the last immunization. Central administration of recombinant IL4

recapitulated the effects of *M. vaccae* on Cd200r1 and Mrc1 mRNA expression. *M. vaccae* reduced basal levels of genes (Nlrp3 and Nfkbia) involved in microglial priming; thus, we explored the effects of *M. vaccae* on stress-induced hippocampal microglial priming and HMGB1, which mediates priming. We found that *M. vaccae* blocked stress-induced decreases in Cd200r1, increases in the alarmin HMGB1, and priming of the microglial response to immune challenge. Furthermore, *M. vaccae* prevented stress-induced increases in anxiety-like behavior. The present findings suggest that *M. vaccae* enhances immunomodulation in the CNS and mitigates the neuroinflammatory and behavioral effects of stress, which may underpin its capacity to impart a stress resilient phenotype.

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Poster

408. Stress and the Brain: Neuroimmunology

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 408.03/UU4

Topic: F.04. Stress and the Brain

Support: ONRG (N00014-17-S-B001)

Title: Immunoregulatory effects of mycobacterium vaccae are dependent on the route of application

Authors: *S. O. REBER[1], M. AMOROSO[1], T. ELESLAMBOULY[1], M. DANDAMUDI[1], E. KEMPTER[1], C. A. LOWRY[2], D. LANGGARTNER[1]
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Abstract: Immunodysregulation and subsequent chronic low-grade inflammation are risk factors for the development of stress-related somatic and psychiatric disorders, including inflammatory bowel disease (IBD) and posttraumatic stress disorder (PTSD). Thus, immunoregulatory approaches that counterbalance basal and/or stress-induced immune activation are expected to be protective in this context. In support of this hypothesis, we recently demonstrated that repeated subcutaneous preimmunization with a heat-killed preparation of immunoregulatory *Mycobacterium vaccae* (*M. vaccae; NCTC 11659*) protected mice against stress-induced general anxiety, social anxiety, spontaneous colitis, and aggravation of dextran sulfate sodium (DSS)-induced colitis in a mouse model of PTSD. To induce a PTSD-like phenotype, the chronic subordinate colony housing (CSC) paradigm, which is based on the repeated psychosocial traumatization (i.e., social defeat) in combination with chronic subordination of four male CSC mice towards a dominant resident male conspecific, was used. In the current study we employed

the CSC paradigm (start Day 1) to test whether 0.1 mg/ mouse/ week *M. vaccae* has protective effects in CSC and/or respective single housed control (SHC) mice when administered repeatedly via the intragastric (i.g.) or intranasal (i.n.) route prior to CSC on Days -21, -14 and -7. Thus, we assessed the coping behaviour during (Days 1, 8, 15) and anxiety-related behaviour at the end (Day 19: elevated plus-maze; Day 20: open-field/novel object; Day 21: social preference avoidance test) of CSC exposure. Moreover, inflammatory parameters following 7 days (Days 21-28) of DSS treatment were measured. Interestingly, while both repeated i.n. and i.g. administration of *M. vaccae* affected stress coping during CSC, only repeated i.g. administration of *M. vaccae* had colitis protective effects in SHC mice and ameliorated CSC-induced aggravation of DSS colitis. Both repeated i.g. and i.n. administrations increased anxiety-related behaviour in SHC and/or CSC mice. Currently, we are testing if i.g. administration of *M. vaccae* also has protective effects when applied during stressor exposure. Taken together, these data broaden the framework for developing bioimmunoregulatory approaches based on microorganisms known as 'Old Friends' for the treatment and/or prevention of stress-related disorders.

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Poster

408. Stress and the Brain: Neuroimmunology

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Topic: F.04. Stress and the Brain

Support: CIHR Grant GSD-148222
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Title: Oral exposure to a neuroactive bacterial strain leads to acute activation of specific brain regions and modulates chronic social stress-induced behavioural deficits

Authors: *A. BHARWANI[1], K. CHAMPAGNE-JORGENSEN[1], F. M. MIAN[1], M. G. SURETTE[2], J. BIENENSTOCK[3], P. FORSYTHE[2]
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Abstract: The gut microbiota and the brain are engaged in persistent bidirectional interplay—a phenomenon that influences host neural function and behaviour. Indeed, administration of a neuroactive bacterial strain reduces anxiety- and depression-like behaviours, while exposure to chronic social stress causes complex structural changes in the gut microbial community.

However, it is currently unclear whether the microbiota influences stress-associated behavioural and neural changes, and the candidate brain regions that respond to bacterial signals remain unknown. Using a neuroactive bacterial strain, *Lactobacillus rhamnosus* JB-1^{TM}, we investigated whether gut-brain signalling modulates the behavioural changes induced by chronic social stress, and mapped JB-1 induced c-fos immunoreactivity in select brain regions.
Male C57BL/6 mice were orally administered 10^9 colony-forming units (CFU) of JB-1 for 28 days and were subjected to chronic social defeat stress during the final ten days of treatment. Mice were assessed for alterations in social, exploratory, and anxiety-like behaviours using multiple behavioural assays. 16S rRNA sequencing was used to analyze structural changes in the microbiota community. In a second set of experiments, stress-naïve male Balb/c mice, which exhibit behavioural changes following long-term bacteria administration, were administered a single dose of 10^9 CFU of JB-1 before being sacrificed two hours later to map acute JB-1-induced c-fos expression.
Chronic stress induced various behavioural deficits—reduced sociability and exploration, and increased anxiety-like behaviour—that were partially corrected with bacteria administration. Stress exposure induced complex alterations in the microbiota profile, none of which were prevented by treatment. We also present immunofluorescence data of quantified c-fos expression in various brain regions at two hours following JB-1 administration.
These findings demonstrate the ability of bacteria to modulate gut-brain signalling independently of structural changes in the microbiota, and to correct specific behavioural deficits induced by chronic stress. Additionally, these data identify candidate brain regions that respond to bacteria-derived signals and which may be of further interest for functional analysis. Collectively, these results reveal further evidence of gut-brain signalling, and a potential role for microbe-based adjuncts or therapies as a novel strategy in the treatment of psychiatric conditions.

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Poster

408. Stress and the Brain: Neuroimmunology

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Title: High dimensional characterization of immune compartments in a mouse model of social defeat stress

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Abstract: Social stressors, such as aggression are major risk factors for neuropsychiatric disorders, including major depressive disorder (MDD). While it is well established that chronic social stress leads to profound changes in the immune system (e.g. increased levels of pro-inflammatory cytokines), which elicit behavioral changes relevant to MDD, the detailed mechanisms of how the peripheral immune system acts on the brain (and *vice versa*) remains to be elucidated. The present project aims to characterize the different immune cell populations in both blood and brain, and investigate the role of blood-brain barrier endothelial cells as an important interface between these two compartments in the murine chronic social defeat (CSD) model. Using mass cytometry (CyTOF), we performed single cell, high-dimensional analysis of blood in stress susceptible, resilient and unstressed control mice. We show that CSD leads to a significant stress effect, altering leukocyte subpopulation frequencies of both the myeloid and lymphoid lineage. Interestingly, stress susceptible and resilient mice show similar peripheral changes, suggesting that there may be cell intrinsic differences in leukocyte subpopulations or in the way in which inflammatory signals access the brain circuitry to affect depression-like behaviors. Investigating the mechanisms underlying interactions between the peripheral immune and central nervous systems in a mouse model relevant to MDD will yield important insights into the etio-pathophysiology of MDD and may lead to potential novel therapeutic targets.

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Poster

408. Stress and the Brain: Neuroimmunology

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Program #/Poster #: 408.06/UU7

Topic: F.04. Stress and the Brain

Support: NSF IOS 1257679
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Title: Neural and peripheral inflammation markers in adult rats exposed to adolescent social stress

Authors: E. T. GRAACK, 57069[1], J. L. SCHOLL[1], M. A. WEBER[1], M. SATHYANESAN[1], G. L. FORSTER[2], S. S. NEWTON[1], *M. J. WATT[2]
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Abstract: Retrospective human studies show that early life adversity is associated with elevated plasma C-reactive protein (CRP) levels and other markers indicative of immune dysregulation, which appear to increase risk of mental and physical illness later in life. Animal models demonstrate that stressors experienced very early in life result in elevated systemic immune markers in adulthood, associated with psychological and cognitive disruptions. However, whether chronic stress restricted to adolescence has long-lasting effects on basal or stress-induced peripheral and central immune makers is not as well understood. Adolescent social defeat is an animal model of social stress that replicates many of the aspects of peer bullying during a critical period of brain and behavior maturation. Adolescent social defeat in male rats results in learning/memory deficits and enhanced drug seeking in adulthood along with disruptions to cortical and accumbal dopamine function. Here we applied the same model to examine immune markers in basal and stress-challenged conditions in previously defeated male rats. Adolescent rats (postnatal day [P]35-39) were exposed to daily social defeat or control handling and were allowed to develop to adulthood (P60+) undisturbed. Brains and plasma were collected from adult rats immediately following either 20 minutes of restraint stress or in the absence of stress (n = 10-12 per group). Plasma corticosterone, CRP and cytokine levels were determined by ELISA while hippocampal proinflammatory cytokine mRNA was quantified. Main effects of adolescent defeat or restraint stress were observed such that restraint stress increased plasma cortisol levels equally in defeated and control rats, but CRP levels were increased by adolescent social defeat irrespective of restraint stress. Findings suggest long-lasting effects of adolescent social stress on basal immune markers that have been directly implicated in poorer psychological and physical health outcomes in later life.

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Poster

408. Stress and the Brain: Neuroimmunology

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Program #/Poster #: 408.07/UU8

Topic: F.04. Stress and the Brain

Support: MH114049-01

Title: The effect of IL-1βin the BLA on enhanced fear memory

Authors: *A. KULP[1], B. LOWDEN[1], J. KRZOSKA[1], M. HAUCK[1], M. RUSS[1], D. MEHTA[1], D. BARNARD[2], J. D. JOHNSON[3]
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Abstract: Following a traumatic event, individuals have an enhanced memory of the context of the event (location, people, etc.). This is evolutionary advantageous since it allows the individual to avoid the dangerous environment in the future; however, some individuals have too strong of a memory to a fearful event and is debilitating. Currently, it is unclear what causes the enhanced memory at the molecular level. The leading mechanism proposed is through the activation of the stress response, particularly the sympathetic nervous system (SNS). When stressed, the SNS utilizes norepinephrine binding to its receptor, beta-adrenergic receptors (βAR). Following, there is an influx of interleukin-1β (IL-1 β), a proinflammatory cytokine, which has evidence to be a sufficient molecule for inducing the enhanced fear memory: 1) Rodents given repeated stress show enhanced IL-1 β production in the amygdala; 2) Low levels of IL-1β added through intracerebroventricular injection facilitate memory processes; 3) Rodents with a stressful past have enhanced fear memories. Therefore, it is reasonable to hypothesize that IL-1β facilitates the enhanced fear memory following chronic stress.To answer this question, male rats were administered 0.5 µL of IL-1β in the basolateral amygdala, a brain region important for fear memory association:1) 0.0 ng/ µL; 2) 0.01 ng/ µL; 3) 0.1 ng/ µL; 4) 1.0 ng/ µL after the fear training. Surprisingly, the data suggest IL-1β dampens fear memory in a dose dependent manner. Future studies will investigate whether blocking IL-1 β enhances fear memory.

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Poster

408. Stress and the Brain: Neuroimmunology

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Program #/Poster #: 408.08/UU9

Topic: F.04. Stress and the Brain

Title: Gender-specific gene expression profiling of the prefrontal cortices of social defeat stress model mice

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Abstract: The prevalence of depression is higher among female population, and some of clinical features of depression is known to be gender-relevant. Although molecular mechanisms underlying the pathogeneses of depression have been intensively investigated utilizing the postmortem brains from depressed patients and multiple animal models of depression, gender

difference has rarely been taken into account. Since functional imaging and postmortem brain studies suggested the prefrontal cortex (PFC) abnormalities in patients with depression, gender difference in microarray-based gene expression profiling of PFC was evaluated utilizing the social defeat stress mouse model, which is widely used as an animal model for depression in neuroscience researches. Gene ontology analyses were used to investigate molecular and cellular pathways potentially involved in depression-linked behavior. Mitochondrion-associated genes were significantly enriched among the differentially expressed genes in the PFC between male mice susceptible to social defeat stress and control male mice, whereas nucleus-associated genes were significantly enriched among differentially expressed genes in the PFC between susceptible and control female mice. Besides, two hormonal molecules were extremely increased only in social defeat-susceptible female mice, whereas they were decreased in social defeat-susceptible male mice. The findings was validated by real-time PCR studies. The data suggests that these gender-specific gene expression profiling can underlie the pathogenesis of depression.

Disclosures: M. Sakai: None.

Poster

408. Stress and the Brain: Neuroimmunology

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Alfred P. Sloan Foundation (grant number G-2015-14165); CAL (PI)

Title: Effects of immunization with the environmental bacterium mycobacterium vaccae and chronic circadian disruption on the microbiome and metabolome of inbred male C57BL6/N mice

Authors: *C. L. CHO[1], J. D. HEINZE[1], S. A. SAGO[1], D. M. KIENZLE[1], A. GONZÁLEZ[2], F. VARGAS[2], D. SCHMIDT[1], M. C. FLUX[1], D. G. SMITH[1], A. I. ELSAYED[1], D. A. DUGGAN[1], J. D. JONES[1], L. T. TRAN[1], P. H. SIEBLER[1], R. S. THOMPSON[1], M. H. VITATERNA[3], F. W. TUREK[3], M. FLESHNER[1], P. C. DORRESTEIN[2], R. KNIGHT[2], K. P. WRIGHT[1], C. A.

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Abstract: Previous studies in our laboratory demonstrate that a heat-killed preparation of the immunoregulatory and anti-inflammatory environmental bacterium *Mycobacterium vaccae NCTC* 11659 is a potentially useful countermeasure that protects against negative outcomes of chronic psychosocial stress. These protective effects included ameliorations of stress-induced decreases in alpha (phylogenetic) diversity and increases in beta diversity of the gut microbiome, chemically induced colitis in a model of inflammatory bowel disease, anti-CD3-stimulated release of proinflammatory cytokines from freshly isolated mesenteric lymph node cells *ex vivo*, and anxiety-like behavior. Here we utilized a 2 x 2 experimental design in 3 cohorts of juvenile male C57BL/6N mice (postnatal day (pnd) 28; $N = 45$) to determine if immunization with *M. vaccae* is an effective countermeasure in a murine model of chronic circadian disruption (CDR). Mice were implanted on pnd 36 with telemetric recording devices for monitoring 24-h rhythms of core body temperature and locomotor activity (LA). We immunized (3 x weekly immunizations beginning on pnd 43) adolescent male mice with a heat-killed preparation of *M. vaccae* or vehicle and evaluated the gut microbiome and fecal metabolome using 16S rRNA gene sequencing and untargeted LC/MS/MS, respectively, before and during 8 weeks of CDR (pnd 42, 56, 91, and 105). Briefly, CDR consisted of weekly 12-h light reversals of the normal 12:12h light:dark cycle every 7 days for 8 weeks (pnd 64-120). Single-housed mice immunized with vehicle and maintained on a non-manipulated 12-h light:dark cycle (lights on at ZT0 = 7:00am, lights off at ZT12 = 7:00pm) for 8 weeks were used as controls. Immunization with *M. vaccae* increased LA measures during the active dark phase prior to the onset of CDR. Gut microbiome diversity and community structure were remarkably consistent within individuals across time. Neither *M. vaccae* immunization ($F = 1.3799$, $p = 0.152$, 121 residuals, PERMANOVA; pnd 56-105) nor CDR ($F = 1.2045$, $p = 0.26$, 82 residuals, PERMANOVA; pnd 91-105) altered alpha- or beta-diversity of the gut microbiome. We show instead that differences in microbial community structures in inbred mice are highly dependent on the cohort of mice received from the commercial vendor ($F = 16.495$, $p < 0.001$, 120 residuals, PERMANOVA; pnd 42-105) and cluster differently in measures of centroid dispersion ($F = 3.4056$, $p = 0.029$, 81 residuals). Analysis of the fecal metabolome is ongoing. These findings demonstrate the importance of using animal models that are not only genetically identical, but also housed in identical conditions from birth for use in -omics studies.

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Poster

408. Stress and the Brain: Neuroimmunology

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Topic: F.04. Stress and the Brain

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SynaNet H2020 Twinning Action (GA-692340)

Title: Effects of a nootropic and immunomodulator drug in a mice model of social isolation

Authors: *D. C. MELO MAGALHÃES[1,2,3], M. MAMPAY[3], G. SHERIDAN[3], C. A. VALENTE[1,2], A. M. SEBASTIÃO[1,2]
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Abstract: Social isolation (SI) has psychiatric outcomes, such as anxiety and depression. However, biomolecular mechanisms underlying how this negatively impacts health are poorly understood.In this work, we hypothesize that SI, as a psychological stressor, will activate neuroendocrine pathways and will ultimately lead to a chronic systemic inflammation. This can cause alterations in the crosstalk of the immune system and CNS. Subsequently, activation of neuroinflammatory pathways and learning/memory impairment causes a development and/or continuous anxiety- and depressive-like behavior. This work aimed to study if the modulation of the crosstalk between the systemic immune system and CNS enhances cognition in SI mice and ameliorates neuroinflammation. Thus, a mouse model of SI was used and the effect of Fingolimod (FTY720), a nootropic and an inhibitor of the peripheral immune cell trafficking, upon SI-induced anxiety-like behaviour was evaluated. Young and adult C57BL/J6 mice were divided into 4 groups. Two groups were SI for three weeks, with FTY720 treatment or DMSO (designated as control (CTL)). The remaining two groups were grouped house (GH) with 4-6 animals per cage, and submitted to the same two conditions. The treatment was given through drinking water *ad libitum*, since FTY720 is lipophilic. The number of animals in this first battery of tests ranged between 6-8. All statistical analyses were performed through two-way ANOVA, followed by Bonferroni's Comparison Test. First, behaviour was evaluated through appropriate tests. Depression was assessed through Forced Swim Test and anxiety by Open Field. Young mice SI-FTY720 showed a decrease in immobility time, when compared with CTL. Morris Water Maze was used to evaluate learning, during 8 trials, and spatial reference memory through a probe trial in the last day. However, neither SI nor FTY720 had an impact on learning and memory. Following the behavior tests, a molecular approached was performed, specifically

ELISA, to evaluate neuroinflammatory markers in this SI model. Protein levels of IL-1β in hippocampal tissue, showed a decrease with FTY720 in SI groups. However, only in adult mice, a statistical significance was obtained. In summary, in young mice FTY720 showed a significant improvement in anxiety- and depressive- behavior, as well as a tendency for decreased levels of IL-1β. These results point to a relevant role of FTY720 in SI young mice. However, further confirmation is required and a replication of this series must be conducted.

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Poster

408. Stress and the Brain: Neuroimmunology

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Topic: F.04. Stress and the Brain

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Title: How does the presence of exogenous opioids affect linear relationships between inflammatory cytokines and mu-opioid receptor availability?

Authors: *A. K. BAKER[1], A. V. BAKIAN[1], P. R. BURGHARDT[3], E. L. GARLAND[1], J.-K. ZUBIETA[1], T. LOVE[2,1]
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Abstract: Objective. Chronic pain is one of the most prevalent and pernicious health problems in the US. Opioids are the frontline treatment for chronic pain, contributing to alarmingly high and rapidly increasing rates of opioid misuse. Opioids alter pain through numerous pathways, including inflammatory cytokine signaling in the immune system and μ-opioid neurotransmission in the endogenous opioid system—pathways also implicated in the reward circuitry undergirding addiction. In examining the downward spiral from chronic pain to opioid misuse, it is prudent to examine relationships between these systems. **Methods**. This secondary analysis examined data obtained from a sample of chronic non-neuropathic back pain (CNBP) patients (N=66) and healthy controls (N=28). We examined bivariate relationships between CRP, IL-8, IL-18, & IL-1RA and μ-opioid binding potential (BP) in the amygdala (AMG), hippocampus (HIP) and nucleus accumbens (NAc), at baseline and during acute pain. **Results**. **Baseline.** Among controls, CRP was significantly negatively correlated with μ-opioid receptor BP in each region of interest (L AMG: r = -.83, p < .05; R AMG: r = -.87, p < .05; L HIP: r = -.91, p < .01; R HIP: r = -.78, p < .05; L NAc: r = -.88, p < .01, R NAc: r = -.94, p < .01). There were no significant correlations between cytokines and μ-opioid BP in any region in the CNBP

group. **Pain Challenge.** Among controls, there were no significant correlations between cytokines and change in μ-opioid BP during experimental pain. Among CNBP patients, IL-8 and IL-18 were correlated with ΔBP in the right NAc (r = .68, p < .05 and r = .80, p < .01, respectively), and IL-1RA was correlated with ΔBP in the left AMG (r = .55, p < .05) and bilateral HIP (L: r = .57, p < .05; R: r = .62, p < .05). **Opioids vs. No Opioids: Baseline.** Among those without exogenous opioids in urine, CRP was correlated with μ-opioid BP in the bilateral NAc (L: r = -.70, p < .01; R: r = -.56, p < .05). Patients with exogenous opioids in urine exhibited correlations between IL-8 and μ-opioid BP in the right AMG (r = .91, p < .05) and between IL-18 and μ-opioid BP in the right AMG (r = .92, p < .05) and bilateral HIP (L: r =.88, p < .05; R: r = .96, p < .01). **Opioids vs. No Opioids: Pain Challenge.** Among patients without exogenous opioids, IL-1RA was correlated with ΔBP in the left AMG (r = .73, p < .01) and left HIP (r =.60, p < .05). Those with exogenous opioids exhibited no significant correlations between cytokines and change in μ-opioid BP. **Discussion.** Results suggest the presence of relationships between inflammatory markers and the endogenous opioid system. Also, that exogenous opioids alter the manner in which the immune and nervous systems interact in the context of persistent pain.

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Poster

408. Stress and the Brain: Neuroimmunology

Location: SDCC Halls B-H

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Program #/Poster #: 408.12/UU13

Topic: F.04. Stress and the Brain

Support: NIH/NIAID U19AI067773
NIH/NIGMS R25GM060507
LLU Initiative for Maximizing Student Development

Title: Developing a radiation biodosimeter using exosomal miRNA isolated from murine brain tissue

Authors: *F. OYEFESO[1], N. NISHIYAMA[1], A. GONDA[2], N. WALL[2], M. PECAUT[1]
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Abstract: Exosomes are small vesicles (30-150 nm) released from the cell by exocytosis during normal cell function. They have been reported to carry proteins, viral components, and nucleic acids. Further, they may have functionally diverse roles including cell-cell communication, disease transfer, and cell waste management. Still, much is unknown about the specific function

of exosomes and isolating exosome from excised tissue is a relatively new research approach compared to traditional techniques. We are investigating the possibility of using the content of exosomes as a dose-specific radiodosimeter, focusing specifically on exosomal miRNA of brain tissue. We will compare the exosomal miRNA signature found in brain tissue with a similar profile characterized in the blood. We believe this will ultimately allow us to quickly assess any potential risks for radiation-induced neural tissue damage due exposure during cancer treatment, combat situations, or nuclear disasters (e.g. Fukushima & Chernobyl). Our parameters for this research are sex (male and female), whole-body gamma-radiation dose (0, 2, and 4 Gy), and time after exposure (4 and 48 hours).
As a first step, we are testing two independent methods for isolating exosomes from brain tissue to determine which will give us a higher yield and greater sample purity. We begin by homogenizing tissue from a mouse hemi-brain and using step-wise centrifugation to remove large cell components. Using one protocol, this step is followed by ultracentrifugation to pellet exosomes. The ultracentrifugation steps require a sucrose gradient (layered 2.5 M, 1.3 M, and 0.6) to purify exosomes apart from cell debris and other extracellular vesicles (Vella et al., 2018). Following the alternative protocol, the step-wise centrifugation is also followed by ultracentrifugation. This protocol requires a sucrose gradient with six layered 2-ml solutions starting from 0.25 M sucrose in 0.35 M increments (Perez-Gonzalez et al., 2012). After resuspending the exosome pellets, we have compared the isolation methods by quantifying exosome size, quantity, and purity using Nanoparticle Tracking Analysis (Malvern Panalytical). The research is ongoing, following completion of exosome isolation we will proceed to isolating total RNA to characterize miRNA type and quantity.

Disclosures: F. Oyefeso: None. **N. Nishiyama:** None. **A. Gonda:** None. **N. Wall:** None. **M. Pecaut:** None.

Poster

408. Stress and the Brain: Neuroimmunology

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 408.13/UU14

Topic: F.04. Stress and the Brain

Support: CIHR
NSERC
CGS Vanier

Title: The effects of early life stress on adult depressive-like behaviour, intestinal and blood-brain barrier permeability, and brain-immune interactions

Authors: *J. SZYSZKOWICZ[1,2], S. BARNETT BURNS[1,2], I. KIM[2], M.-C. AUDET[3], G. TURECKI[2,1], G. N. LUHESHI[2,1]

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Abstract: Background & Hypothesis: Early life stress (ELS) significantly increases the risk for adult psychopathology, including depression and suicide; however, the mechanisms of this vulnerability remain unclear. The gut microbiome (the bacteria, viruses, and eukaryotes that reside in our intestinal tract) may be a key player in this regard as it regulates many physiological processes related to depression including, hypothalamic-pituitary-adrenal (HPA) axis homeostasis and brain-immune interactions.
Hypothesis: ELS confers long-lasting changes in gut microbiome composition, intestinal and blood-brain-barrier permeability, and promotes pro-inflammatory brain-immune interactions, ultimately resulting in an increased risk for depression.
Methods: We are using male and female C57Bl/6J mice exposed to early life (P2-P9) limited bedding in which we are assessing depressive-like behaviours, HPA axis homeostasis, intestinal and blood-brain barrier permeability, brain-immune interactions, and gut microbiome composition. Concurrently, we are collecting post-mortem intestinal tissue from depressed suicides or controls in which we will repeat relevant measures of intestinal permeability, cytokine expression, and gut microbiome composition.
Results & Significance: Preliminary results from our animal studies suggest that early life limited bedding produces sex-specific deficits in social behaviour along with decreased hypothalamic expression of corticotrophin releasing hormone (CRH) along with decreased expression of claudin-5 in both the hypothalamus and colon of these animals, suggesting altered HPA axis and blood-brain and intestinal barrier disruption respectively. These early observations are indicative of important changes in the multiple systems (brain, immune, gut) targeted for analysis as part of our studies and add support to our hypothesis that ELS triggers an integrative response that may be initiated by fundamental alterations in the microbiome.

Disclosures: J. Szyszkowicz: None. **S. Barnett Burns:** None. **I. Kim:** None. **M. Audet:** None. **G. Turecki:** None. **G.N. Luheshi:** None.

Poster

408. Stress and the Brain: Neuroimmunology

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Program #/Poster #: 408.14/UU15

Topic: F.04. Stress and the Brain

Support: Fondation du CHU Excellence Scholarship
Canada Research Chair in Neuroimmune Plasticity in Health and Therapy
NSERC Grant

Title: Phenotypic features of glucocorticoid receptor-deficient microglia in a mouse model exposed to chronic stress

Authors: *K. PICARD[1], K. BISHT[1], K. P. SHARMA[1], C. LIMATOLA[2], L. MAGGI[2], I. BRANCHI[3], M.-E. TREMBLAY[1]
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Abstract: Microglia, the resident immune cells of the brain, are active participants in the plastic remodeling of neuronal circuits. They are also very sensitive to changes in their environment, notably resulting from the exposure to chronic stress, well-known for predisposing to neuropsychiatric disorders and neurodegenerative diseases. Upon stress exposure, corticosterone released by the adrenal gland binds to glucocorticoids receptors (GR), throughout the body, and mainly within the hippocampus, amygdala and prefrontal cortex among the brain. Corticosterone in physiological concentrations promotes the formation of dendritic spines, while in high concentrations, it rather induces their elimination. GR receptors are abundantly expressed by microglia, but their roles are not well known. We hypothesize that deletion of microglial GR in adult mice exposed to chronic unpredictable stress would alter the homeostatic functions of microglia, particularly at synapses.
We generated mice in which microglia are selectively deficient in GR. In particular, CX3CR1-CreER mice were crossed with GR-floxed mice. The resulting mice (microglial GR-KO) received tamoxifen at adulthood. We evaluated their behavior using the Intellicage system, which randomly exposes mice to different stressors in addition to monitoring their behavior. The mice were housed 12 per cage (6 controls and 6 microglial GR-KO). They were habituated to the cages for two weeks, then exposed to chronic stress or left undisturbed for another two weeks. The stress paradigm revealed a greater vulnerability to stress in the microglial GR-KO mice. These mice were then perfused with aldehydes. Brains were cut using a vibratome and sections containing the hippocampus were immunolabeled with the marker Iba1, expressed by myeloid cells, and Tmem119, which is specific to microglia, thus allowing to differentiate microglia from peripheral myeloid cells. Using confocal analyzes, we observed no changes in the density and distribution of microglia in the hippocampus of microglial GR-KO mice and their controls, whether exposed to stress or not. There were also no changes in the infiltration of peripheral myeloid cells. Analyzes are in progress to evaluate possible changes in microglial morphology. Complementary observations will be made by electron microscopy, to analyze the ultrastructure of microglia and characterize their interactions with synapses.
Our study will provide novel insights into the specific contribution of microglial GRs in the adaptation of the brain and behavior to chronic stress. This understanding could ultimately lead to the development of treatments reducing the negative effects of stress.

Disclosures: K. Picard: None. **K. Bisht:** None. **K.P. Sharma:** None. **C. Limatola:** None. **L. Maggi:** None. **I. Branchi:** None. **M. Tremblay:** None.

Poster

408. Stress and the Brain: Neuroimmunology

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Program #/Poster #: 408.15/UU16

Topic: F.04. Stress and the Brain

Support: Ohio State University startup funds to KML
Ohio State University graduate fellowship to AIS
NIH R21 MH105826 to KML
NARSAD Young Investigator Award to KML

Title: Early life stress effects on mast cell degranulation and blood-brain barrier function depend on the severity and chronicity of stress

Authors: *A. I. SAULSBERY[1], C. E. PAGE[2], L. H. NELSON[2], C. M. DODSON[2], K. M. LENZ[1]
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Abstract: Early-life adversity in humans has been associated with vulnerability to adult mood and anxiety disorders. The causes of this vulnerability are not fully understood. In adult rats, chronic stress compromises blood-brain barrier (BBB) integrity and promotes depression- and anxiety-like behavior (Menard et al., 2017; Weber, Godbout, & Sheridan, 2017). We seek to determine whether stress increases BBB permeability in the developing brain via mast cell activity, and whether sex differences in BBB function exist at baseline or in response to early life stress. Mast cells are innate immune cells that colonize the developing brain and release vasodilators, histamine, serotonin, and proteases through a constitutive process called degranulation. Mast cells also regulate vascular permeability in the periphery and secrete cytokines and prostaglandins independent of degranulation during inflammogenic insults. Male and female Sprague-Dawley rats underwent one of several stressors: acute maternal separation stress (MSS) on postnatal day (P) 2; brief (15 min) daily MSS (e.g., handling) from P4-11, 3hrs/day of MSS from P1-7, or no stress. Brains from the acute MSS and brief daily MSS experiments were sectioned and stained with toluidine blue at stressor completion to visualize mast cells. Mast cells were counted and classified as granulated or degranulated. The proportion of degranulated mast cells did not differ between acutely stressed and control rats, although acute stress reduced the number of degranulated mast cells relative to control. Brief daily MSS increased total mast cells (but not degranulated mast cells) in the hippocampus relative to control. Regardless of stress, females in this experiment had a lower proportion of degranulated mast cells than males. Rat pups subjected to 3hrs/day of MSS were killed on P7. Hippocampi and hypothalamuses were rapidly dissected from these rats. In ongoing experiments, we are assessing expression of BBB protein genes and mast cell-related genes across stress conditions.

We predict that 3hr/day MSS during the first week of life will decrease expression of BBB protein genes, such as those for claudin-5, occludin, and tight junction proteins. Increased BBB permeability could alter brain development and increase vulnerability to depression- and anxiety-like behavior by facilitating trafficking of pro-inflammatory cytokines and peripheral immune cells into the brain. This work will help determine how early life stress creates vulnerability to adult psychological disorders, possibly enhancing early intervention efforts following early-life adversity.

Disclosures: A.I. Saulsbery: None. **C.E. Page:** None. **L.H. Nelson:** None. **C.M. Dodson:** None. **K.M. Lenz:** None.

Poster

408. Stress and the Brain: Neuroimmunology

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Topic: F.04. Stress and the Brain

Support: NIH Grant R01-MH0104344
Veteran's Administration VISN 22 Mental Illness Research, Education, and Clinical Center.

Title: Altered photoperiod exposure induces a neuroinflammatory response in mice

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Abstract: BACKGROUND: Seasonal variations in daylight can influence switching between mania and depression in patients with bipolar disorder (BD), i.e., more frequent depression episodes in the fall/winter vs. mania episodes in the spring/summer. Exposure to a winter-like short active (SA) photoperiod induces depression-like behavior plus elevated corticosterone levels (glucocorticoid hormone involved in stress response). Exposure to a summer-like long active (LA) photoperiod induces a risk-prone behavioral profile. Stress can regulate inflammation and certain cytokines can directly influence and alter brain structures important in emotional regulation. The current study assessed whether similarly altered photoperiod exposure induced neuroinflammatory changes in mice.
METHODS: Female C57BL/6J mice were exposed to either: 1) normal active (NA; 12:12 L:D; n=5), 2) short active (SA; 19:5 L:D; n=6), or 3) long active (LA; 5:19 L:D; n=4) photoperiod for two weeks. On day 14, brains and trunk blood were collected to analyze cytokine levels by enzyme-linked immunosorbent assay (ELISA). Cytokines assessed include interleukin (IL)-1β, IL-6, tumor necrosis factor alpha (TNFα), IL-1 receptor antagonist (IL-1Ra), and IL-10. C-

reactive protein (CRP) levels were also assessed.
RESULTS: A main effect of photoperiod for plasma IL-10 levels ($F_{(2,10)} = 6.9$, $p<0.05$) was observed, with elevated levels in LA-exposed mice vs. NA- and SA-exposed mice. No other significant group differences were seen in plasma cytokine or CRP levels. There were no significant group differences in brain cytokine levels or brain CRP levels; however, nonsignificant elevations in brain IL-6, TNFα, and IL-1Ra were observed.
DISCUSSION: Two weeks of altered photoperiod exposure induces elevations in proinflammatory cytokines in the mouse brain. While SA- and LA-induced increases in brain IL-6 and TNFα were not significant, elevated proinflammatory cytokines can disrupt neurotransmitter systems (e.g. serotonin, dopamine). Brain regions involved in emotional regulation, including the anterior cingulate cortex, hippocampus, and amygdala, are also particularly affected by proinflammatory cytokines. Neuroinflammatory changes, therefore, may contribute to the behavioral profiles observed in mice after altered photoperiod exposure. Finally, IL-1Ra levels tend to increase after IL-1 levels to terminate an inflammatory response. Assessing neuroinflammation at the beginning of altered photoperiod exposure will determine whether the current data represent the termination of a greater initial photoperiod-induced neuroinflammatory response.

Disclosures: M.A. Kwiatkowski: None. **Z.A. Cope:** None. **J. Deslauriers:** None. **J.W. Young:** None.

Poster

408. Stress and the Brain: Neuroimmunology

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Program #/Poster #: 408.17/UU18

Topic: F.04. Stress and the Brain

Support: NSERC RGPIN-2016-06146

Title: Chronic social defeat in male mice elicits long-lasting changes to intestinal barrier integrity and pro-inflammatory cytokine activation

Authors: A. D. SANTOS[1,2], N. D. OSBORNE[2,3], M. SEDRAK[1], H. ANISMAN[1,2], *M.-C. AUDET[4,1,2,3]
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Abstract: Pro-inflammatory activation resulting from socially stressful experiences or immune insults has been associated with the pathogenesis of depressive illnesses. In mouse models, social stressors known to elicit depressive- and anxiety-like behaviours also increase circulating and

brain expression of pro-inflammatory cytokines, which has been suggested to stem from perturbations of gut microbiota composition and intestinal membrane integrity. The aim of the current study was to examine, in male mice, the long-lasting effects of a chronic social stressor on intestinal barrier integrity and pro-inflammatory cytokine expression along the gut-brain axis. Male C57BL/6 mice experienced a social defeat stressor (10-minute exposure to an aggressive resident mouse, followed by sensory contact for 24 hours) or a control condition (sensory contact with a non-aggressive resident for 24 hours) for 10 consecutive days. Five weeks later, blood, intestinal tissue from the jejunum, and brain tissue from the hippocampus were collected to determine plasma corticosterone levels and mRNA expression of the pro-inflammatory cytokines interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF)-α, the tight junction proteins occludin-1, zonula occluden-1, claudin-2, and the neurotrophin brain-derived neurotrophic factor (BDNF). Compared to non-stressed mice, chronically defeated mice had increased mRNA expression of IL-6, IL-1β, and TNF-α in the jejunum, but comparable expression of these cytokines in the hippocampus. Jejunal occludin-1 expression was reduced in mice that had been chronically defeated whereas the other tight junction proteins were unaffected. Finally, BDNF expression in socially stressed mice was decreased in the hippocampus. These findings demonstrate that chronic social defeat elicits long-lasting inflammatory activation and changes to barrier integrity in the jejunum. Perturbations of the intestinal membrane may increase the likelihood of microbial translocation into the intestinal mucosa and circulation, which may then drive pro-inflammatory activation along the gut-brain axis.

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Poster

408. Stress and the Brain: Neuroimmunology

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 408.18/UU19

Topic: F.04. Stress and the Brain

Title: Regional examination of glucocorticoid receptors and endogenous opioid signaling genes in self injurious behavior

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Abstract: Abstract
The DSM-V defines Non-suicidal self-injury (NSSI) as the deliberate infliction of physical harm to one's own body without suicidal intent. While NSSI is by definition, not an attempt at suicide,

it is strongly associated with future suicide ideation. Self-injurious behavior (SIB) occurs in approximately 1-4% of the adult human population, with higher rates of incidence in the adolescent and institutionalized populations. With such a prevalent impact on our society, it is important to understand the underlying mechanisms that may lead a person to self-harm. Disruptions in endogenous opioid signaling and stress regulation have been proposed as potential contributing factors in NSSI and SIB. SIB also occurs in a low percentage of captive monkeys; it is an endogenously occurring model of SIB in humans in the context of captive animals. Rhesus Macaque monkeys are evolutionarily and physiologically similar to humans, share 93% of their DNA with humans. To study SIB, we used 8 sex-matched pairs of rhesus macaques, eight who exhibited self-injurious behavior (SIB) and eight controls, to examine alterations in gene expression. We examined genes in the endogenous opioid pathway as well as the glucocorticoid and mineralocorticoid receptors. The brain regions chosen are those closely linked to reward reinforcement and stress adaptation. Thus far, our findings suggest no uniform changes in gene expression of the mu-opioid receptor, mineralocorticoid receptor, glucocorticoid receptor, β-endorphin precursor molecule proopiomelanocortin (POMC), or the dynorphin A and B precursor prodynorphin in the regions tested. We did observe that females with SIB had reduced amounts of mu-opioid receptor in the amygdala ($p<0.001$), and a trend towards decreased prodynorphin in the Orbitofrontal cortex ($p=0.083$). Males with SIB had a significant increase in glucocorticoid receptor expression in the amygdala ($p<0.05$), whereas females with SIB had a significant decrease in mineralocorticoid receptors in the amygdala ($p<0.01$) and a significant decrease in glucocorticoid receptors in the hippocampus ($p<0.01$). It is our aim to expand these studies with six additional macaque brains from males with SIB that have been collected for mRNA extraction. Our data provides further evidence that regulation of stress responses, particularly in the amygdala, is playing a role in the behavioral expression of SIB. Previous studies have also identified reactive changes in astrocytes of animals exhibiting SIB. We have therefore collected brains from 13 additional rhesus macaques, 10 who exhibited SIB, to examine morphological changes in astrocytes via immunohistochemistry.

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Poster

408. Stress and the Brain: Neuroimmunology

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Program #/Poster #: 408.19/UU20

Topic: F.04. Stress and the Brain

Support: IOER Funds

Title: Relationship between microbiome convergence and stress susceptibility

Authors: *J. GIANUZZI[1], M. LAMPETER[1], L. YUAN[2]
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Abstract: JG and ML contributed equally.
Stress and stress-related disorders have been linked to both genetic and environmental factors. The gut microbiome has been suggested to exert influence on physiology and stress-related behaviors in humans, termed the gut-brain axis. This investigation aims to quantify the relationship between stress susceptibility/resilience and microbiome changes. Both cognitive and microbiome changes are experienced by individuals subject to new stressors. The association between microbiome states and stress resilience is of great current interest. We evaluate the relationship between cognitive resilience and microbiome convergence over time in newly matriculated medical students (n=31) who are subjected to novel stressors during their first semester of medical school. Using both subjective (Patient Health Questionaire-9, PHQ9, and Cohen's Perceived Stress Survey, PSS) and objective (Cortisol Level) assessments, participants were divided into stress susceptible and resilient groups. Their fecal samples were collected at different time points. Bacterial genomic DNA was extracted and amplicons representing the V4 region of 16s rRNA genes were amplified and sequenced. We use QIIME2 and PiCRUST for taxonomic and functional comparisons, respectively. Those include comparison of microbiome percent observed convergence (POC) between stressor resilient vs. susceptible groups. Our goal is to test whether specific microbiome changes are associated with stress resilient or susceptible groups Thorough understanding of intricacies of the gut-brain axis have potential to influence treatment of stress-related disorders and uncover new directions for genomic analyses of gut microbiota in parallel with human genomic analysis.

Disclosures: J. Gianuzzi: None. **M. Lampeter:** None. **L. Yuan:** None.

Poster

408. Stress and the Brain: Neuroimmunology

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Topic: F.04. Stress and the Brain

Support: NIMH Grant MH-109165
NIMH Grant MH099482
NIDCR Training Grant DE014320

Title: Glutamatergic neuronal IL-1R1 is necessary for paired fighting stress-induced social withdrawal and working memory deficits

Authors: *D. J. DISABATO[1,2], D. NEMETH[2], X. LIU[2], B. OLIVER[3], J. GODBOUT[1,3], N. QUAN[2,3]
[1]Neurosci., [2]Biosci., [3]Inst. for Behavioral Med. Res., The Ohio State Univ., Columbus, OH

Abstract: Chronic stress is associated with an increase in prevalence of mental health complications such as anxiety and depression. We have previously reported that chronic social stress causes microglial activation, monocyte infiltration into the brain, and increased interleukin-1β (IL-1β) signaling in association with prolonged anxiety-like behavior. We hypothesized that IL-1β signaling in the brain was the key player in this stress-mood disorder interaction. In the present study, we used a modified paired fighting (PF) social stress paradigm to induce social stress. To examine the significance of IL-1 signaling in these processes, we used a global IL-1 receptor 1 (IL-1R1) reporter mouse (IL-1R1$^{GR/GR}$) and a global IL-1R1 knockout, the IL-1 receptor restore model (IL-1R1$^{r/r}$). Following exposure to six days of PF, both IL-1R1$^{GR/GR}$ mice and IL-1R1$^{r/r}$ mice had increases in neutrophils and Ly6C^{hi} reactive monocytes in circulation, but only IL-1R1$^{GR/GR}$ mice had an increased percentage of peripherally-derived leukocytes in the brain after PF. IL-1R1$^{GR/GR}$ mice alone displayed general withdrawal in a social interactivity test with juvenile C57BL/6 mice, and a percent decrease in spontaneous alternations in the Y-maze, a measure of functional working memory. IL-1R1$^{r/r}$ mice did not display these changes. Because of the importance for glutamatergic signaling in working memory tasks, we repeated the PF protocol with vGlu2Cre-IL-1R1$^{f/f}$ mice in which IL-1R1 has been deleted only on glutamatergic neurons. Following PF, vGlu2Cre-IL-1R1$^{f/f}$ mice show increased leukocytes in circulation, monocyte infiltration, and microglial morphological alterations similar to Cre^{-} WT controls. However, vGlu2Cre-IL-1R1$^{f/f}$ mice do not display behavioral changes after PF. Taken together, these results show that PF-induced social withdrawal and working memory deficits are dependent upon glutamatergic neuronal IL-1R1 signaling.

Disclosures: D.J. Disabato: None. **D. Nemeth:** None. **X. Liu:** None. **B. Oliver:** None. **J. Godbout:** None. **N. Quan:** None.

Poster

408. Stress and the Brain: Neuroimmunology

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Program #/Poster #: 408.21/UU22

Topic: F.04. Stress and the Brain

Support: NIH Grant R01 MH-109165

Title: Structural and functional cytoarchitecture of IL-1R1-expressing system in the brain

Authors: *X. LIU[1], D. NEMETH[2], D. B. MCKIM[1], O. BERDYSZ[1], G. GORANTLA[1], J. P. GODBOUT[3], J. F. SHERIDAN[4], L. ZHU[4], N. QUAN[5]

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Abstract: IL-1R1 signaling is important for the pathogenesis for almost all CNS diseases. Here, we demonstrate the cell-type specific IL-1R1 expression patterns in the brain using a knockin reporter. The functions of IL-1R1 are then investigated by selective reciprocal deletion or expression of IL-1R1 on specific cell types. Endothelial IL-1R1 is found to mediate sickness behavior, leukocyte recruitment, impaired neurogenesis, and inflammatory microglial activation, whereas ventricular IL-1R1 is found critical for monocyte recruitment and non-inflammatory microglial activation. In contrast, the astrocytic IL-1R1 signaling dampens inflammatory cytokines, while neuronal IL-1R1 can promote microglial activation. In addition, IL-1 stimulates its own production in microglia indirectly through endothelial IL-1R1. Collectively, these findings describe the structural and functional cytoarchitecture of the brain IL-1R1 expressing system and lay a foundation for the dissection and identification of IL-1R1 signaling pathways in the pathogenesis of numerous CNS diseases.

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Poster

408. Stress and the Brain: Neuroimmunology

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 408.22/VV1

Topic: F.04. Stress and the Brain

Support: Conacyt CB221653 to MA

Title: Social defeat-induced interleukin 6-dependent behavioral changes in c57bl/6 mouse

Authors: *M. ATZORI[1], J. VARGAS-MIRELES[2], E. ESQUIVEL RENDON[3], P. ACOSTA-MARES[2], R. D. CUEVAS OLGUIN[2], M. MIRANDA-MORALES[4], G. RODRÍGUEZ-ESCOBEDO[2], S. ROSE-JOHN[5]
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Abstract: Social stress is an important trigger for neuropsychiatric disease. The pro-inflammatory cytokine interleukin 6 (IL-6) has been positively correlated with the onset of numerous stress-triggered neuropsychiatric conditions including schizophrenic psychoses and depression. This study sought to assess the IL-6 dependence of behavioral changes elicited by

social stress. To investigate this issue, we compared the behavior of wild type animals (WT) with that of genetically modified mice in which IL-6 central trans-signaling was blocked by overexpression of a soluble version of the IL-6 transducer glycoprotein 130 by the promoter of the astrocytic marker Glial Fibrillary Acidic Protein (GFAP-sgp130Fc, TG). C57BL/6 mice where submitted to a well-established chronic Social Defeat (SD) protocol, consistent in a 10 min exposure every day during 10 days to a preselected aggressive CD1 mouse. Four experimental groups were used for the experiments: control WT, SD-exposed WT, control TG, and SD-exposed TG mice. Both WT and TG SD-exposed animals were further divided into SD-resilient (R) and SD-sensitive mice (S), according to their response to a peculiar Open Field test measuring the Corner Preference, an index of sensitivity to SD. SD greatly reduced the time in the zone of contact with the CD1 mouse, and increased the time in the corner and corner preference in the Open Field test, equally in WT and TG animals. WT and TG animals displayed similar frequency of R vs. S animals (32% for WT, vs. 41% TG), suggesting that functional IL-6 central trans-signaling is not necessary for SD effectiveness in the induction of the fear response. On the contrary, SD decreased time in the center and time in the closed arms in the Plus Maze test, as well as the sucrose preference and the Porsolt forced swim time-to-immobility, only in WT but not in TG mice. Our results suggest that while central IL-6 trans-signaling may not be not necessary for the induction of the fear response, it may be selectively involved in other emotional behaviors like anxiety, depression, and anhedonia.

Disclosures: M. Atzori: None. **J. Vargas-Mireles:** None. **E. Esquivel Rendon:** None. **P. Acosta-Mares:** None. **R.D. Cuevas Olguin:** None. **M. Miranda-Morales:** None. **G. Rodríguez-Escobedo:** None. **S. Rose-John:** None.

Poster

408. Stress and the Brain: Neuroimmunology

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 408.23/VV2

Topic: F.04. Stress and the Brain

Support: NIMH IRP

Title: Revealing repair roles for inflammatory monocytes in neurovascular dysfunction caused by chronic social defeat

Authors: *M. L. LEHMANN[1], C. CROSSEN[2], S. L. KIGAR[3], M. HERKENHAM[4]
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Abstract: Psychosocial stressors, which contribute to the development of affective disorders in humans, induce central and peripheral immune pathway signaling that is increasingly thought to be relevant to the pathophysiology of depression. Previously we showed that 14 days of chronic social defeat (CSD), a model of psychosocial stress, triggered a change in blood-brain barrier (BBB) permeability coupled with the leakage of intravascular substances into brain parenchyma, i.e., microhemorrhages. Peripheral monocyte subtypes that express chemokine receptor CCR2 have been implicated in the pathogenesis of several different disease processes, including vascular permeability and numerous neuropathologies associated with inflammation and microhemorrhage. We wanted to understand how $CCR2^+$ cells contribute to BBB pathologies that occur in response to CSD. We found that CSD induced pronounced declines in behavioral tests of sociability coupled with scattered microhemorrhages in brain, but it had no effect on either the anatomical distribution or density of $CCR2^+$ monocytes adhered to vasculature. However, a substantial elevation in numbers of adhered $CCR2^+$ cells was detected if mice were given seven days to recover from CSD stress in the homecage environment. Next, we asked if microglia influence $CCR2^+$ cell trafficking to brain vasculature during or after CSD. Microglia were ablated by oral administration of the CSF-1R inhibitor PLX5622. Mice treated with PLX5562 and then exposed to CSD showed substantially elevated numbers of $CCR2^+$ cells associated with the vasculature; however, there was no apparent anatomical specificity. In contrast, animals that had undergone CSD stress with intact microglia showed no elevated recruitment. The inability of stress to increase $CCR2^+$ cell recruitment to brain diminishes the likelihood that monocytes play a causal role in the induction of neurovascular pathology during CSD. However, the elevation in $CCR2^+$ cell recruitment during recovery from stress highlights a potential role for monocyte/macrophages in mediating neurovascular repair. Lastly, the global trafficking pattern suggests that events causing $CCR2^+$ cell recruitment are not causally related to regional neuronal or microglia activation. Phenotyping of $CCR2^+$ cells is ongoing. Further investigation will examine peripheral factors responsible for BBB pathology in stressed mice.

Disclosures: M.L. Lehmann: None. **C. Crossen:** None. **S.L. Kigar:** None. **M. Herkenham:** None.

Poster

408. Stress and the Brain: Neuroimmunology

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 408.24/VV3

Topic: F.04. Stress and the Brain

Support: NIA R01 AG051902
OSU Dean's Distinguished University Fellowship
NIDCR T32 DE014320

Title: Microglia are extrinsically primed by the aged microenvironment

Authors: *S. M. O'NEIL, K. G. WITCHER, D. B. MCKIM, J. P. GODBOUT
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Abstract: Microglia, the resident innate immune cells of the CNS, are responsible for propagating inflammatory signals from the periphery to the brain, where they drive the behavioral sickness response. With normal aging, these cells develop a pro-inflammatory, "primed" profile with increased expression of inflammatory mediators. Moreover, secondary immune challenge causes an exaggerated and prolonged neuroinflammatory response mediated by primed microglia in the aged brain. A critical question is if this age-associated profile can be reversed. Recent studies show CSF1R antagonism results in profound elimination of microglia in adult mice. Therefore, we hypothesized CSF1R antagonist-mediated depletion of microglia in the aged brain would result in repopulation of new, unprimed microglia. Here we provide novel evidence that microglia in the brain of adult and aged mice were robustly eliminated following 3-week oral administration of CSF1R antagonist PLX5622. When the CSF1R antagonism was stopped, microglia repopulated the adult and aged brain at the same rate and efficiency with new cells no longer burdened with lipofuscin, the hallmark lipid debris of aging. FAC-sorting and RNA-Seq analysis of microglia revealed these new microglia took on the same primed mRNA profile as the cells they replaced. Moreover, RNA-Seq analysis of the brain provided evidence of reactive astrogliosis in aged mice independent of microglial depletion/repopulation. Lastly, peripheral innate immune challenge still caused an exaggerated microglial inflammatory response in the aged brain with prolonged behavioral deficits. These data indicate the local microenvironment of the aged brain significantly influences the profile of repopulating microglia. Taken together, aged microglia can proliferate and repopulate the CNS, but the resulting "new" microglia still adopt a pro-inflammatory profile characteristic of aging.

Disclosures: S.M. O'Neil: None. **K.G. Witcher:** None. **D.B. McKim:** None. **J.P. Godbout:** None.

Poster

408. Stress and the Brain: Neuroimmunology

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 408.25/VV4

Topic: F.04. Stress and the Brain

Support: NIMH Grant R01-MH093473
NIMH Grant R01-MH097243

Title: Social defeat of female mice recapitulates neuroimmune and behavioral responses detected in males

Authors: *W. YIN, D. B. MCKIM, N. R. GALLAGHER, Y. WANG, J. P. GODBOUT, J. F. SHERIDAN
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Abstract: Repeated Social Defeat (RSD) has been used extensively to study the biological and behavioral responses to chronic psychosocial stress in pre-clinical rodent models. In this model, an aggressive intruder is introduced into a cage of resident mice with an established dominance hierarchy, and subsequently attacks and defeats the residents. RSD in mice successfully recapitulates key immunologic findings from humans with anxiety or mood disorders, including increased circulating IL-6 and proinflammatory monocytes. However, studies using RSD generally exclude female mice due to difficulty eliciting aggression among female cohorts. The inclusion of females in murine studies of chronic psychosocial stress is an important consideration because anxiety and mood disorders are highly prevalent among the human female population. Here, we used a modified aggressor, an estrogen receptor α (Esr1)-Cre mouse injected with a conditional Gq-DREADD expressed in the ventrolateral part of the ventromedial hypothalamus (VMHvl), to socially defeat female mice. Upon injection of a designer drug, the ER+ neurons in the VMHvl are activated, producing indiscriminate aggression. Male Esr1-Cre (DREADD) aggressors attacked female C57BL/6 mice and elicited subordinate behaviors. Following six consecutive days of defeat by DREADD aggressors, female mice exhibited anxiety-like behavior and social avoidance. Social defeat in females activated neurons and microglia in regions of the brain associated with threat appraisal, including the amygdala, prelimbic cortex, and hippocampus. Social defeat also caused a peripheral immune response characterized by enhanced myelopoiesis, monocyte release and trafficking to the spleen and brain, and elevated plasma IL-6. Social defeat also increased IL-1β expression in the brain. These findings resemble previous studies conducted in male mice, suggesting that the peripheral immune response to defeat stress is similar across both sexes. Modified DREADD aggressors may be an important means to include female mice in future studies using repeated social defeat stress.

Disclosures: W. Yin: None. **D.B. McKim:** None. **N.R. Gallagher:** None. **Y. Wang:** None. **J.P. Godbout:** None. **J.F. Sheridan:** None.

Poster

409. Biological Rhythms and Sleep: Entrainment and Phase Shifts

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 409.01/VV5

Topic: F.08. Biological Rhythms and Sleep

Support: National Eye Institute Grant EY008117
National Institute on Deafness and other Communication Disorders Grant DC007864

National Institute of General Medical Sciences Grant GM102965
National Institute of General Medical Sciences Grant GM107405
National Science Foundation GRFP Fellowship

Title: Novel photoreceptors entrain the circadian clock and modulate uv light-evoked behaviors in *Drosophila*

Authors: *L. BAIK[1], J. NI[2], Y. RECINOS[1], J. A. CHEVEZ[1], C. MONTELL[3], T. C. HOLMES[1]
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Abstract: Animals' ability to anticipate and adapt to daily changes in the environment through their internal circadian clock is crucial for survival. The circadian clock is entrained by daily oscillation of day/night cycles of light. In *Drosophila*, three distinct phototransduction pathways entrain the circadian clock: 1) CRYPTOCHROME (CRY) and 2) internal opsin-based RHODOPSIN7 (RH7) and 3) external opsin-based photoreceptors. We show that while mutation of either CRY or RH7 induces minor defects, loss of both CRY and RH7 leads to severe impairment in photoentrainment. We show that CRY and RH7 are expressed in a subset of the brain circadian neurons including the pigment dispersing factor (PDF)-positive lateral ventral neurons (LNv). CRY and RH7 mediate electrophysiological response to different spectral peaks of short wavelength light in large-LNvs (l-LNv). Reciprocally, the circadian clock regulates UV light-evoked attraction/avoidance behavior. Rhythmic UV light avoidance is crucial for avoiding potentially harmful environment conditions and may prevent desiccation. Here we show that the circadian clock and circuit modulates the valence and time-of-day dependent rhythm of UV light avoidance/attraction behavior. Flies lacking CRY or RH7-based phototransduction have attenuated UV light avoidance. Circadian mutant flies lacking core clock genes lack time-of-day dependent changes in UV light avoidance behavior. Non-genetic environmental disruption of the circadian clock by constant UV light exposure leads to complete loss of rhythmic UV light avoidance behavior. Flies with ablated or electrically silenced LNvs have attenuated avoidance response to UV light. We conclude that core circadian proteins, photoreceptors, and the pacemaker lateral ventral neurons regulate both the timing and the valence of UV light avoidance/attraction. Circadian modulated sensory responses contribute adaptively to behaviors in *Drosophila* to limit risk of environmental exposure during the hottest part of the day.

Disclosures: L. Baik: None. **J. Ni:** None. **Y. Recinos:** None. **J.A. Chevez:** None. **C. Montell:** None. **T.C. Holmes:** None.

Poster

409. Biological Rhythms and Sleep: Entrainment and Phase Shifts

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 409.02/VV6

Topic: F.08. Biological Rhythms and Sleep

Title: Temperature entrainment of two different circadian rhythms in the flesh fly

Authors: *R. RAGSDALE, K. JOPLIN, D. MOORE
Biol. Sci., East Tennessee State Univ., Johnson City, TN

Abstract: It is well known that 24-hour day-night (light-dark) cycles can entrain the circadian rhythms of most species possessing circadian clocks. However, much less understood are other environmental cycles that may synchronize (entrain) the internal clock with the outside world. Potential non-photic time cues (zeitgebers) include daily cycles of temperature, food availability, and social signals. The goal of this project is to evaluate the efficacy of temperature cycles administered during the pupal and adult stages, ranging from 1°C to 10°C in amplitude, as potential zeitgebers for two different circadian rhythms, eclosion and locomotor activity, in the flesh fly (*Sarcophaga crassipalpis*). Both rhythms were monitored in male and female flies, using infrared motion detectors, under precisely controlled 24-h temperature cycles (12 h thermophase, 12 h cryophase) in constant darkness. In both sexes, our results show clear entrainment of eclosion (a once-in-a-lifetime event) and locomotor activity (reflecting daily sleep-wake rhythms) in response to temperature cycles at amplitudes of 2.5 (n= 28), 5 (n= 54), and 10^{o} C (n= 102). Both rhythms adopted a fixed phase position of acrophase (peak of the rhythm) within 1-2 h after the onset of thermophase. However, at 1^{o} C amplitudes (n= 26), the phase position occurred several hours earlier, during mid-cryophase, suggesting a continued influence of a light-dark cycle experienced two weeks prior to eclosion during the larval phase. Thus, the 1^{o} C amplitude cycles may be near the threshold for detection by the entrainment pathways communicating with the circadian clock. Interestingly, the entrainment profiles are remarkably different for light and temperature cycles, suggesting that these two zeitgebers activate different behavioral programs. Finally, the endogenous free-running periods under different constant temperatures yield a Q_{10} of 0.989, indicating an exceptionally precise level of temperature compensation. Our results provide conclusive evidence that temperature is a strong circadian zeitgeber in flesh flies, thereby expanding the known repertoire of environmental cues these organisms use to sync their internal clock with the world around them. These findings also set the stage for future experiments designed to explore the interactions between light and temperature entrainment mechanisms - these zeitgeber interactions almost certainly occur in nature but have received little or no attention.

Disclosures: R. Ragsdale: None. **K. Joplin:** None. **D. Moore:** None.

Poster

409. Biological Rhythms and Sleep: Entrainment and Phase Shifts

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 409.03/VV7

Topic: F.08. Biological Rhythms and Sleep

Support: NIH GM102965
NIH GM107405

Title: Social jet lag evokes *Drosophila* circadian neural network desynchrony

Authors: *C. E. NAVE[1], L. ROBERTS[2], J. D. ESTRELLA[2], N. PERVOLARAKIS[3], P. J. SHAW[4], T. L. LEISE[5], T. C. HOLMES[2]
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Abstract: Weekly weekday-to-weekend shifts in sleep-wake cycles produce social jet lag (SJL), driven by artificial photic cues which shift our biological clocks in a manner similar to travel across time zones. We compared whole circadian circuit responses to un-shifted day/night and SJL-shifted cycling at single cell-resolution in cultured adult *Drosophila* brains for 11-days using real-time bioluminescence recordings of *per* gene cycling. Un-shifted circadian circuits show highly synchronous oscillation. In contrast, 3hr SJL weekend shifts significantly dampens oscillator synchrony and rhythmicity in most circadian circuit neurons for multiple days during and after shifts, while a small circadian subset robustly increase synchrony in response to 3hr SJL shifts. SJL shifts cause transient defects in memory and learning and sleep stability between 5-6 days of the week. Similarities in animal circadian biology suggest that SJL-induced defects are likely applicable to humans.

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Poster

409. Biological Rhythms and Sleep: Entrainment and Phase Shifts

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 409.04/VV8

Topic: F.08. Biological Rhythms and Sleep

Support: ANII (Uruguay), Grant FCE_3_2016_1_126178
CSIC (UDELAR, Uruguay)

Title: Social and environmental influences in circadian rhythmicity of electric behavior

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[3]Unidad Bases Neurales de la Conducta, [4]Inst. de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay

Abstract: Animal behavior is synchronized with environmental clues resulting in the synchronization of conspecifics activity. As biological rhythms are part of the integrative control of the brain, it is fundamental to explore its interaction with environmental and social influences. Nocturnal arousal in pulse type gymnotiform fish is associated to an increase in the basal rate of the electric organ discharge (EOD-BR). This characteristic electric behavior is modulated by social context through the neuropeptide arginine-vasotocin (AVT). Environmental influences on rhythmic behavior are tightly related to the melatonin system throughout vertebrates. In *Gymnotus omarorum*, isolated animals in laboratory settings, with a 12:12 light-darkness photoperiod, show a melatonin dependent-AVT independent early nocturnal increase in EOD-BR. In nature, nocturnal EOD-BR doubles its daily values decaying towards sunrise, despite the constant darkness registered under water. Wild animals are synchronized in their circadian rhythm, a trait that is lost when field individuals are recorded in isolation. With the aim of understanding the joint action of environmental and social influences on the nocturnal increase in electric behavior, isolated animals were independently treated with intraperitoneal melatonin (n=6, 1µg/g) and AVT (n=6, 1µg/g) during daytime. Both neurohormones increase the discharge rate, albeit differently, with no effects of their respective blockers (Luzindole, Manning Compound). Melatonin exerts a greater longer lasting effect; whereas AVT mediated increase is transient and mild. This AVTergic effect mimics an AVT mediated increment in the discharge rate of the central pacemaker nucleus commanding EOD-BR. Field experiments were performed to assess the role of Melatonin and AVT regulation when animals are under the influence of natural environmental and social stimuli. Animals in social context (n=6) maintain a circadian rhythmicity of EOD-BR even when treated with the melatonin antangonist Luzindole (n=4, ip, 2µg/g) known to successfully interfere with the nocturnal increase in EOD-BR of isolated lab setting recorded fish. Treated animals also show greater synchronization than isolated animals further supporting the importance of social stimuli as a timing signal.

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Poster

409. Biological Rhythms and Sleep: Entrainment and Phase Shifts

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 409.05/VV9

Topic: F.08. Biological Rhythms and Sleep

Support: Interdisciplinary Environmental Toxicology Program - Environmental Toxicology Fellowship
Neuroscience Program, University of Illinois at Urbana-Champaign

Title: Two models of circadian disruption modify locomotor activity and circadian clock gene expression in the suprachiasmatic nucleus of male and female Long-Evans rats

Authors: *K. HATCHER[1,2], R. C. BALACHANDRAN[1], L. MOLINA[3], A. CHU[4], M. L. SIEG[2], Y. PATEL[3], P. EUBIG[1], M. M. MAHONEY[1]
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Abstract: The endogenous circadian timekeeping system synchronizes rhythmic physiology and behavior with the environment. In mammals, this system is controlled by the suprachiasmatic nucleus (SCN). Exogenous factors, such as light at night and shift work, can modulate circadian rhythms. Circadian disruption (CD) by exposure to these exogenous factors influences outputs of the circadian system, including behavior, and the underlying physiology of the SCN. In this study, we assessed the impacts of two models of CD on locomotor activity, estrous cyclicity, and SCN clock gene expression in male and female Long-Evans rats. After an initial training period, animals performed the Five-Choice Serial Reaction Time Task, a cognitively demanding task, for 21 days. Animals were tested under one of three conditions: 4-hours after lights-off with no exposure to ambient light (Control), 4-hours after lights-off and exposure to ambient light during testing (Light at night [LAN] model), and 4-hours after lights-on during the animal's resting phase (Shift Work model). Animals were individually housed, and locomotor activity was quantified using automated infrared activity monitors. Estrous cyclicity was monitored daily using non-invasive vaginal lavage. Following testing, the SCN was removed for qPCR analysis at four different time points: 4 and 8 hours after lights-on (i.e. Zeitgeber Time [ZT] 4 and ZT8), and 4 and 8 hours after lights-off (i.e. ZT16 and ZT20). After 3 days of testing, Shift Work females exhibited reduced total activity, but as testing progressed they maintained similar total activity patterns as controls. Interestingly, males from both CD groups exhibited reduced total activity for the duration of testing compared to controls. Beginning after 12 days of testing, Shift Work males and females and LAN males had reduced anticipatory locomotor activity in the 3-hours before the task when compared to controls. Additionally, beginning 3 days after testing, Shift Work males and females exhibited decreased nocturnal activity, exhibiting more than 50% of their total locomotor activity during the light phase. In females, estrous cycle length was similar across groups, however both CD groups exhibited increased time spent in the estrus stage and decreased time spent in metestrus and proestrus stages. Circadian clock gene expression of *Bmal1*, *Clock*, *Per1*, *Per2*, *Cry1*, *Cry2*, *Avp*, and *Rorα* was analyzed at all four ZTs. We found effects in clock gene expression in a sex-, time point-, and gene-specific manner in both CD models compared to control. Together, these data suggest that two models of CD can differentially impact outputs and physiology of the circadian timekeeping system.

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Poster

409. Biological Rhythms and Sleep: Entrainment and Phase Shifts

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 409.06/VV10

Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant GM117650
Vanderbilt International Scholarship Program

Title: DNA methylation modulates period aftereffects of light entrainment without affecting transient phase shifts

Authors: *S. KIM, D. G. MCMAHON
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Abstract: Light signals can induce acute changes in the phase and enduring changes in intrinsic period (i.e. cycle length) of the circadian clock in the mammalian brain, as short- and long-term plasticity respectively. Although phase shifts are known to arise by transient photic induction of the Period genes, the mechanism underlying how light can drive long-lasting changes in the circadian clock remains elusive. A previous study showed that DNA methylation mediates the period aftereffects of entrainment to non-24 hour light cycles (i.e. T-cycles; Azzi et al., 2014, Nature Neuroscience 17, 377-382.) To determine if DNA methylation is a fundamental mechanism for driving light entrainment, we further tested whether DNA methylation is involved in the circadian clock resetting by single light pulses in constant darkness. We found that pharmacological inhibition of DNA methyltranferases with RG108 prior to and during the light pulse did not alter the phase shift of the endogenous locomotor activity rhythm but did attenuate the light-driven period aftereffects of these single pulses. Our findings suggest that DNA methylation is not necessary for acutely phase shifting the circadian clock, but is critical for driving enduring period aftereffects of photoperiodic light entrainment.

Disclosures: S. Kim: None. **D.G. McMahon:** None.

Poster

409. Biological Rhythms and Sleep: Entrainment and Phase Shifts

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 409.07/VV11

Topic: F.08. Biological Rhythms and Sleep

Support: NSERC

Title: An animal model of social jetlag: The role of endogenous oscillators on hippocampal-dependent learning and memory

Authors: J. M. CLEARY, L. M. LEWIS, K. B. VIGUERS, K. A. JONES, A. W. NEWMAN, T. T. S. CASSELL, D. M. SKINNER, *C. M. THORPE
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Abstract: Students tend to shift their weekend sleep onset and wakeup times, resulting in differences between weekday and weekend sleep schedules, or “social jetlag.” Social jetlag disrupts the light-entrainable oscillator (LEO). Animal models have previously shown that photoperiod shifting in rodents elicits circadian arrhythmia similar to human shift workers. However, there is a dearth of research examining social jetlag in animal models as is experienced by students. The current study investigated the interaction between the LEO and the food-entrianable oscillator (FEO) by implementing a novel animal model of social jetlag, the “student light manipulation (SLM).” To determine the impact of social jetlag on learning and memory, both retention and acquisition were investigated. First, while receiving one meal (FEO access) or many meals (no FEO access) per day, rats were trained on both hippocampal-dependent (HD) and hippocampal-independent (HI) tasks under a 12:12 light-dark cycle, and then exposed to a period of SLM. There were no retention differences between SLM and control rats, yet rats with FEO access retained the HD tasks better than rats without FEO access. Next, while receiving one meal or many meals per day, rats were exposed to the SLM while being trained on both HD and HI tasks. There were no differences in acquisition between SLM and control rats. However, rats with FEO access acquired the HD task faster than rats without FEO access. Currently, there is little known about the anatomical location of the FEO. Such findings are indicative of the need to further investigate the role of the FEO in learning and memory, and its independence from the SCN.

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Poster

409. Biological Rhythms and Sleep: Entrainment and Phase Shifts

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 409.08/VV12

Topic: F.08. Biological Rhythms and Sleep

Support: NIH NIA 5P01AG009975-18

Title: Recurring circadian disruption alters circadian clock sensitivity to resetting

Authors: *M. E. HARRINGTON[1], T. L. LEISE[3], A. GOLDBERG[3], J. MICHAEL[3], G. MONTOYA[3], S. SOLOW[3], P. MOLYNEUX[2], C. B. SAPER[4], R. VETRIVELAN[5]
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Abstract: Modern life can involve repeated disruptions to daily light cycles. We have shown that one phase advance of the light:dark (LD) cycle can temporarily disrupt synchrony of the suprachiasmatic nucleus (SCN) neural circadian rhythm. Persistent disruption to the circadian system through light at night and phase resetting can be modeled by exposure to a 20 h light cycle, a day length that mice are unable to entrain to. We examined brain and peripheral tissues in mice exposed to more than a month of a repeating 20h light cycle (LD10:10). Control animals were housed under LD12:12. We measured locomotor activity and body temperature rhythms *in vivo*, and rhythms of PER2::LUC bioluminescence in tissues cultured *ex vivo*. Housing in LD10:10 induced disruptions to rhythms in response to recurrent exposure to light in subjective night. Bioluminescent measures *ex vivo* showed results that varied across tissues. Rhythm phase was strongly reset by dissection in SCN tissue from mice housed under LD10:10, while smaller phase shifts were observed in SCN from control LD12:12 mice. White adipose tissue (abdominal, mesenteric, inguinal fats) was reset by dissection regardless of light pre-treatment. Diet (high fat vs normal chow) and age (3-7 mos vs 19-22 mos) did not appear to moderate these effects. We conclude that exposure to circadian disruption might desynchronize SCN neurons, increasing network sensitivity to disruption. Dissection can induce large (as in fat tissues) or small (as in SCN) effects on phase even in mice from LD12:12. We interpret these results as suggesting that tissues from highly synchronized SCN networks, as, for example, with increased daily exercise or after short photoperiod exposure, would be less sensitive to these resetting effects, and tissue with weakened coupling, as, for example, with disruption of neuronal VIP signaling, will show larger resetting effects. Exposure to occasional light at night on a recurring weekly basis disrupts circadian rhythms and alters sensitivity of resetting.

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Poster

409. Biological Rhythms and Sleep: Entrainment and Phase Shifts

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 409.09/VV13

Topic: F.08. Biological Rhythms and Sleep

Support: CONACYT-254264

Title: Effect of circadian synchronization to a hedonic stimulus on the sleep pattern and memory of the adult rat

Authors: *C. PEÑA-ESCUDERO[1], S. PRIEGO-FERNÁNDEZ[1], A. A. CORONA-MORALES[2], F. GARCÍA-GARCÍA[1]
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Abstract: Sleep is a circadian behavior/phenomenon entrained by the light-dark cycle, which is the best characterized synchronizer. Other signals, such as periodic food intake, can also entrain molecular, physiological and behavioral circadian rhythms. Adult rats that receive a single palatable meal (PM, such as a chocolate bar) restricted to a specific time each day, develop an increased arousal and locomotor activity prior to scheduled presentation of the PM, called anticipatory behavior (AA). The entrainment by this hedonic stimulus during the animal resting period could lead to disruption of the wake-sleep cycle. The objective of the present study was to determine whether PM entrainment modifies the sleep pattern and consequently it affects the declarative memory in Wistar rats. For this purpose, eight adult male Wistar rats (300-400 g) were implanted with electrodes for sleep conventional recording. Then rats were housed in individual cages under light/dark cycle (12/12; lights on at 16:00 h, defined as zeitgeber time 0 [ZT0]) with free access to food and water. After 7-10 days of recovery, basal recording of the sleep-wake cycle was measured with free access to food and water. Then, rats had access to a PM (chocolate bar, 5 g) at ZT 6 for eight days, with continuous sleep recordings and food and water *ad libitum*. We defined AA when wakefulness time 15 minutes prior to PM time was statistically higher than the same period in *ad libitum* conditions, at least in two consecutive days. After 9 days of PM entrainment, rats were evaluated in the object recognition test to measure declarative memory. For this test, eight additional rats not entrained to PM were included as control group. The results showed that at day 8, there was an increase in wakefulness time and a decrease in NREM time 45 minutes prior to PM, compared to the basal recordings. Animals expressing AA also showed lower REM sleep time one hour before PM. Wakefulness and NREMS times showed fluctuations in the first half of the active period of the rat, although total REM time did in a 24-h cycle did not change. EEG power spectral during NREM sleep and declarative memory did not show significant differences between control and PM groups.In conclusion, our results suggest that PM induces the presence of a timing system that leads the subject to eat during their resting period without altering the homeostatic components of the sleep-wake cycle, so that the cognitive process, such as declarative memory, is not affected.

Disclosures: C. Peña-Escudero: None. **S. Priego-Fernández:** None. **A.A. Corona-Morales:** None. **F. García-García:** None.

Poster

409. Biological Rhythms and Sleep: Entrainment and Phase Shifts

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 409.10/VV14

Topic: F.08. Biological Rhythms and Sleep

Support: NIH R01 GM117650-01
NIH NINDS F31 NS096813
NSF GRFP

Title: Photoperiodic encoding in the SCN: Interaction between cycle length and photoperiod in altering circadian properties

Authors: *M. TACKENBERG[1], D. G. MCMAHON[2]
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Abstract: Photoperiod is a factor in mood disorders and other non-communicable diseases, encouraging investigation of neural response to seasonal light exposure. These responses include changes to circadian properties such as phase angle of entrainment and free-running period of locomotor behavior and clock gene rhythms. Many of these changes outlast the photoperiod itself, indicating that photoperiodic information is encoded within the brain. While the mammalian master clock, the suprachiasmatic nucleus (SCN), is likely a primary site of photoperiodic encoding, how that encoding is induced remains poorly understood. We have examined the mechanisms of induction of photoperiodic encoding in the SCN by differentiating the after-effects of sustained exposure to non-equinox photoperiods and non-24-hour day lengths on *ex vivo* SCN PER2::LUC rhythms and locomotor behavior. We use an unsupervised clustering method for image analysis to explore underlying subregional changes to the phase and period of the SCN in these altered light conditions. Additionally, we examined the effects of pharmacological manipulation of $GABA_A$ signaling on free-running period *ex vivo* and have employed VIP-driven channelrhodopsin to explore the sufficiency of activity within these neurons in inducing photoperiodic after-effects. We found that while both T cycle duration and photoperiod length have significant influences on the free-running period and locomotor behavior duration (alpha) in DD, period changes are predominantly influenced by T cycle length and alpha changes are predominantly influenced by photoperiod length. In T24, bicuculline alters the period of the PER2::LUC rhythm in short and long photoperiod SCN slices, and extended activation of VIPergic SCN neurons by optogenetic stimulation induces a long photoperiod phenotype in short photoperiod mice *in vivo*. Our results so far indicate that photoperiod and T cycle have influence over persistent period and alpha changes, with photoperiod-induced changes being sensitive to $GABA_A$ blockade and inducible by VIPergic neuron stimulation.

These experiments aim to improve our understanding of how continued exposure to a particular photoperiod induces changes within the SCN, as well as how those changes may be altered.

Disclosures: M. Tackenberg: None. **D.G. McMahon:** None.

Poster

409. Biological Rhythms and Sleep: Entrainment and Phase Shifts

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 409.11/VV15

Topic: F.08. Biological Rhythms and Sleep

Support: Medical Research Foundation New Investigator Grant

Title: The central clock synchronizes the liver under ad lib feeding conditions

Authors: *M. P. BUTLER[1,2], A. KUKINO[2], X. XIE[3], A. M. BERMAN[2], H. E. CALCAGNO[2]
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Abstract: Objective and rationale. Time-restricted food synchronizes circadian clocks in peripheral organs, but the synchronizing role of natural eating behavior is not known. Therefore, we tested whether naturalistic patterns of food intake, without long fasting intervals, were sufficient to phase shift and entrain the liver clock. Methods. PER2::LUC mice (B6.129S6-*Per2*^{tm1Jt}/J, Jackson Laboratory; n=11-12 adult males) were housed individually in cages with custom automatic feeders that dropped 20 mg pellets (S0163, Bio-Serv, Flemington, NJ) either when a pellet was removed from the trough (Ad Lib) or on a specified schedule. Circadian rhythm phase was measured longitudinally in the liver and submandibular gland by in vivo imaging 6 times in 24h under isoflurane anesthesia. An average feeding profile across the day was first obtained under Ad Lib feeding; this pattern was then imposed on mice by presenting food every 90 min and varying the meal size. In experiment 1, liver phase was assessed under this imposed schedule in a light-dark cycle, then after shifting the food schedule by 12h, and finally after restricting food availability to the light phase. In experiment 2, mice were acclimated to the imposed schedule and then released into constant darkness with the same feeding schedule. Circadian phase was measured every 2-3 weeks thereafter and assessed as a function of imposed meal schedule and as a function of the free-running circadian locomotor activity rhythm. Results. In experiment 1, peak PER2::LUC bioluminescence in the liver occurred 8h into the dark phase in both Ad Lib and imposed feeding conditions. Reversing the feeding schedule caused a significant but small 2h advance, suggesting that the liver is principally entrained by light under these conditions. In experiment 2, liver phase in constant darkness was best predicted by feeding schedule in half of the mice and best predicted by locomotor activity patterns in the other half. Conclusions. Natural patterns of eating are a weak zeitgeber for the

liver; under natural patterns of food intake, the central circadian clock retains its ability to entrain peripheral organs.

Disclosures: M.P. Butler: None. **A. Kukino:** None. **X. Xie:** None. **A.M. Berman:** None. **H.E. Calcagno:** None.

Poster

409. Biological Rhythms and Sleep: Entrainment and Phase Shifts

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 409.12/VV16

Topic: F.08. Biological Rhythms and Sleep

Support: FDN 143337

Title: Neural mechanisms linking hypernatremia and hyperthermia to circadian time

Authors: *C. GIZOWSKI[1], C. A. ZAELZER[2], C. W. BOURQUE[3]
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Abstract: Our brain encloses an inner clock, the suprachiasmatic nucleus (SCN), which optimizes physiological functions according to the body's differing needs at various times of day (e.g. during wake vs sleep). When clock timing goes awry (e.g. due to age, jet lag, or shift work), this can result in significant health issues such as an increase the risk of cancer, mental illness and cardiovascular disease. Clock time is encoded by the action potential firing rate of vasopressin neurons in the SCN and my previous work in mice showed that this activity can activate thirst neurons to promote anticipatory water intake that protects against dehydration during sleep. While clock time is normally adjusted by daylight onset, it can also be regulated by non-photic stimuli through unknown mechanisms. In this project, I examined if hypernatremia and hyperthermia can acutely regulate clock time. The organum vasculosum lamina terminalis (OVLT) is a preoptic nucleus that contains neurons capable of detecting these conditions via the osmo- and heat-sensitive transduction channel dn-Trpv1. I therefore examined if OVLT neurons can modulate SCN clock neurons. Histological and tracing experiments showed that dehydration-activated dn-Trpv1 OVLT neurons project to the SCN. Moreover, single cell RT-PCR experiments showed these cells express GAD65, a marker of GABAergic neurons. Preliminary results suggest GABA excites SCN vasopressin (VP) neurons during wake time, when SCN electrical activity is low. Electrophysiological analysis in slices further revealed that a hypernatremic stimulus delivered to the OVLT significantly increases the frequency of spontaneous GABA synaptic currents and triggers an anticipatory shift in the onset of electrical activity in SCN clock neurons. These data show that the SCN not only drives circadian rhythms, but also receives important physiological signals that can mediate non-photic adjustments in clock time and possibly adapt organisms to dynamic environments.

Disclosures: C. Gizowski: None. **C.A. Zaelzer:** None. **C.W. Bourque:** None.

Poster

409. Biological Rhythms and Sleep: Entrainment and Phase Shifts

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 409.13/VV17

Topic: F.08. Biological Rhythms and Sleep

Title: The effect of photoperiod on the estrous cycle length and stability in the C57 mice

Authors: *M. ORTEGA VILLEGAS[1], M. FUENTES-CANO[2], P. DURAN[3]
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Abstract: The master clock in mammals, located in suprachiasmatic nucleus, impose phase and period to peripheral oscillators. This hypothalamic structure is part of reproductive axis, were entrain every structure of the axis to generate the physiological responses that require a temporal signal to give continuity to the estrous cycle on female. This process is regulated by many signals, and some of them can be modified by photoperiod. The most important signal for photoperiodic response is driven by melatonin, which code the night length that changes along the year. Previously we observed that, in Mongolian gerbil (a model that presents seasonal reproduction), the photoperiod alters estrous cycle regularity, both under short day, lengthening diestrous, and under long day, lengthening diestrous and estrous phases. The aim of the present study is to analyse if photoperiod alters estrous cycle regularity in a model that does not show seasonal reproduction. Female mice C57 were exposed to 14:10, 8:16 and 16:8 photoperiods. The estrous cycle was monitored by cytology via vaginal smears between ZT4 and ZT5, for 15 days. Under 14:10 photoperiod cycles were regular with an average length of five days, meanwhile under 16:8 photoperiod average length was 5.8 days and under 8:16 average length was 7.5 days, in both short and long days photoperiods estrous phase was lengthened. It has been reported prolonged exposition to light increase estrogens levels and secretion, promoting the vaginal estrous stands by more than a day, but it cannot explain the estrous lengthening under 8:16 photoperiod. Estrous cycle irregularity decreases fertilization chances and is related to an increase of abnormalities in early embryonic development, chromosomal aberrations, failure of implantation and embryonic death, that reflects the desynchronization in the central and peripheral events that are regulating endocrine and reproductive function in females, in this case is generated by photoperiod, and is not limited to models with seasonal reproduction. Next step is to register circadian locomotor activity that has been shown a correlation to estrous cycle under each photoperiod, in order to see a possible effect at hypothalamic level.

Disclosures: M. Ortega Villegas: None. **M. Fuentes-Cano:** None. **P. Duran:** None.

Poster

409. Biological Rhythms and Sleep: Entrainment and Phase Shifts

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 409.14/VV18

Topic: F.08. Biological Rhythms and Sleep

Support: FC-2017

Title: Maternal malnutrition impact entrainment in adult offspring for water intake and locomotor activity rhythms

Authors: D. J. BUSTAMANTE-VALDEZ[1], J. S. GONZALEZ-RUANO[1], M. FUENTES-CANO[2], *P. DURAN[1]
[1]LBAE, Biología Celular, Facultad de Ciencias, UNAM, Ciudad de Mexico, Mexico; [2]Facultad de Ciencias, UNAM, Mexico City, Mexico

Abstract: Malnutrition is a widely health problem, it impacts not only developmental countries but higher economies, hypercaloric-hipoproteinic diets as the “cafeteria diet” are been linked to overweight, metabolic syndrome, diabetes and other metabolic disruptions from childhood to adulthood. However, there is limited research about its effect on the intrauterine environment and the critical periods of brain development, Hypoproteinic maternal malnutrition has been reported to diminish brain cells, produce cognitive deficits, and trigger irreversible physiological or metabolic changes; mainly the circadian expression for sleep-wake, water intake and locomotor activity rhythms, have been linked to a neuro-anatomo-functional alteration on the hypothalamic suprachiasmatic nuclei (SCN), the circadian clock on most physiological and behavioral responses. The coordination for circadian biological rhythms energy utilization, and storage is physiologically linked to a healthy food homeostasis in mammals. In this study, the juvenile offspring of dams under a “cafeteria diet” protocol during gestation and lactation was recorded, to analyze the water intake and locomotor activity circadian rhythms entrainment and incidentally the SCN integrity. Sprague-Dawley adult female rats were randomly divided in 2 nutritional protocols: control (CO) given rodent lab diet (5001 purina Chow 4.15kcal) and Cafeteria diet (CD-6.32kcal) maintained under 12/12 light-dark cycle, temperature room 22-24°C and food and water *ad libitum* three weeks before mating, during gestation and lactation. After weaning all offspring was sexed and maintained under rodent lab diet, and water and food ad libitum Postnatal body weight and size were recorded. At 7 weeks of age, the circadian protocol started as follows: 3 weeks under 12:12 Light-Dark (LD) cycle, 3 weeks constant darkness (DD) and 3 weeks re-entrainment to LD. Water intake and locomotor activity were recorded using a infrared sensors system. Food and water intake were monitored trhought the protocol. circadian parameters as amplitude, period, mesor, % of rhythm, transitory days to reintraiment were analized. The results showed, that malnourished female offspring present

lower body weight but same size as controls, Food intake for males diminished during constant darkness. For both locomotor and water intake rhythms the CD group displayed difficulty to re-entrain, more transitory days were required, period was reduced and the activity increased just before the lights on. At the end of the recordings, a 12 h cycle seems to appear, suggesting a splitting, as reported for hypoproteinic malnourished rats in other study.

Disclosures: D.J. Bustamante-Valdez: None. **J.S. Gonzalez-Ruano:** None. **M. Fuentes-Cano:** None. **P. Duran:** None.

Poster

409. Biological Rhythms and Sleep: Entrainment and Phase Shifts

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 409.15/VV19

Topic: F.08. Biological Rhythms and Sleep

Support: NSF grant IOS1456706

Title: Use of excitatory and inhibitory DREADDs to evaluate the role of raphe nuclei-to-infralimbic cortex projections in the circadian regulation of the infralimbic cortex

Authors: *H. K. STRNAD, J. RAVENEL, A. E. CONCHA, S. P. SHARMA, M. J. HARTSOCK, R. L. SPENCER
Behavioral Neurosci., Univ. of Colorado At Boulder, Boulder, CO

Abstract: Circadian rhythms drive biological functions in a manner that parallels the roughly 24-hour cycle of light and dark on Earth. The suprachiasmatic nucleus (SCN) synchronizes its clock gene oscillatory expression with the environmental light/dark cycle. This rhythm is then communicated throughout the rest of the brain and periphery to optimize physiological functioning at appropriate times of day. Rhythmic clock gene expression outside of the SCN has been established, and that expression requires an intact SCN. However, not much is known about the specific mechanism by which the SCN communicates to extra-SCN cells. We have found that appropriately timed daily circadian patterns of corticosterone (CORT) hormone secretion are necessary for normal rhythmic clock gene expression in the infralimbic cortex (IL; Woodruff et al. Endo 157:1522-34, 2016). However, those studies suggest that CORT is not the sole entrainment factor. Serotonergic neurons from the raphe nuclei (RN) have been shown to contribute non-photic regulation of circadian rhythms via innervation of the SCN, demonstrating the capacity to directly regulate expression of clock genes. RN neurons have a wide range of projections throughout the brain including the prefrontal cortex. The current project utilized Designer Receptors Exclusively Activated by Designer Drugs (DREADD) to characterize DRN projections to the IL and their role in circadian regulation of the IL. A dual virus intersectional approach was used to express excitatory DREADDs in only RN neurons that project to the IL.

All rats underwent stereotaxic surgery for infusion of (retro)AAV-pmSyn1-EBFP-CRE into the IL. One week later AAV8-hSyn-DIO-hM3Dq was infused into the RN. After allowing one week for recovery from surgical procedures, rats were injected with Clozapine-*N*-Oxide (CNO) to activate the DREADD receptors and were perfused 1.5 hours later. Cells in the dorsal, ventrolateral, and ventromedial regions of the RN show successful DREADD expression as indicated by an mCherry reporter fusion protein. Some expression was also seen in the intermediate and median nuclei. FOS immunoreactivity was used to assess the ability of CNO to activate neurons expressing the DREADD as well as increases in cell activity at the IL projection site. Ongoing studies will use the same intersectional approach utilizing an inhibitory virus (AAV-hSyn-DIO-hM3Di) and then examine the extent to which activation or inhibition of this pathway at different times of day modulates diurnal variation in IL neural activity.

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Poster

409. Biological Rhythms and Sleep: Entrainment and Phase Shifts

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 409.16/VV20

Topic: F.08. Biological Rhythms and Sleep

Support: Nyenhuis Grant, Hope College
Neuroscience Program, Hope College
Psychology Department, Hope College

Title: Mixed mood state behaviors and circadian dysfunction following homocysteic acid treatment: Potential animal model for bipolar disorder

Authors: L. EVERT[1], G. MOYA[1], G. FOGO[2], N. ROZEMA[1], R. FELTON[1], S. PLOWMAN[1], T. STAUB[1], L. A. CHASE[1], *A. J. GALL[3]
[2]Neurosci., [3]Psychology, [1]Hope Col., Holland, MI

Abstract: Bipolar disorder is a neuropsychiatric disease characterized by cyclical fluctuations of mood states between states between mania and depression. Circadian rhythm abnormalities and inconsistent sleep patterns are two common symptoms of bipolar disorder (Millar, Epsie, & Scott, 2004). Elevated levels of homocysteine, or hyperhomocysteinemia, in the blood or cerebrospinal fluid commonly occurs in patients with neuropsychiatric illnesses, including bipolar disorder (Bell et al., 1992; Boushey, Beresford, Omenn, & Motulsky, 1995). Homocysteic acid (HCA), an endogenous metabolite of homocysteine, has been implicated as a harmful neurotoxin and agonist of NMDA receptors. We have previously shown that postnatal administration of HCA (from postnatal day 3 to 21) in Sprague Dawley rats results in both

mania-like and depressive-like behaviors, suggesting that this may serve as a novel animal for bipolar disorder. The purpose of the present study was to characterize any circadian abnormalities that may be present in HCA-treated rats, as sleep and circadian dysfunction are common symptoms of bipolar disorder. In addition, we also characterized the developmental onset of the mania-like and depressive-like behaviors in this model. Prior to puberty, we found that HCA-treated rats exhibited no manic-like behaviors and only a trend toward depressive-like behaviors. After puberty, however, HCA-treated rats presented a mixed mood-state of both manic-like and depressive-like behaviors, along with significant dysfunction in the circadian clock. Specifically, both the free-running period and the amplitude of the rhythm were significantly reduced following HCA treatment. We are currently using microarray analyses to determine differences in circadian gene expression levels between HCA treated animals and controls. Additionally, we are examining the therapeutic role of lithium for reversing the circadian disruptions exhibited by the HCA treated animals. Altogether, the findings of the present study provide strong evidence in support of the HCA model's face validity for bipolar disorder, allowing us to better understand the mechanisms underlying the development of this disease.

Disclosures: L. Evert: None. **G. Moya:** None. **G. Fogo:** None. **N. Rozema:** None. **R. Felton:** None. **S. Plowman:** None. **T. Staub:** None. **L.A. Chase:** None. **A.J. Gall:** None.

Poster

409. Biological Rhythms and Sleep: Entrainment and Phase Shifts

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 409.17/VV21

Topic: F.08. Biological Rhythms and Sleep

Support: CONACYT PEI-91/2013
IMMIS-RHV2017

Title: Approach to the circadian cycle regulation in c58 mice, a autism model

Authors: *L. TÉLLEZ-AZTORGA[1], E. IBARRA-CORONADO[2], I. OLIVER-DOMINGUEZ[2], J. C. ROJAS-CASTALLEDA[5], R. HARO VALENCIA[6], A. PÉREZ-TORRES[3], V. RODRÍGUEZ-MATA[2], J. HERNÁNDEZ-FALCON[2], K. MENDOZA-ANGELES, 01400[4], P. DURAN[1]
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Abstract: One of the most frequent neurocognitive entities in pediatric population is Autistic Spectrum Disorder (ASD). Because this entity becomes apparent since the early childhood, it is necessary to diagnose and establish therapeutic measures as soon as it is detected. Epidemiological studies have shown a high prevalence of ASD but there is a lack of adequate tools for the diagnosis. ASD is a complex entity that includes alterations in sleep, circadian rhythms and cognition, and there is a great need for biological models that fulfill most of the human alterations, in order to understand the biological basis of this disorder. Some murine models have been developed, among them the c58/j strain that shows repetitive behaviors, no other studies have been validated this model for ASD. The goal of this work was study the locomotor circadian rhythm of c58/j strain in order to validate it as an ASD murine model. We recorded locomotor activity in individual cages under 12:12 LD cycles during three weeks of continuous recordings then they were submitted to continuous darkness (DD) for 3 more weeks. We analyzed locomotor activity by means of cosinor with which we found differences between the control and the c58/j strains. In 12:12. In the 12:12 L/O scheme we found that in the c58/j strain the amplitude is greater than in the control strain, while the acrophase is smaller, no notable changes were observed regarding the mesor. During the continous dark, the strain c/58j showed higher values in the mesor and amplitude, while the acrophase still maintains lower values in comparison to the control strain. Our data suggest that there is a difference in the expression of the rhythm of locomotor activity, which may be comparable to the human condition.

Disclosures: L. Téllez-Aztorga: None. **E. Ibarra-Coronado:** None. **I. Oliver-Dominguez:** None. **J.C. Rojas-Castalleda:** None. **R. Haro Valencia:** None. **A. Pérez-Torres:** None. **V. Rodríguez-Mata:** None. **J. Hernández-Falcon:** None. **K. Mendoza-Angeles:** None. **P. Duran:** None.

Poster

409. Biological Rhythms and Sleep: Entrainment and Phase Shifts

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 409.18/VV22

Topic: F.08. Biological Rhythms and Sleep

Support: CIHR MOP142458

Title: Evaluation of the circadian system in a VPA-induced model of autism spectrum disorder

Authors: *S. FERRARO, N. DE ZAVALIA, S. AMIR
Ctr. for Studies in Behavioural Neurosci., Concordia Univ., Montreal, QC, Canada

Abstract: Autism Spectrum Disorder (ASD) is a pervasive developmental disorder characterized by restrictive patterns of behaviour and deficits in social interaction and communication. Often

accompanied by co-morbid disorders, a staggering number of juvenile ASD patients exhibit sleep-wake cycle disturbances. Research has shown that these irregular sleep-wake cycles exacerbate ASD symptomology and further impair social performance. Although irregular sleep-wake cycles have been identified in ASD individuals, little has been done to assess the contribution of the circadian system to these findings. The objective of this study is to phenotypically characterize the circadian system in an animal model of autism. Valproic acid (VPA), a commonly prescribed anti-epileptic drug, administered on day 12.5 of gestation results in offspring which exhibit ASD-like behaviours. Male offspring are introduced to running-wheel apparatuses post-weaning and undergo various circadian challenges, including baseline 12:12 light-dark (LD) cycles, constant dark, constant light and phase advance/delay protocols. Baseline activity analysis revealed a significant increase in the total amount of activity bouts during the light phase in VPA-treated animals compared to controls (control: 1.1 ± 0.1795, VPA: 1.917 ± 0.2865 $p < 0.05$). Moreover, VPA-treated animals show greater distribution of wheel-running behaviour between light and dark phases, while controls show greater confinement of this activity to the dark phase (control: 0.07550 ± 0.01609, VPA: 0.1724 ± 0.01506. $p < 0.0001$). Further investigation revealed a later running-activity offset in VPA-animals ($p < 0.0005$). Constant light analysis revealed a greater amount of running activity in VPA animals ($p < 0.05$) , and an increase in the number of days to reach arrhythmicity (control: 12.89 ± 0.5638, VPA: 23.93 ± 2.069. $p < 0.0005$). Together, these results suggest differential light perception in VPA-treated animals, and suggest alterations in the entrainment capacity of the SCN. Moreover, these results lend to the validity of the VPA-induced model of ASD, as these findings recapitulate what is seen in human populations.

Disclosures: N. de Zavalia: None. **S. Amir:** None.

Poster

409. Biological Rhythms and Sleep: Entrainment and Phase Shifts

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 409.19/WW1

Topic: D.07. Vision

Title: Environmental light influences small intestine microbiota and metabolism through immune system

Authors: *I.-C. LEE, S.-K. CHEN
Life Sci., NTU, Taipei, Taiwan

Abstract: Recently, external light effect on mammalian circadian clock through ipRGCs in retina, which is also influence peripheral circadian oscillation and regulate several physiological hormone homeostasis. In previous studies, while absence of light-dark cycle might cause to the obesity, insulin resistance and gut microbiota oscillation abnormally change in mouse model.

However, how does environmental light influence the gut microbiota and its mechanism still unknown. In this study, we determined the transcriptome expression in mouse intestine tissue under dim light at night (LAN) and investigated what kind of genes would be involved in this pathway and how's its function within external light. In our preliminary results, we found that LAN group mice expressed lower nuclear receptor subfamily 1(Nr1d1) which involved in toll-like receptor 4(TLR4) signal pathway and impact the immune regulation compare to the mice with regular normal light-dark cycle.
Keyword: ipRGCs, intestine metabolism, light-dark cycle, immune, obesity

Disclosures: I. Lee: None. **S. Chen:** None.

Poster

409. Biological Rhythms and Sleep: Entrainment and Phase Shifts

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 409.20/WW2

Topic: F.03. Neuroendocrine Processes

Support: NIH Grant R21CA202745
NIH Grant R01NS092388

Title: Light at night exacerbates metabolic dysfunction in a polygenic mouse model of type 2 diabetes mellitus

Authors: *K. L. RUSSART[1], S. CHBEIR[1], R. J. NELSON[3], U. MAGALANG[2]
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Abstract: Electric lighting is beneficial for modern society; however, in recent years it is becoming apparent that light at night is not without biological consequences. Several studies have reported deleterious effects on health and behavior in humans and nonhuman animals. Exposure of nondiabetic mice to dim light at night (dLAN) impairs glucose tolerance, whereas a return to dark nights (LD) reverses this impairment. Reduced glucose tolerance is a characteristic of type 2 diabetes mellitus (T2DM); thus, we predicted that exposure to dLAN will exacerbate the metabolic abnormalities in a mouse model of T2DM. We exposed male TALLYHO/JngJ (TH) mice, a polygenic mouse model of T2DM, to LD or dLAN (40 lux) in 2 separate experiments. After 8 weeks, intraperitoneal glucose tolerance testing (ipGTT) was performed, and following recovery, we conducted intraperitoneal insulin tolerance testing (ipITT). In Experiment 1, all mice were then returned to dark nights for 4 weeks, and the ipITT was repeated. In Experiment 2, tissues were collected for analysis after recovery from the ipITT. TH mice housed in dLAN had increased body weight, impaired glucose tolerance, and higher insulin resistance than mice housed in LD. Insulin resistance was improved when mice were returned to LD for 4 weeks. Additionally, more mice developed T2DM and survival rate decreased when

housed in dLAN. dLAN worsens the metabolic abnormalities in a polygenic rodent model of T2DM and these effects were reversed upon return to dark nights. The decreased survival in the TH mice exposed to dLAN is an unexpected result that requires further investigation. Limiting light at night may be a simple intervention that could benefit humans with T2DM, but would require additional studies.

Disclosures: K.L. Russart: None. **S. Chbeir:** None. **R.J. Nelson:** None. **U. Magalang:** None.

Poster

410. Molecular Biology and Physiology of Clocks

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 410.01/WW3

Topic: F.08. Biological Rhythms and Sleep

Support: NIH R21 CA202745

Title: Disruption of endogenous circadian rhythms by exposure to light at night accelerates pancreatic tumor growth in mice

Authors: *S. CHBEIR[1], K. L. G. RUSSAT[1], W. H. WALKER, II[1], D. M. PROCA[2], A. C. DEVRIES[3], R. J. NELSON[4]
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Abstract: Intact circadian rhythms are critical for optimal physiological and behavioral functions. The widespread adoption of electric lighting world-wide has led to significant exposure to artificial light at night (LAN), which results in disruption of circadian rhythms. Exposure to LAN is strongly correlated with increased prevalence of obesity, metabolic disorders, and certain types of cancer. Pancreatic ductal adenocarcinoma (PDAC) is among the most aggressive cancers with poor prognosis and short post diagnosis survival rates in which K-Ras mutations account for ~90-95% of cases. The objective of this study was to explore whether dysregulation of circadian rhythms by LAN accelerates PDAC development in high-risk patients using a PDAC mouse model bearing K-Ras-G12D and P53−R270H mutation alleles (KPC). We hypothesized that exposure to LAN accelerates tumorigenesis by dampening the expression of the circadian gene Period-2 (*Per2*) in the pancreas, thereby disinhibiting cellular proliferation and exacerbating inflammatory responses to tumor formation. Six week old male KPC mice and littermate controls were randomly assigned to either dark nights (LD group: 14 h light-150 lux/10 h dark-0 lux) or a LAN group (14 h light-150 lux/10 dim white light-40 lux) for 8 weeks (n=10-11 per group). KPC mice had significantly increased body weight, pancreas mass, and elevated rates of neoplastic transformation resulting in increased incidence of solid tumors. More than 80% of the LAN group had both pancreatic intraepithelial neoplasia (PanIN) grade 3

lesions, and invasive ductal adenocarcinoma, whereas only 30% of the LD group had detectable tumor growth. These results were accompanied by reduced expression of *Per2* in the hippocampus and pancreas of KPC and WT mice in LAN, as well as increased expression of the cell cycle gene *Cyclin D* in the pancreas of KPC mice. Furthermore, LAN exacerbated the inflammatory responses to tumor growth in KPC LAN mice by increasing serum IL6 and pancreatic gene expression of *IL-6*, *TNFα*, and *NFkB*. In conclusion, LAN induces disruption of circadian clock function resulting in a sequence of events that promote cell proliferation and elevated inflammation, leading to a feed-forward process that enhances tumorigenesis, tumor growth, and early onset PDAC.

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Poster

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Topic: F.08. Biological Rhythms and Sleep

Support: Conacyt 243298
FAI 2017
CA-UASLP 254

Title: The loss of melatonin circadian rhythm promotes tumor growth and angiogenesis

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Abstract: The development of new blood vessels is essential to sustain the metabolic demand of tumor cells and it is associated to the increases expression of several pro-angiogenic genes, including *VEFG*, *EGF*, *PDGF*, *TNF-α*, *ANG-1*. Previous studies performed in our laboratory showed that, in rats inoculated with glioblastoma C6 cells (tumor bearing, TB), the Circadian Desynchronization (CD) induced by constant light (LL) promotes tumor growth and increases tumor vascularization, in comparison to non-desynchronized (LD) rats, suggesting that CD enhances the pathological process. To investigate the mechanisms that underlie this outcome, we focused on melatonin, which is a hormone released in a circadian fashion, whose rhythm is flattened by exposure to LL and which physiologically participates to the regulation of cell cycle and angiogenesis. We found that tumor growth and angiogenesis did not increase when TB

animals and under LL were administered with a daily dose of exogenous melatonin, which suggests that this hormone might have an inhibitory effect over endothelial proliferation. To investigate the role of CD and melatonin in cancer development, we compared the hepatic mRNA expression of cell cycle-regulating genes (*p53*, *p21*, *Cyclin E*), clock genes (*Per2*, *Bmal1*, *Rorα*, *Rev-erbα*) and pro-angiogenic genes (*VEFG*, *EGF*, *PDGF*, *TNF-α*, *ANG-1*) of following groups of rats: (1) LD; (2) LD-TB; (3) LL; (4) LL-TB; (5) LL-TB + melatonin. Results showed that the rhythms of p53, p21, Cyclin E and Per2 mRNAs were lost in all the groups under LL, and only *Per2* recovered rhythmicity after melatonin administration. VEGF displayed a phase delay of ~12 h in LL-TB, and ~18 h in both LL and LD-TB, with respect to the LD controls. LL and LL-TB showed increased levels of TNFα, while LL-TB also presented higher levels of ANG-1 in comparison with animals under LD. In the cancer tissue extracted by animals that were injected with C6 cell, neither the rhythm nor the levels of p53, Cyclin E and Per2 mRNAs were affected by LL, while melatonin administration decreased their concentration. Interestingly, VEGF y PDGF mRNAs were down-regulated by LL exposure and normal levels restored in melatonin-administered animals. All this evidence indicates that the loss of melatonin rhythm that has been reported in murine models and individuals exposed to CD might enhance tumor growth by promoting the loss of control over the cell cycle and pro-angiogenic genes expression.

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Poster

410. Molecular Biology and Physiology of Clocks

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Topic: F.08. Biological Rhythms and Sleep

Support: FONCYT PICT 2013 No. 021
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SECyT UNC 2016
FONCYT PICT 2016 No. 0187

Title: Temporal control of metabolism in proliferative glioblastoma cancer cells with differential time-regulated chemotherapy susceptibility

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Abstract: Circadian clocks driving transcriptional/translational rhythms in gene expression are even present in immortalized cell lines whereas metabolic rhythms conducted by an ancestral metabolic/redox clock can persist in enucleated cells or in the absence of transcription. Disruption of circadian rhythms by modern life (continuous illumination, shift work, jet lag, etc.) may cause metabolic disorders and higher cancer risk; however, little is known about clock functioning in tumor cells. Here we evaluated expression of clock and clock-controlled genes, redox metabolisms (reactive oxygen species (ROS), and levels and peroxiredoxin cycles) and cell susceptibility to the chemotherapeutic treatment of bortezomib in cultures of glioblastoma T98G cells. For this, cells were kept under proliferation (with serum), synchronized with dexamethasone (100 nM) (time 0) and collected at different times. In these cells, mRNA expression for clock- (*Bmal1, Per1, Rev-erbα)* and glycerophospholipid synthesizing enzyme-genes did not display circadian rhythmicity or fluctuated with a shortened period (τ=16 h) whereas the redox metabolism exhibited a 12 h- rhythm in ROS levels and longer peroxiredoxin oxidation cycles (τ=30 h). Moreover, cell viability significantly changed over time after bortezomib (500 nM) chemotherapy. Cell viability and redox state rhythms were altered when *Bmal1* expression was knocked down by CRISPR/Cas 9 technology showing different amplitudes, phases or periods than wild type cells. Nevertheless, observations indicate that an intrinsic metabolic clock continues to function in proliferating cells, controlling diverse metabolisms and highlighting differential states of tumor suitability for more efficient, time-dependent chemotherapy. Also, we may infer that cross-talk between the oscillators takes place in tumor cells to ensure tumor growth and survival over time. Furthermore, regardless of the precise nature of the link between them, molecular and metabolic oscillators work in unison to maintain cellular homeostasis.

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Poster

410. Molecular Biology and Physiology of Clocks

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Program #/Poster #: 410.04/WW6

Topic: F.08. Biological Rhythms and Sleep

Support: T32 DA017637

Title: Quantification of Ox2R mRNA during baseline, oral nicotine, and withdrawal conditions

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Abstract: Nicotine and withdrawal from chronic nicotine induce changes in reported and observed sleep quantity and architecture. The mechanisms responsible for the changes are not well characterized. The literature suggests a role of the wake-promoting orexinergic neurons of the lateral (LH) and dorsal medial (DMH) hypothalamus. The orexins (A & B) have widespread projections throughout the brain and act on two receptors (OX1R & OX2R). OX2R appears to be the primary receptor mediating the effects of arousal. The present study utilized a mouse model of forced oral nicotine to characterize the effects of nicotine consumption and withdrawal on the expression of OX2R throughout the brain during three conditions: baseline (BL), nicotine day one (N1), and withdrawal day one (WD1). Adult female C57BL/6J mice were individually house and maintained on a 12 hr light/12 hr dark cycle with ad libitum access to food and a 0.2% saccharin drinking solution. In a preliminary experiment, samples were collected at ZT8 (3PM). Pre-nicotine BL samples were collected one week after placement in individual cages. In a separate cohort of animals 200µg/ml of nicotine was added to the .2% saccharin water to produce nicotine dependence. N1 samples were collected at 32 hours after the introduction of nicotine. In a final cohort of animals, nicotine exposure persisted for 14 days and withdrawal was induced by excluding the nicotine from the drinking solution. WD1 samples were collected 32 hours after the removal of nicotine. Samples were coronally sliced at 14µm and a [α-35S]-labeled riboprobe specific to OxR2 was hybridized to the sections. Sections were exposed to phosphor screens for a period of one week and images were captured using a Cyclone phosphorimager. For individual brain regions 2-4 representative samples from consecutive slides were identified and quantified (cpm) using Optiquant software. Preliminary analysis indicates that relative to BL, the N1 and WD1 conditions show a trend towards increased OX2R in the olfactory tubercle (Tu), ventral pallidum (VP), and medial septal nucleus (MS). Additional samples are being collected at ZT0 and ZT12 for analysis to determine if there is diurnal variation in OX2R expression and whether the effect of nicotine and/or withdrawal on OX2R expression is time of day dependent.

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Poster

410. Molecular Biology and Physiology of Clocks

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Program #/Poster #: 410.05/WW7

Topic: F.08. Biological Rhythms and Sleep

Support: CIHR MOP142458

Title: Alcohol-drinking behaviour and preference in striatal Bmal1 knock-out mice

Authors: *N. DE ZAVALIA, P. SOLIS, S. FERRARO, K. SCHÖTTNER, M. BUTTON, J. GOLDSMITH, M. VALYEAR, N. CHAUDHRI, S. AMIR
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Abstract: The neural and molecular mechanisms that mediate chronic alcohol consumption and alcohol use disorders are not well understood. Alcohol exposure affects brain pathways and neuronal circuits related to reward, stress, habit formation, and decision-making, ultimately leading to alcohol dependence/addiction. Specifically, the dorsal striatum is implicated in addiction, 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. Persistent disruption to the circadian environment, regardless of whether the disruption is genetic or environmental, can affect alcohol intake. Genetic polymorphisms of clock genes involved in the generation of circadian rhythms are associated with a pattern of social drinking and alcohol abuse. The aim of this work was to study ethanol consumption 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 projection 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) 3 days of sucrose (2% m/v) access protocol. 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 nucleus (the master circadian clock) or hippocampus of KO compared with WT 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 consumption and alcohol preference between HET and WT male and female mice were observed. No significant differences in sucrose consumption 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. Molecular Biology and Physiology of Clocks

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Topic: F.08. Biological Rhythms and Sleep

Support: Helse Vest grant, ID HV911 893
Faculty of Psychology, University of Bergen, Småforsk, ID 173200-270452; 173200-810298

Title: Extended photoperiod alters the circadian rhythm and expression of synaptic plasticity-associated genes. The impact of blue-enriched light

Authors: *A. R. MARTI[1], S. SKREDE[1,2], J. MRDALJ[1], T. T. PEDERSEN[1], L. H. BJERRUM[1], J. WASLANDER[1,3], V. LYSNE[1], K. M. ERSLAND[1,4], T. E. G. HENRIKSEN[1,5], C. R. BRAMHAM[1], J. GRØNLI[1]
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Abstract: Light affects regulation of circadian rhythmicity and cognition through the blue-light sensitive intrinsically photosensitive retinal ganglion cells (ipRGCs). Here we use a model of extended photoperiod (20h light, 4h dark; 20:4 LD) with two different spectral qualities (white and blue-enriched light) to examine effects on the circadian rhythm of body temperature (BT), and expression of synaptic plasticity markers in the rat brain. For recording of BT rhythm, male rats (n=6/group) were housed in standard 12:12 LD condition, followed by exposure to 7 days of extended photoperiod (20:4 LD) in either white or blue-enriched light conditions, and 14 days recovery in standard 12:12 LD condition. Tissue from a separate set of animals (n=10/group) was collected on exposure day 7 (E7), and recovery day 3 (R3), from anterior cingulate cortex (ACC) and prefrontal cortex (PFC). Tissue samples were analyzed with qPCR to examine expression of immediate early response genes, including brain-derived neurotropic factor (BDNF), the activity-regulated cytoskeleton-associated (Arc) protein, Krueppel-like factor 10 (KLF10) and Neuronal PAS domain protein 4 (NPAS4). Two-tailed student's t-test was used for statistical comparison to 12:12 LD time-matched controls ($p<.05$). The rhythm of BT maintained a 24h period in both blue-enriched and white light conditions during 20:4 LD, although the acrophase was shifted to co-occur with the dark phase. Upon return to 12:12 LD, decreased amplitude and no clear 24h period was observed for several days. At E7, the extended photoperiod, independently of spectral quality, decreased expression of Arc (ACC and PFC; $t's_{(15\text{-}18)}>-3.74$, $p's<.002$) and KLF10 (ACC; $t's_{(17\text{-}18)}>-2.51$, $p's<.02$). NPAS4 was decreased in the blue-light condition only (PFC; $t_{(15)=}-3.53$, $p=.003$). The expression of BDNF was not

significantly changed at E7, but increased at R3 in the blue-light condition only (PFC; $t_{(14)}$=3.80, p=.002). No significant differences in expression of Arc, KLF10 or NPAS4 were observed at R3 compared to controls.The return from extended photoperiod disturbed the circadian rhythm of body temperature. In addition, extended photoperiod affected several markers of synaptic plasticity in brain regions important for mood and cognitive performance. The effect also appears to depend on the spectral quality of light and brain region.

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Poster

410. Molecular Biology and Physiology of Clocks

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Program #/Poster #: 410.07/WW9

Topic: F.08. Biological Rhythms and Sleep

Title: Gene expression in the SCN is altered during timed restricted feeding

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Abstract: The suprachiasmatic nucleus (SCN) of the hypothalamus regulates circadian rhythms of physiology and behavior in mammals. The circadian clock mechanism in the SCN is normally entrained by the daily light/dark (LD) cycle. This regular activity rhythm can be modulated by regular, nonphotic events. In particular, animals respond to periods of restricted food access during the daytime by showing a period of food anticipatory activity, which is thought to represent signaling from a food entrainable oscillator. The response of the circadian clock in the SCN to restricted feeding appears to be both species and strain-specific. In mice, some inbred strains can entrain locomotor activity to normocaloric restricted feeding cycles in constant darkness, while others, such as the C57BL/6J strain, rarely do so. Previous research suggests that C57BL/6J mice do not experience a shift in clock gene expression in the SCN during normocaloric timed restricted feeding, while other brain regions do show changes in clock gene expression. However, such studies were limited and primarily looked at period gene expression. In this series of studies, we sought to measure expression levels of both period and neuropeptide genes in the SCN of C57BL/6J mice on a 4-hour daytime restricted feeding schedule. In addition, clock and neuropeptide genes will be examined in hypothalamic nuclei involved in feeding or reproduction, including the arcuate, dorsomedial, and ventromedial nuclei. In a preliminary experiment, male and female mice were housed in 12:12 LD cycle for two weeks with ad libitum food access. Half of the mice then had food restricted to four hours in the middle of the light phase (ZT 6 to ZT 10), and the other half continued with ad libitum feeding, both for

a period of 10 days. The mice were euthanized at ZT 7 and the brains removed and stored at -80ºC. The SCN was punched out and RNA was extracted. Real-time quantitative PCR was used to analyze expression levels of per1, per2, avp, vip, grp and prok2. To our surprise, preliminary analysis of the data reveals that during restricted feeding at ZT 7 there is an increase in the expression levels of Per1 and Per2 in restricted feeding compared with ad libitum fed controls. No changes were detected in neuropeptide expression, however, a power analysis showed insufficient sample size. A larger, second experiment is examining expression in multiple brain regions across a full 24-hour time course. Changes in the quantities of Per1 and Per2 in the SCN from restricted feeding are contrary to the current conventional wisdom that the SCN in C57BL/6J mice is unaffected by restricted feeding, and may represent changes in amplitude rather than phase.

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Poster

410. Molecular Biology and Physiology of Clocks

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH R01 NS079584
NIH R01 NS094571
Johns Hopkins Woodrow Wilson Fellowship

Title: Identifying novel genes that regulate the circadian timing of sleep

Authors: *K. PARK[1], S. S. LEE[1], Q. LIU[2], I. D. BLUM[1], M. N. WU[1,2]
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Abstract: The circadian clock is an evolutionarily conserved biochemical oscillator that rhythmically modulates physiology and behavior. Investigations conducted in model organisms have unraveled the molecular circadian oscillator, but the output mechanisms by which this clock regulates physiology and behaviors are poorly understood. We previously identified *wide awake* (WAKE), a novel gene important for the circadian regulation of sleep in *Drosophila melanogaster*, and found that the mouse homolog is enriched in the mammalian pacemaker, the suprachiasmatic nucleus (SCN) (Liu et. al, 2014). Therefore, we hypothesize that other molecules critical for circadian output might be expressed in the mammalian SCN and have orthologs in *Drosophila*. To quickly identify these genes in mammals, we conceived of a top-down approach generating a list of candidates based on mammalian SCN expression profiling and then screening behavioral rhythmicity using genetic knockdown in fruit flies.
Using RNA-seq data from mouse SCN, we performed read alignment to quantify gene

transcription levels and identified the 3,230 most highly expressed genes in this data set for further screening. We further expanded this list by culling a list of candidates from the literature, using publicly available microarray and RNA-seq datasets collected at different time points. This initial list of ~4,000 gene candidates was further refined by visually examining mRNA expression using *in situ* hybridization data from the Allen Brain Atlas. Each candidate gene was then categorized based on restricted expression patterns, focusing primarily on genes that were enriched in the SCN but expressed relatively sparsely in the rest of the brain. From these 900 SCN-enriched genes, 700 *Drosophila* homologs were identified.
We are currently performing an RNAi screen of the fly homologs, focusing on phenotypes related to the circadian timing of sleep. So far, 54 out of the 510 genes from the primary screening have been identified as potential candidates whose knockdown affects various aspects of the circadian timing of sleep. Details of the results of this screen and implications for the circadian regulation of sleep in mammals will be discussed.

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Poster

410. Molecular Biology and Physiology of Clocks

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Topic: F.08. Biological Rhythms and Sleep

Support: Kent State University Brain Health Institute Seed Grant

Title: The importance of diurnal corticosterone rhythms in regulating mood

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Abstract: Corticosterone (CORT) is primarily known for controlling glucose metabolism and is released by the adrenal cortex. An intrinsic circadian rhythm of CORT is established due to the increased need for glucose during an animal's active phase than its rest phase. This results in higher levels of CORT during the active phase and lower levels during the resting phase. CORT has also been shown to entrain the rhythm of certain circadian genes to optimize function during specific times of the day. Circadian genes appear to be expressed by the neurons in the limbic brain areas that control emotional responses. Therefore, our hypothesis states that removal of the circadian rhythm of CORT can flatten the circadian gene expression in these limbic brain areas, resulting in disruption of emotional behavior. To test our hypothesis, the first step was to find a dose of CORT that can be administered in an osmotic mini-pump that mimics low basal diurnal levels of CORT. For this, we had four groups (n=3 each): 1) No pump (control); 2) 0.2ug/hr; 3)

1.0ug/hr; 4) 2.0ug/hr dose of CORT. Treatment groups underwent adrenalectomy and pump implant while the control group underwent sham surgery. Blood samples were collected one week after the surgery and an assay will be performed to measure CORT. The next step will be to examine the emotional behavior following the flattening of the diurnal CORT rhythm. For this, we plan to conduct sucrose-preference and forced swim tests during the active phase.

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Poster

410. Molecular Biology and Physiology of Clocks

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Topic: F.08. Biological Rhythms and Sleep

Support: MOST106-2320-B-182-014-
CMRPD1H0071

Title: The Na^+/H^+-exchanger NHE1 regulates extracellular and intracellular pH and $[Ca^{2+}]_i$ in the the rat suprachiasmatic nucleus

Authors: Y.-S. CHEN[1], P.-C. CHENG[1], H.-Y. LIN[1], R.-C. CHENG[1], *R.-C. HUANG[2]
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Abstract: The master circadian clock in the suprachiasmatic nucleus (SCN) is densely packed with neurons and has higher metabolic activity than extra-SCN areas in the anterior hypothalamus. In this study we tested the hypothesis that H^+ produced during energy metabolism may be extruded by the Na^+/H^+ exchanger (NHE) to maintain a more alkaline intracellular pH (pHi) and cause a more acidic extracellular pH (pHe) in the SCN in hypothalamic slices. We used ion-selective electrodes to measure the pHe values in hypothalamic slices containing the SCN and ratiometric pH imaging to investigate the pHi in reduced (mini-slice) SCN preparations. In hypothalamic slices bathed in 10 mM HEPES-buffered solution a standing acidification of ~0.3 pH units was recorded with pH-sensitive microelectrodes in the SCN but not extra-SCN areas. The NHE blocker amiloride alkalinized the pHe with an IC_{50} of 30 µM. Results obtained between day and night revealed similar levels of standing extracellular acidification and similar changes in the pHe evoked by 100 µM amiloride. RT-PCR showed the expression of mRNA for plasmalemmal-type NHE1, NHE4, and NHE5 isoforms in the SCN. The NHE1-specific antagonist cariporide alkalinized the pHe with an IC_{50} ~0.1 µM and at 1 µM acidified the pHi, suggesting that constitutive activation of NHE1 extrudes H^+ to maintain a more alkaline pHi and cause a more acidic pHe in the SCN. To determine the possible role of NHE1 in the regulation of $[Ca^{2+}]_i$, ratiometric Ca^{2+} imaging was employed to investigate the

effect of cariporide on glutamate-evoked Ca^{2+} responses in the SCN neurons. The results indicated that cariporide had mixed inhibitory and stimulatory effects on the basal $[Ca^{2+}]_i$ but inhibited glutamate-evoked Ca^{2+} rise. Real-time PCR and western blotting showed no day-night variation in NHE1 mRNA and protein levels. Immunofluorescent staining revealed punctated immunoreactivity for NHE1 around the soma and in neuropil, with moderate levels of colocalization with markers for afferent inputs, serotonin transporter and neuropeptide Y. Together the results indicate that constitutive activation of NHE1 extrudes H^+ to regulate both pHi and pHe and may play a role in the regulation of glutamatergic signaling in the rat SCN.

Disclosures: Y. Chen: None. **P. Cheng:** None. **H. Lin:** None. **R. Cheng:** None. **R. Huang:** None.

Poster

410. Molecular Biology and Physiology of Clocks

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 410.11/WW13

Topic: F.08. Biological Rhythms and Sleep

Support: NIH R01NS082413-06

Title: Characterizing clock gene expression in the substantia nigra pars compacta

Authors: *L. GOODE[1], H. H. HUYNH[2], S. BOAS[2], J. R. PAUL[3], R. M. COWELL[4], K. L. GAMBLE[5]
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Abstract: Though the expression of the core circadian molecular clock genes has been well characterized in the central circadian oscillator, the suprachiasmatic nucleus, less is known about clock gene expression in other brain regions and cell populations. One such brain region is the substantia nigra pars compacta (SNc). In particular, very little is known regarding clock gene expression within the dopaminergic neurons of the SNc. These neurons of the SNc release dopamine onto striatal neurons important for coordinating normal movement. Additionally, they exhibit pace-making properties thought to be responsible for the tonic release of dopamine. The molecular clock regulates the intrinsic firing of action potentials in the pace-making neurons of the suprachiasmatic nucleus, and thus, exploring the molecular clock within dopaminergic neurons of the SNc may provide insight into regulation of their pace-making activity. Real-time imaging of organotypic brain slice cultures containing SNc taken from Per2-Luciferase reporter mice reveals strong PER2::LUC expression rhythms that persist for several circadian cycles. Detection and visualization of individual neurons in these organotypic cultures show that

PER2::LUC oscillates at the single-cell level. Moreover, we used RNAScope single-molecule fluorescent in-situ hybridization, and found that Per2 mRNA expression in tyrosine hydroxylase-containing neurons in the SNc changes over 12hr light:12hr dark cycle, peaking in the early night. Future research aims to elucidate the role of the molecular clock in regulating these neurons' normal physiology and function. Given that dopaminergic SNc neurons are vulnerable to metabolic dysfunction and neurodegeneration, these studies are important for understanding underlying mechanisms of Parkinson's disease, which also show circadian variation in symptomology.

Disclosures: L. Goode: None. **H.H. Huynh:** None. **S. Boas:** None. **J.R. Paul:** None. **R.M. Cowell:** None. **K.L. Gamble:** None.

Poster

410. Molecular Biology and Physiology of Clocks

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 410.12/WW14

Topic: F.08. Biological Rhythms and Sleep

Support: NIH R01NS091234

Title: Somatostatin regulates photoperiodic changes in circadian behavior and master clock function

Authors: *D. A. MAY[1], M. ARZBECKER[2], T. INDA[2], E. HERFF[2], J. A. EVANS[2]
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Abstract: Seasonal changes in the environment modulate daily rhythms of behavior and physiology, but the neural mechanisms that underlie these adaptations remain unclear. Previous research has found that seasonal lighting conditions influence somatostatin expression in the hypothalamus, which has been postulated to be driven by changes in the master clock of the suprachiasmatic nucleus (SCN). Here we test that long days increase somatostatin (SST) neurons in the SCN and that SST signaling regulates circadian clock function. Using a genetic labeling approach, we find evidence that long days activate *de novo* somatostatin transcription in a reserve pool of SCN neurons. Similar effects are observed in the paraventricular and periventricular nuclei. Further, we find that loss of somatostatin alters circadian behavioral responses to long day photoperiods and other environmental lighting conditions. Last, we find that inhibition of somatostatin signaling disrupts coupling among SCN neurons that have been reorganized into a polarized network state by long days. Collectively, these results suggest that somatostatin signaling contributes to light-driven plasticity of circadian behavior and master clock function.

Disclosures: D.A. May: None. **M. Arzbecker:** None. **T. Inda:** None. **E. Herff:** None. **J.A. Evans:** None.

Poster

410. Molecular Biology and Physiology of Clocks

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 410.13/XX1

Topic: F.08. Biological Rhythms and Sleep

Support: GM076430
EY027202

Title: *In vivo* physiology of the perihabenular nucleus

Authors: *L. LAZZERINI OSPRI[1], H. ZHAO[1], S. HATTAR[2]
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Abstract: Light can affect mood independently of circadian and sleep alterations. A newly-discovered circadian oscillator in the dorsal thalamus, the perihabenular nucleus (PHb), mediates these direct effects of light. As its physiology was entirely unknown, we set out to characterize the PHb's activity in mice in vivo. PHb neurons were transfected with the fluorescent Ca^{2+} sensor GCaMP6. Specificity of transfection to the PHb was ensured by conditional expression of the GCaMP6 vector based on connectivity. An optical fiber was implanted above the PHb to deliver excitation light and collect emitted fluorescence, in a photometry setup where the test mouse could move and behave freely in its cage for multiple-day recordings.
In a standard equinoctial light cycle (T24), we found that the PHb responded to out-of-phase light presentation: Light pulses at night elicited biphasic Ca^{2+} transients, characterized by short (90 ms) onset latency, a 2 s plateau phase followed by more intense activity, and a slow decline back to baseline over a variable interval lasting 5-30 s. The PHb also detected the regularly-scheduled lights-on event (daybreak) at ZT00, which elicited a similar biphasic response, but of smaller amplitude and with faster decay. The PHb also responded to the regular lights-off event at ZT12 with a modest, slow Ca^{2+} transient with onset 1 s after nightfall and peak within 5 s. "Spontaneous" (i.e. photic-stimulus-independent) activity was also evident with sporadic, low-amplitude peaks occurring throughout the day and night. Median GCaMP fluorescence exhibited a daily rhythm, with the peak in the photophase. After an animal was switched to an aberrant light cycle, LD 3.5:3.5 (T7), known to cause depression-like behavior, the PHb showed a generalized activity elevation, with Ca^{2+} transients after the light-to-dark and dark-to-light transitions, and a progressive disruption of its daily rhythm. We conclude that the PHb seems to be especially suited to encode information about dawn and dusk timing, consistent with a role in photoperiod calculation, and that depressogenic light regimens can stimulate its activity.

This is the first physiological characterization of a newly-discovered thalamic nucleus involved in mood regulation by light exposure.

Disclosures: L. Lazzerini Ospri: None. **H. Zhao:** None. **S. Hattar:** None.

Poster

410. Molecular Biology and Physiology of Clocks

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 410.14/XX2

Topic: F.08. Biological Rhythms and Sleep

Support: NIH R01NS091234

Title: vasopressin modulates circadian behavior by setting phase and period of scn neurons

Authors: *K. E. ROHR, J. EVANS
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Abstract: The suprachiasmatic nucleus (SCN) is a neuronal network that programs daily rhythms in behavior and physiology. Intercellular signaling among SCN neurons is required to maintain daily rhythms in behavior and adjust them to the environment. A subset of SCN neurons produces arginine vasopressin (AVP), which typically viewed as an important SCN output. In addition, the SCN expresses receptors for AVP, but whether AVP modulates SCN function remains unclear. Here, we tested the influence of AVP signaling on SCN function and circadian behavior. To test the role of AVP signaling on SCN clock function, we examined the molecular rhythms of SCN neurons using real-time bioluminescence imaging. To manipulate AVP signaling, we used a pharmacological blockade of AVP receptor signaling (V1 antagonism).
Whereas the SCN under typical conditions displays molecular rhythms that are close to 24 h, V1 antagonism increased SCN period by at least 1 h. Additionally, V1 antagonism caused SCN neurons to display similar times of peak protein expression, which increased homogeneity of molecular timing across the network. The magnitude of response to V1 antagonism varied across the anteroposterior SCN and neurochemically distinct subregions of the network, suggesting that AVP signaling differentially modulates the molecular clock of neuronal subgroups. Consistent with pharmacological results in vitro, AVP knockdown mice display longer free-running period and differences in the plasticity of circadian waveform under constant darkness. Together, these results indicate that AVP signaling modulates the period and phase relationships of SCN neurons, which has functional consequences for how the master clock communicates time of day to downstream tissues and responds to environmental changes.

Disclosures: K.E. Rohr: None. **J. Evans:** None.

Poster

410. Molecular Biology and Physiology of Clocks

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Topic: F.08. Biological Rhythms and Sleep

Support: R01 MH103361/MH/NIMH NIH
R01 NS066345/NS/NINDS NIH
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Title: Circadian regulation of phosphoprotein enriched in astrocytes (PEA-15) expression and function within the mouse suprachiasmatic nucleus

Authors: K. WHEATON[1], S. ATEN[2], L. S. QUEIROZ[1], K. SULLIVAN[2], J. OBERDICK[2], K. OBRIETAN[2], *K. R. HOYT[1]
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Abstract: Disruption of the circadian timing system has profound detrimental effects on health and cognition and has been linked to an array of human pathologies. The suprachiasmatic nucleus (SCN) of the hypothalamus (often referred to as the 'master clock') generates timing cues that coordinate cellular oscillator populations throughout the brain as well as peripheral organ systems. This phasing is tightly controlled by photic input signals from the retina and resetting of the clock by photic input is regulated by the p44/42 mitogen-activated protein kinase (MAPK) pathway. Here, we investigated cellular mechanisms that regulate ERK (the key effector kinase of the MAPK pathway) phosphorylation and its cellular localization within the SCN. To this end, we examined the expression and function of phosphoprotein enriched in astrocytes (PEA-15), a scaffold protein that regulates both the activation state of ERK and its subcellular localization. Using immunohistochemical labeling and Western analysis, we found that PEA-15 is highly enriched within the mouse SCN and PEA-15 expression oscillates with a circadian rhythm. Notably, the peak in PEA-15 expression was antiphase to the well-characterized circadian oscillation in ERK activity. PEA-15 phosphorylation at serine-104 was induced by photic stimulation. This phosphorylation event triggers the dissociation of ERK from PEA-15 and the translocation of ERK to the nucleus. We confirmed both PEA-15 binding to ERK and the dissociation of ERK from PEA-15 upon photic stimulation via co-immunoprecipitation experiments in SCN lysates. In total, these studies reveal PEA-15 to be neuronally enriched, clock-gated and light responsive in the SCN. Given its high expression in the SCN, coupled with its role as a critical regulator of MAPK signaling, PEA-15 may be well positioned to contribute to the functional fidelity of signaling networks that regulate SCN clock timing and entrainment. Current work is focused on the examination of clock timing in PEA-15 null mice.

Disclosures: K. Wheaton: None. **S. Aten:** None. **L.S. Queiroz:** None. **K. Sullivan:** None. **J. Oberdick:** None. **K. Obrietan:** None. **K.R. Hoyt:** None.

Poster

410. Molecular Biology and Physiology of Clocks

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 410.16/XX4

Topic: F.08. Biological Rhythms and Sleep

Title: Microglia dynamics in the suprachiasmatic nucleus across circadian time

Authors: *L. G. CHASTAIN, K. A. HALTER, R. A. PROSSER
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Abstract: Microglia, the brain's resident immune cells, have been shown to play important non-immune roles in regulating synapses and maintaining neuronal function. Circadian rhythms are regulated by the suprachiasmatic nucleus (SCN) of the hypothalamus, but specific mechanisms underlying circadian clock regulation are not completely understood. Data on the role of microglia in circadian rhythms are scarce, but a few studies have shown significant time of day differences in microglial immune response, and microglia show circadian rhythms in pro-inflammatory factor expression, suggesting that microglia undergo circadian changes in function. Our studies aim to investigate whether microglia undergo changes in phenotype in the SCN as a function of endogenous circadian rhythms. To address this issue, we used immunohistochemical characterization of microglia cells in the SCN of adult male C57Bl/6 mice at three distinct circadian times: Zeitgeber Time (ZT) 6, ZT 16, and ZT 23 (where ZT 0 corresponds to lights on and ZT12 corresponds to lights off). Hypothalamic blocks of mouse brain tissue (n=3) were collected at the three timepoints, sectioned at 25 µm thickness and stained for IBA-1 (a microglia-specific marker) using immunofluorescence. Slides were imaged on an immunofluorescent microscope to assess fluorescent intensity, microglia cell number, and microglia cell phenotype in the SCN region at the three times. As microglia transition from a quiescent to an active state, their morphology changes from a ramified state (small cell body and many filopodial extensions) to an amoeboid state (larger cell body, less extensions). We characterized microglia in the SCN as either amoeboid, partially amoeboid, ramified, or partially ramified according to the number and complexity of their processes. Differences between timepoints were analyzed by one-way ANOVA followed by Newman-Keuls post-tests. There was no significant difference in microglia number in the SCN across circadian time, but there was a significant increase in partially ramified microglia at ZT 16 and ZT 23 compared to ZT 6. In addition, there was a trend for increased partially amoeboid microglia in the SCN at ZT 6 compared to ZT 16 and ZT 23. Finally, there was an increase in IBA-1 immunofluorescence in the SCN at ZT 23 as compared to ZT 6 in the SCN. Thus, microglia undergo morphological

changes in the SCN as a function of circadian time, and these changes may indicate dynamic alterations in function or activation. Follow up studies are addressing whether microglial interactions with SCN neurons are altered across circadian time and whether microglia play a role in modulating SCN neuronal regulation of circadian rhythm.

Disclosures: L.G. Chastain: None. **K.A. Halter:** None. **R.A. Prosser:** None.

Poster

410. Molecular Biology and Physiology of Clocks

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 410.17/XX5

Topic: F.08. Biological Rhythms and Sleep

Title: Calcium-binding protein calreticulin is co-expressed with neurogenesis marker SOX2 in the adult mouse suprachiasmatic nucleus

Authors: T. M. BIRKHOLZ, D. H. BELIGALA, A. DE, *M. E. GEUSZ
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Abstract: The role of multiple calcium-binding proteins such as calbindin and calretinin in the hypothalamic suprachiasmatic nucleus (SCN) has been frequently examined. Here we describe the expression pattern of the multifunctional calcium-binding protein calreticulin (calregulin or calreg) that is known to buffer endoplasmic reticulum (ER) Ca^{2+}. Cytosolic Ca^{2+} mobilization driven by Ca^{2+} release from ryanodine-sensitive internal stores has been shown to modify gene transcription in the SCN and shift the phase of the circadian clock. Calreg is a major regulator of ER Ca^{2+} levels and has been shown to interact with ryanodine receptors of the ER to influence Ca^{2+} release, yet its function within the SCN is largely unknown. To identify calreg protein distribution in the SCN we performed immunocytochemistry and confocal microscopy of mouse brain sections. Imaging revealed positive calreg expression within both shell and core SCN sub-regions with relatively higher expression in the ventral SCN. A large number of calreg-positive cells (85%) showed co-localization with SOX2 protein, a regulator of embryonic and adult neurogenesis that is known to be expressed in the SCN. The SCN expresses several proteins that are more often found in stem cells or during neurogenesis, and confirming this we identified stem cell marker OCT4 in 53% of SCN cells. Neural stem cells have been shown to express integrins that are known to serve in cell-cell communication and cell adhesion with the extracellular matrix. Overexpression of calreg has been shown to influence integrin-mediated epithelial-mesenchymal transition, suggesting that it also contributes to morphological changes and stemness in the SCN. Furthermore, co-localization of calreg with SOX2 indicates that calreg may have a unique function in SCN stem-like cells. Localization of calreg with neuronal marker NeuN indicated that some of the calreg expression was in SCN neurons. Calreg expression was primarily within the ventral SCN where retinal axons project to SCN neurons, suggesting that

calreg could serve in this pathway of light signals to the clock that maintains its entrainment to the daily rhythms of the external environment.

Disclosures: T.M. Birkholz: None. **D.H. Beligala:** None. **A. De:** None. **M.E. Geusz:** None.

Poster

410. Molecular Biology and Physiology of Clocks

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 410.18/XX6

Topic: F.08. Biological Rhythms and Sleep

Support: MRC UK DTP Studentship
BBSRC BB/M02329X/1

Title: Circadian timekeeping in the brainstem: Ticking and talking in the area postrema and nucleus of the solitary tract

Authors: *L. CHROBOK[1], R. C. NORTHEAST[2], H. D. PIGGINS[2]
[2]Fac. of Life Sci., [1]Univ. of Manchester, Manchester, United Kingdom

Abstract: Major components of energy balance exhibit circadian variation. In the brainstem, the area postrema (AP) is a sensory circumventricular organ which plays well documented roles in cardiovascular and visceral reflexes. Immediately adjacent to the AP is the nucleus of the solitary tract (NTS), a key relay structure for enteroceptive signals. Together these nuclei create a complex which acts a powerful gateway for homeostatic signals through the brainstem. Here, using single cell bioluminescent imaging, we report our novel findings of clear circadian rhythmicity and spatiotemporal patterning of *Per2*, a core canonical clock gene, in the AP and NTS. To monitor cyclic changes in PER2 *ex vivo*, bioluminescence signals were recorded from brain slice explant cultures obtained from male mPER2::LUC mice. Highly robust single cell oscillations were readily detected in both the AP and adjacent NTS. Spatiotemporal analysis revealed higher amplitude oscillations in the cell-dense AP compared with the NTS, where rhythmic cells were more sparsely distributed. At the whole tissue level, the peak in the NTS rhythm was delayed by approximately 2 hours compared to that of the AP (n=9). Rhythmic bioluminescence was sustained for up to 7 days in culture and could be resynchronised and prolonged for up to a further 14 days by forskolin. Single cell analysis demonstrated that individual rhythmic cells in the NTS were more robust than corresponding cells in the AP, but the phase dispersal of rhythmic NTS cells was broader than that of rhythmic AP cells. To gain insight into the role of signalling between the AP and the NTS in maintenance of oscillations, we mechanically disconnected these two nuclei (n=3) which led to an increased phase dispersion of rhythmic NTS cells. This indicates that the phase coupling of NTS cells is dependent on intact neural connectivity to the AP. Additionally, the application of 0.5 μM TTX resulted in

pronounced phase desynchronization of single cell oscillators in both structures throughout the pharmacological blockage of action potential-dependent neuronal communication. This diminished the rhythmicity of the AP/NTS at the whole tissue level, which recovered on removal of TTX from the media. This study is the first to demonstrate self-sustained single cell oscillations in PER2 bioluminescence in the AP and the NTS with evidence of neuronal interplay between the structures in the maintenance of these rhythms. When compared to other extra-suprachiasmatic nucleus oscillators, the AP/NTS complex exhibit highly robust rhythmicity, therefore our findings place it as a potentially important component of an extended neural circadian system.

Disclosures: R.C. Northeast: None. **H.D. Piggins:** None.

Poster

410. Molecular Biology and Physiology of Clocks

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Topic: F.08. Biological Rhythms and Sleep

Support: MRC UK DTP Studentship
BBSRC BB/M02329X/1

Title: Robust cycling of circadian clock genes in the area postrema/nucleus of the solitary tact complex accompanies daily changes in permeability to blood-borne signals

Authors: R. C. NORTHEAST[1], L. CHROBOK[2], *H. PIGGINS[3]
[1]Fac. of Biology, Medicine, and Hlth., Univ. of Manchester, University of Manchester, United Kingdom; [2]Fac. of Biology, Medicine, and Hlth., Univ. of Manchester, Manchester, United Kingdom; [3]Univ. Manchester, Manchester, United Kingdom

Abstract: The area postrema (AP) is a sensory circumventricular organ (CVO) located in the brainstem that senses blood-borne chemical signals. Although the AP lacks a blood-brain barrier (BBB), a specialized cellular barrier between the AP and the adjacent nucleus of the solitary tract (NTS) regulates the entry of circulating substances into the brain. This barrier is composed of vimentin/GFAP+ve glial processes connected by tight junction proteins including zona occludens-1 and occludin. Little is known about the role of the intrinsic circadian clock in the AP/NTS complex and how it might regulate their key functions. Here we investigated rhythmic gene expression in these areas as well as the possibility of daily changes in the permeability of the NTS to blood-borne signals. In brainstem slice cultures obtained from mPER2::LUC mice, we detected sustained PER2 bioluminescence oscillations at the level of single cells in both the AP and NTS (n=6). Confocal imaging of fixed brain sections from *Per1::*Venus mice (n=3) showed *Per1* expression in numerous cells of the AP/NTS complex. Moreover, circadian qPCR

measurements from AP and NTS sections (CT0, CT6, CT12 and CT18; n=4-5) indicated robust *Per2* cycling, peaking at CT12, with *Bmal1* oscillation reversed in phase in both structures. Intra-cardiac perfusions with Evans Blue dye (EB; does not penetrate a functionally healthy BBB) were performed at two time points, early day (ZT1; n=5) and early night (ZT13; n=4). Surprisingly, at ZT13, EB staining was found not only in the BBB-lacking AP, but also penetrating into the adjacent NTS (particularly its medial subregion); whereas at ZT1, all EB staining was restricted to AP only. Further immunohistochemical staining against glial markers in the AP/NTS border showed decreased density of GFAP-positive but not vimentin-positive processes in the early subjective night (CT13, n=5) compared to early subjective day (CT1, n=4). Additionally, *zona occludens-1* in the AP demonstrated circadian variation across 4 time points (CT0, CT6, CT12, CT18; qPCR) with the lowest expression at CT12. As expected, EB was contained in the other sensory CVOs, the subfornical organ and vascular organ of lamina terminalis, and with no accompanying circadian change in glial markers unlike the AP/NTS. Taken together these results reveal novel temporal control unique to the glial border in the AP/NTS complex. This study is the first to demonstrate robust circadian rhythmicity of the core molecular clock in the AP/NTS complex *ex vivo*. We also highlight a potential role for the local circadian clock in shaping daily changes in the permeability of the brainstem to blood-borne signals.

Disclosures: R.C. Northeast: None. **L. Chrobok:** None. **H. Piggins:** None.

Poster

410. Molecular Biology and Physiology of Clocks

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 410.20/XX8

Topic: F.08. Biological Rhythms and Sleep

Support: NNSF 31671221

Title: A Bmal1-ablation macaque monkey model for circadian-related disorders

Authors: *H.-C. CHANG[1], Y.-C. TANG[2]
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Abstract: Circadian dysfunction has long been recognized as a risk factor causing metabolic abnormalities, psychiatric disorders and aging. To investigate the circadian-related disorders in a physiological comparable manner to humans, we generated a circadian disrupted non-human primate model to monitor the associations to clinic findings. By ablating BMAL1, a crucial circadian transcription factor, with CRISPR/Cas9 editing in cynomolgus monkeys, we obtained monkeys showing higher nocturnal activities and depression phenotypes at preadolescent age. Deterioration of internal circadian program in the mutants was revealed by arrhythmic hormonal

levels and a sensitized response to constant illuminating regimen. Ablation of BMAL1 led to transcriptional programs elevated toward inflammatory and stress responses. Further transcription network analysis revealed significant correlations clustered to human sleep deprivation, major depressive disorder and aging data, suggested the *Bmal1* model is valuable in examining circadian disturbed outcomes in a collective manner. The new model offered a perspective option to circadian-oriented interventions for psychological and chronic diseases.

Disclosures: H. Chang: None. **Y. Tang:** None.

Poster

410. Molecular Biology and Physiology of Clocks

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 410.21/XX9

Topic: F.08. Biological Rhythms and Sleep

Support: NSF Grant 1256105

Title: Connectome of the mouse SCN: New insight into the relation of the peptides VIP to AVP

Authors: *S. VARADARAJAN[1], M. TAJIRI[1], R. JAIN[1], R. HOLT[1], Q. AHMED[1], J. LESAUTER[1], R. SILVER[2]
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Abstract: A brain clock, constituted of ~20,000 peptidergically heterogeneous neurons, is located in the hypothalamic suprachiasmatic nucleus (SCN). While many peptidergic phenotypes have been identified, little is known about the connections among these neurons in mice. We first sought to identify contacts among major peptidergic cell types in SCN using triple-label fluorescent immunocytochemistry. To this end, contacts among VIP, gastrin-releasing peptide (GRP), and calretinin (CALR) cells of the core, and arginine vasopressin (AVP) and met-enkephalin (ENK) cells of the shell were analyzed. While reciprocal connections are made among the other phenotypes tested, VIP fibers make many appositions with AVP neurons, but contacts in the reverse direction are sparse. To better understand the impact of VIP-to-AVP communication, we next explored the SCN in VIP deficient mice (VIP-KO). In these animals, AVP expression is markedly reduced in the SCN, but it is not altered in the paraventricular and supraoptic nuclei. Surprisingly, in VIP-KO mice, the number of AVP appositions onto other peptidergic phenotypes is not reduced. Colchicine administration restored numbers of AVP neurons to that of wild-type littermates. The results support the hypothesis that AVP synthesis is reduced in neurons of VIP-KO animals. In summary, the various SCN peptidergic phenotypes of the SCN core and shell make largely reciprocal connections. The results highlight a unique mostly unidirectional core-to-shell communication by VIP-to-AVP. Furthermore, the results point to an important role for VIP in AVP regulation, in a manner specific to the SCN.

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Poster

411. Sleep: Molecules Cells and Drugs

Location: SDCC Halls B-H

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Program #/Poster #: 411.01/XX10

Topic: F.08. Biological Rhythms and Sleep

Support: NIH T32MH020068

Title: Adenosine receptors and recovery sleep in *C. elegans*

Authors: *B. MAHAMA, A. C. HART
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Abstract: The purpose of this study was to investigate the role of adenosine receptor, *ador-1*, signaling on recovery sleep in *C. elegans*. Poorly understood homeostatic mechanisms drive recovery sleep after extended wakefulness, altering the next sleep period. Studies with humans and mice implicate adenosine buildup and adenosine receptor signaling in this recovery sleep. However, *Drosophila* and other mouse studies suggest adenosine receptor signaling is not needed for recovery sleep. I propose to examine this conflict by studying the consequence of *ador-1* loss of function in *C. elegans* recovery sleep. I developed a simple system that perturbs sleep during L4/A lethargus and showed that recovery sleep in this system is dependent on daf-16, as reported in the literature (PMID: 23477722, PMID: 25474127, PMID: 29523076). Compared to wildtype animals that increase their total sleep after deprivation, preliminary data suggests *ador-1* loss of function animals decreased their total sleep after deprivation. These results imply that adenosine receptor signaling in *C. elegans* may be important for their ability to respond to disrupted sleep. In the future, I will probe specifics of the signaling pathway by testing the effects of caffeine, a known adenosine receptor antagonist. Then, I will determine which neurons require *ador-1* function for recovery sleep. These studies will help us understand how animals recover after sleep loss.

Disclosures: B. Mahama: None. **A.C. Hart:** None.

Poster

411. Sleep: Molecules Cells and Drugs

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 411.02/XX11

Topic: F.08. Biological Rhythms and Sleep

Support: The University of California Institute for Mexico and the United States (UC MEXUS)
Consejo Nacional de Ciencia y Tecnología (CONACyT; Grant: CN-17-19)
Escuela de Medicina, Universidad Anáhuac Mayab (Grant: PresInvEMR2017)

Title: Sleep goes nuclear: Role of histone methylation/demethylation inhibition in sleep modulation

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Abstract: Abstract
Histone activity has been described as a key element in epigenetic mechanisms. Moreover, histone methyltransferases and demethylases have been identified as contributing factors in the development of multiple health disturbances, especially cancer. However, the neurobiological role of histone demethylases in behaviors, such as sleep, remains unknown. Thus, we investigated the effects of the systemic administration of histone demethylation inhibitor GSK-J1 or the histone methylation inhibitor DZNep in sleep-wake cycle during the lights-on or the lights-off period of rats. We found that systemic injection of GSK-1 (5, 10 or 20mg/Kg, i.p.) when injected at the beginning of the lights-on period induced no statistical changes in total time of W, SWS or REMS. However, if administered at the beginning of the lights-off period, GSK-J1 decreased wakefulness (W) and enhanced slow wave sleep (SWS) as well as rapid eye

movement sleep (REMS). In opposition, DZNep (5, 10 or 20mg/Kg, i.p.) exerted a dose-dependent increase in W as well as a decrease in SWS and REMS during the lights-off period with no changes observed in lights-on period. Thus, histone demethylation inhibition provoked sleep whereas histone methylation inhibition induced alertness. Our study reports by the very first time the role of epigentical elements such as histones in sleep modulation.
This work was supported by The University of California Institute for Mexico and the United States (UC MEXUS) and Consejo Nacional de Ciencia y Tecnología (CONACyT; Grant: CN-17-19) and Escuela de Medicina, Universidad Anáhuac Mayab (Grant: PresInvEMR2017) given to E.M-R.

Disclosures: G. Arankowsky-Sandoval: None. **N. Barbosa Rocha:** None. **A. Barciela Veras:** None. **S. Machado:** None. **H. Budde:** None. **E. Murillo-Rodríguez:** None.

Poster

411. Sleep: Molecules Cells and Drugs

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Program #/Poster #: 411.03/XX12

Topic: F.08. Biological Rhythms and Sleep

Support: R01 MH 099544
Carver Chair in Neuroscience

Title: Sleep deprivation induces alterations in RNA splicing and changes in gene expression within the hippocampus

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Abstract: Sleep deprivation has become a common problem in modern society due to work demands, the use of personal electronics and lifestyle choices. Sleep deprivation results in numerous adverse health issues including the exacerbation of mood disorders and neurodegenerative diseases. We and others have shown that sleep deprivation causes deficits in cognitive function and performance, with hippocampus-dependent memory particularly susceptible to the effects of sleep deprivation. Previously, we found that a single 5 hour period of sleep deprivation impacted hippocampal gene expression (Vecsey et al., 2012). However, little is known about the effects of sleep deprivation on mRNA processing and splicing. The current research furthers our understanding of this question using genome-wide deep RNA sequencing to identify the impact of sleep deprivation on gene expression and mRNA splicing. Wildtype

C57BL/6J male mice were sleep deprived using a gentle handling method that has previously been shown to disrupt sleep without inducing stress effects (Vecsey et al., 2013). Immediately after sleep deprivation (or at the same circadian time point for non-sleep deprived controls), the hippocampus was removed, flash frozen and RNA extracted. Following rRNA depletion with the Ribo-Zero kit (Illumina), RNA libraries were prepared using the TruSeq Stranded Total RNA Library Prep Gold kit (Illumina). Sequencing was performed on a HiSeq4000 with 150bp paired-end reads. Differential RNA expression between the sleep deprived (n=4) and control (n=4) groups was computed with statistical models accounting for unwanted sources of variation. Gene set enrichment analysis was performed on gene ontology terms and KEGG pathways. Differential expression of genes of interest following sleep deprivation was verified in independent biological replicates using Q-PCR. Consistent with previous research (Vecsey et al, 2012; Wang et al 2010), we found that sleep deprivation significantly affected gene expression of *Rbm3*, an RNA binding protein. Specifically, we found that sleep deprivation significantly increased the abundance of the long isoform of *Rbm3* and significantly decreased levels of the short *Rbm3* transcripts. Furthermore, we found genes in two pathways related to regulation of mRNA splicing up-regulated following sleep deprivation ($P = 8.3 \times 10^{-4}$). These results strongly suggest that sleep deprivation affects RNA splicing as well as overall gene expression. This study sets the stage for future work investigating the role of sleep in RNA processing and understanding of the molecular consequences of acute sleep deprivation.

Disclosures: M.E. Gaine: None. **S. Chatterjee:** None. **E. Bahl:** None. **J.J. Michaelson:** None. **L.C. Lyons:** None. **T. Abel:** None.

Poster

411. Sleep: Molecules Cells and Drugs

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Program #/Poster #: 411.04/XX13

Topic: F.08. Biological Rhythms and Sleep

Title: Sleep- related electrophysiological activity of cortical cultures on MEA

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Abstract: Over the last few years, it has been proposed that electrophysiological markers for sleep cycles can be also derived from in vitro neuronal networks, similar to those present in vivo. To this end, a lot of efforts in current sleep science aims at understanding to which extent an in vitro system can recapitulate the essential features of a sleeping brain. In this context, cortical cultures coupled to Micro-Electrode Arrays (MEAs) can represent a simplified and easily accessible in vitro model used for studying principles of neurodynamics and neural coding.

Although neuronal assemblies plated on MEAs show spontaneously synchronized, low frequency firing patterns, which could resemble the slow wave oscillations that characterize non-REM (NREM) sleep in vivo, the lack of the awake state counterpart limited the investigation of the main physiological aspects of sleep. To this end, in a previous work we stimulated our in vitro neuronal networks using the cholinergic agonist Carbachol (CCh, 20 µM) to suppress the sleep features. We observed for the first time that CCh treatment affected both the high and the low frequency components of the signal, causing a suppression of the classical sleep-like properties of activity. Starting from this finding, we were then interested in understanding how the electrophysiological signal changed in pathologies that affect sleep. Prader-Willi syndrome (PWS) is a rare neurodevelopmental disorder that is associated with a paternally-expressed genomic imprinting defects within the human chromosome region 15q11-13. One of the candidate genes is the small nucleolar ribonucleic acid-116 (SNORD116). PWS patients are often affected by sleep-wake disturbance associated with alteration during REM sleep, including abnormalities in theta waves. The same identical sleep/EEG defects were found using mutant mice (PWS) carrying a deletion of Snord116 at the orthologous locus. Then we wanted to answer one main question: can our model recapitulate the essential features in a pathological model of sleep? More specifically, is it possible to observe the same abnormalities in a simplified and accessible model in vitro? We performed preliminary experiments using cortical cultures of PWS mice and we applied Carbachol (CCh, 20 µM) to suppress the sleep waves. We found the same difference in the theta waves upon CCh administration, suggesting the idea that our model can be used to study and manipulate sleep properties in a very controlled way. MEA recordings coupled to cortical cultures seem to represent a possible model to investigate the essential features of sleep in both physiological and pathological conditions.

Disclosures: I. Colombi: None. **M. Pace:** None. **V. Tucci:** None. **M. Chiappalone:** None.

Poster

411. Sleep: Molecules Cells and Drugs

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Support: The University of California Institute for Mexico and the United States (UC MEXUS)
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Title: Cannabidiol enhances acetylcholine levels from basal forebrain in rats

Authors: *K. M. ROMERO CORDERO[1,2], G. ARANKOWSKY-SANDOVAL[3], N. BARBOSA ROCHA[4,5], A. BARCIELA VERAS[4,6], S. MACHADO[4,7,8,9], H. BUDDE[4,10,11,12], E. MURILLO-RODRIGUEZ[2,4]

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Abstract: *Cannabis sativa* is a plant that contains more than 500 components, of which the most studied are Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD). Several studies have indicated that CBD displays neurobiological effects, including wake promotion. In this regard, experimental evidence shows that injections of CBD enhance wake-related compounds, such as monoamines (dopamine, serotonin, epinephrine, and norepinephrine). Despite that CBD modulates several neurochemicals linked with wakefulness modulation, no clear evidence is available regarding the effects of CBD on additional wake-related compound such as acetylcholine (ACh). Here, we determine that systemic injections of CBD (0, 5, 10 or 30mg/Kg, i.p.) at the beginning of the lights-on period, increased the extracellular levels of ACh measured by microdialysis and HPLC means. These findings indicate that CBD promotes ACh accumulation in the basal forebrain, a brain region related to wake control.

Disclosures: K.M. Romero Cordero: None. **G. Arankowsky-Sandoval:** None. **N. Barbosa Rocha:** None. **A. Barciela Veras:** None. **S. Machado:** None. **H. Budde:** None. **E. Murillo-Rodriguez:** None.

Poster

411. Sleep: Molecules Cells and Drugs

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Program #/Poster #: 411.06/YY1

Topic: F.08. Biological Rhythms and Sleep

Support: Teva Parmaceuticals project P24450

Title: Does pitolisant suppress cataplexy through the H3 receptor?

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Abstract: Narcolepsy is a sleep disorder characterized by excessive daytime sleepiness (EDS), fragmented sleep/wake and cataplexy (C), the emotionally-triggered loss of muscle tone. Pitolisant (PIT), an inverse agonist at histamine H3 receptors that was approved by the EMA as a narcolepsy therapeutic in 2016, has been reported to decrease both EDS and C symptoms. Here, we tested its effects compared to the selective H3 inverse agonist irdabisant (IRD) and to the wake-promoting drug modafinil (MOD) in a conditional mouse narcolepsy model, *orexin/tTA; Tet-O diphtheria toxin A* mice (DTA mice), where hypocretin/orexin neurons are ablated in the absence of doxycycline in the diet.

PIT (10, 20 and 40 mg/kg), IRD (3, 10 and 30 mg/kg), and MOD (32, 64 and 128 mg/kg) were administered IP to DTA mice during the dark period at ZT18. C, sleep/wake parameters, core body temperature (T_b), and locomotor activity (LMA) were compared to vehicle treatment (0.5% methylcellulose /0.2% Tween 80 in deionized water). EEG, EMG, T_b, LMA were recorded via telemetry along with video recordings of behavior and assessed for 6 h post-injection. EEG and EMG recordings were scored in 10 s epochs for waking (W), rapid eye movement sleep (REM), non-rapid eye movement sleep (NR) and C.

Although both PIT and IRD had strong wake-promoting effects and all 3 compounds increased W in a dose-related manner, only PIT at 40 mg/kg significantly decreased C across the 6 h recording period. Both PIT and IRD significantly decreased NR and REM; MOD effects were limited to decreased NR. Sleep/wake consolidation measures revealed that the increase in W following PIT occurred via an increase in W bout duration and a decrease in the number of W bouts, indicative of a highly consolidated W periods. The increased W following IRD and MOD occurred via increases in both W bout duration and the number of W bouts. EEG power analysis revealed different spectral profiles during W for the 3 compounds. PIT at 40 mg/kg produced large variations across the power spectrum: power within the delta range and from ~21-40 Hz increased while power between ~5-19 Hz and ~42-100 Hz decreased. These effects gradually attenuated but were still evident during the last recording h. In addition, PIT caused abnormal EEG synchrony in 5 of 8 mice. The high dose of IRD resulted in a large increase in power between 30-60 Hz which lasted for ~4 h. MOD had very little effect on the W power spectrum. Our data show that PIT increased W and, at the highest dose, reduced C in a mouse model of narcolepsy. However, since the selective H3 receptor inverse agonist IRD only increased W, the PIT-mediated effects on C may occur through a non-H3 mechanism.

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Poster

411. Sleep: Molecules Cells and Drugs

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Topic: F.08. Biological Rhythms and Sleep

Support: IRG Grant 205443
ANR Grant 17-CE16-0024

Title: Brain state dependent modulation of neuronal firing and membrane potential dynamics in the somatosensory thalamus during natural sleep

Authors: *N. L. URBAIN[1], N. FOURCAUD-TROCMÉ[2], P. SALIN[2], L. GENTET[2]
[1]CRNL INSERM U1028, CNRS UMR 5292, Lyon, France; [2]CRNL INSERM U1028, Lyon, France

Abstract: Slow rhythms and spindle oscillations form the hallmarks of non-rapid eye movement sleep (NREMs). The thalamus plays a central role in sleep rhythms in the mammalian brain yet, surprisingly little is known about its function and interaction with local cortical oscillations during NREMs. To uncover the functioning of the thalamocortical loop in naturally-sleeping animals, simultaneous measurements of thalamic and local cortical activity are required. Focusing on the mouse somatosensory system, we investigated during natural NREMs the neuronal correlates of cortical barrel activity in its two corresponding thalamic nuclei, the ventral posterior medial (VPM) and the posterior medial (Pom) nuclei.
We performed extra- and intra-cellular recordings of thalamic relay cells combined with S1 local field potential (S1-LFP) while simultaneously measuring the electroencephalogram and the electromyogram. These recordings allowed us to investigate the dynamics of thalamic subthreshold and spiking activity coupled with cortical oscillations across episodes of NREMs in non-anesthetized mice.
Our data reveal 1) that spindle features evolve throughout NREMs episodes and vary according to the post-NREMs state, 2) distinct modulations of VPM and Pom activity throughout NREMs episodes, 3) a thalamic nucleus-specific phase-locking to cortical slow and spindle waves, and 4) cell-specific subthreshold spindle oscillations in VPM neurons that only partially overlap with cortical spindles.
Taken together, our results suggest that, during natural sleep, the barrel cortex exerts a leading role in the generation and transfer of slow rhythms to the thalamus and reciprocally for spindle oscillations.

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Poster

411. Sleep: Molecules Cells and Drugs

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Topic: F.08. Biological Rhythms and Sleep

Support: KAKENHI(17H06520)
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Title: Pontine neural ensemble dynamics during p-waves in mice

Authors: *T. TSUNEMATSU[1,2], S. SAKATA[3]
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Abstract: Rapid eye movement (REM) sleep is characterized by hippocampal theta waves and phasic sub-second waves in the brainstem, so-called ponto-geniculo-occipital (PGO) waves in cats or pontine (P) waves in rodents. Although the sub-second pontine wave during REM sleep was originally described in 1960s, the underlying mechanism of P-wave genesis during REM sleep is still not fully understood. Using in vivo electrophysiological approaches in mice, we investigated the temporal evolution of P-waves and accompanied neural ensemble dynamics in the pontine. First, to examine how P-waves appear during the sleep-wake cycle, bipolar electrodes were implanted into the medial parabrachial nucleus (MPB) of head-fixed mice. The density of P-waves was maximum at ~ 0.8 Hz during REM sleep, consistent with that in cats and rats. Second, to monitor neural ensemble dynamics during P-wave genesis, we inserted a 4-shank silicon probe, which could cover multiple pontine nuclei simultaneously. While a rich structure of neural activity was apparent across pontine nuclei, we found that P-waves during REM sleep were associated with highly synchronous burst firing across nuclei. In conclusion, our findings suggest that coordinated firing across pontine neurons plays an important role in P-waves as well as REM sleep regulation.

Disclosures: T. Tsunematsu: None. **S. Sakata:** None.

Poster

411. Sleep: Molecules Cells and Drugs

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Program #/Poster #: 411.09/YY4

Topic: F.08. Biological Rhythms and Sleep

Support: BBSRC

Title: Subcortical Sox14 neurons are required for sleep-wake regulation

Authors: *Ç. GELEGEN VAN EIJL[1], O. BROCK[2], P. JAGER[2], S. BRICKLEY[3], G. MOORE[3], A. DELOGU[2]
[1]Dept. of Basic and Clin. Neurosci., [2]Basic and Clin. Neurosci., King's Col. London, London, United Kingdom; [3]Life Sci., Imperial Col. London, London, United Kingdom

Abstract: ***Background***: Sleep is a universal behavior and its disruption is associated with a broad spectrum of mental disorders. Despite recent progress, our understanding of sleep regulation remains limited. Sox14 is a transcription factor that controls neuronal differentiation and maturation in the subcortical brain. We previously showed that Sox14 expression is required to drive development of a network that ensures entrained circadian rhythmicity. More recently, we have mapped the distribution of Sox14 neurons and identified their presence in areas implicated in the regulation of sleep-wake cycles.
Aim: To determine whether subcortical neurons that are defined by the Sox14 marker are implicated in sleep-wake regulation. For this, we investigated sleep architecture in adult Sox14 mutant mice.
Methods: High resolution imaging of whole brains by 2-photon confocal tomography revealed the global distribution of Sox14 neurons in control ($Sox14^{Gfp/+}$) and mutant ($Sox14^{Gfp/Gfp}$) brains. Electroencephalography and electromyography electrodes were implanted in 4-6 months old control and mutant mice (n=16 total). Sleep recording was performed and recordings were analyzed using SleepPro software (Pinnacle Technology, USA). Behavioral tests assessed alterations in cognitive performance of Sox14 mutant mice.
Results: Hypnograms of Sox14 mutant mice indicated a significant increase in time spent in WAKE and a concomitant decrease in NREM and REM vigilance states. REM in particular was dramatically reduced. Increased fragmentation characterized sleep in the Sox14 mutant mice. In agreement with the reduction in sleep time, Sox14 mutant mice failed in a hippocampal-dependent memory task.
Conclusion: Mice deleted for the *Sox14* transcription factor gene show a difference in both macro and micro architecture of their sleep. The Sox14 mutant mice will be a useful tool to study the impact of sleep disruption on brain function (e.g. memory consolidation) and general physiology. The availability of $Sox14^{cre}$ mice will allow for more targeted manipulations of Sox14 neurons to dissect out their relative contribution they make to the observed sleep phenotype.

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Poster

411. Sleep: Molecules Cells and Drugs

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Program #/Poster #: 411.10/YY5

Topic: F.08. Biological Rhythms and Sleep

Title: The effects of sleep deprivation and caffeine on the rodent psychomotor vigilance task (rPVT)

Authors: *M. CREWE, D. HOLT, J. DYCHE
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Abstract: The relationship between sleep deprivation and performance on the Psychomotor Vigilance Task (PVT) and the effects of caffeine to mediate this relationship have been well documented in the human literature. Recently, a version of the PVT that is analogous to the human PVT has been developed for use with rodent models (rPVT). Several studies have measured the effects of sleep deprivation (SD) on rPVT performance, but no studies to date have examined the effect of caffeine to mediate the effects of sleep deprivation as seen in the human literature. The goal of the current study is to determine the effects of sleep deprivation and subsequent caffeine administration on rPVT performance in Wistar rats (N=7). We hypothesized that 6hr/day sleep deprivation would produce significant impairment in rPVT performance, and 10mg/kg i.p. caffeine would be sufficient to mediate the effects of SD on performance. After meeting baseline criterion, animals experienced three conditions for one week each: 6hr/day SD in forced exercise wheels with no caffeine, 10mg/kg i.p. of caffeine (no SD), and 6hr/day SD immediately followed by 10mg/kg i.p. of caffeine. Sleep deprivation and caffeine only conditions were counterbalanced to control for carry-over effects. Performance on the rPVT was measured daily at 15:00. A series of repeated measures ANOVAs were conducted to analyze the effects of each condition on both the primary (mean IRT, percent lapses, & percent of omissions) and secondary (percent correct & percent incorrect) measurements of rPVT performance. Significant ANOVAs were followed-up by pairwise t-tests using a Bonferroni correction. Significant omnibus results were found for mean IRT, percent lapses, percent correct responses, and percent incorrect responses. In general, 6hr/day SD was effective for decreasing performance on the rPVT, but 10mg/kg caffeine was not sufficient to increase performance over baseline or to mediate the effects of sleep deprivation. These results support the hypothesis that 6hr/day will produce significant impairment on the rPVT, but do not support the hypothesis that 10mg/kg i.p. will be sufficient to mediate this effect. These results also do not match patterns observed in the human literature that suggest a caffeine dosage equivalent to two cups of coffee is sufficient to mediate the effects of chronic partial sleep deprivation on PVT performance. Future research in this area will explore dose-response effects of caffeine on rPVT performance following SD as well as the effects of selective adenosine antagonists such as cyclo-pentyl-theophylline.

Disclosures: **M. Crewe:** A. Employment/Salary (full or part-time):; James Madison University. **D. Holt:** A. Employment/Salary (full or part-time):; James Madison University. **J. Dyche:** A. Employment/Salary (full or part-time):; James Madison University.

Poster

411. Sleep: Molecules Cells and Drugs

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Topic: F.08. Biological Rhythms and Sleep

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Title: Activation of adenosine A_{2A} receptors in the olfactory tubercle promotes sleep

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Abstract: Adenosine A_{2A} receptors (A_{2A}Rs) play a major role in adenosine induced-sleep. Studies find that A_{2A}Rs are abundantly expressed in the caudate putamen (CPu), nucleus accumbens (NAc), and olfactory tubercle (OT). Given that A_{2A}Rs in the CPu and NAc regulate sleep, we hypothesized that A_{2A}Rs in the OT might also regulate sleep. First, the role of A_{2A}Rs in the OT was pharmacologically probed by implanting cannulae in bilateral OT and EEG/EMG electrodes in rats (male, SD, 280-320 g), delivering a selective A_{2A}R agonist, antagonist or vehicle, and assessing sleep-wake behaviors. Second, the function of A_{2A}R expressing neurons in the OT ($OT^{A_{2A}R}$ neurons) was chemogenetically determined by expressing hM3Dq in the $OT^{A_{2A}R}$ neurons of mice (male, A_{2A}R-Cre, 22-28 g), giving CNO (i.p., 3 mg/kg), and assessing sleep-wake behaviors. Thirdly, projection sites of $OT^{A_{2A}R}$ neurons were studied by unilaterally

injecting viruses that conditional express hrGFP under the presence of A_{2A}Rs into the OT of A_{2A}R-Cre mice. Finally, properties of connections between $OT^{A_{2A}R}$ neurons and other brain regions were tested by expressing ChR2 in the $OT^{A_{2A}R}$ neurons with the help of *in vitro* electrophysiology. Application of a selective A_{2A}R agonist CGS21680 (0.6 nmol/side) into the OT of rats at 21:00 increased amounts of hourly NREM sleep for five hours compared with the vehicle-treated group, whereas application of a selective A_{2A}R antagonist KW6002 (15.6 nmol/side) at 9:00 increased sleep latency and decreased NREM sleep amounts. A_{2A}R-Cre mice expressing hM3Dq in the $OT^{A_{2A}R}$ neurons treated with CNO at 21:00 showed an increase in hourly NREM sleep amount for three hours. Terminals of $OT^{A_{2A}R}$ neurons were found abundantly in the ventral pallidum (VP) with few scattered in the lateral hypothalamus (LH). Photostimulation (5-ms 473-nm light pulse at 1 Hz) of terminals of $OT^{A_{2A}R}$ neurons at the VP and LH was blocked by the application of a GABA antagonist. Our exploratory research demonstrated that activation of A_{2A}Rs in the OT or $OT^{A_{2A}R}$ neurons increased NREM sleep, probably through inhibitory innervations to the VP and LH. That the OT as one component in the olfactory system regulated sleep may advance the understanding of and encourage new therapies for neurological disorders where sleep disturbances and olfactory dysfunctions are comorbidities. In conclusion, the OT promoted NREM sleep via activation of A_{2A}Rs.

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Poster

411. Sleep: Molecules Cells and Drugs

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Program #/Poster #: 411.12/YY7

Topic: F.08. Biological Rhythms and Sleep

Support: FCE2009_3115- ANII, CSIC-UdelaR y PEDECIBA

Title: *In vitro* model for the study of the role of the mesopontine region in rapid eye movement (REM) sleep and wakefulness

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Abstract: The ponto-mesencephalic junction in the brainstem contains the neuronal circuits that are necessary for the generation of rapid eye movement sleep (REM-S). The latero-dorsal and pedunculo-pontine tegmental nuclei (LDT/PPT), and the nucleus pontis oralis (PnO), have been

proposed to be responsible for the orchestration of the physiologic processes that result in the expression of REM-S, although cellular and synaptic processes that promote the expression of this state remain poorly understood. We carried out the morphological and functional characterization of a slice preparation of rat brainstem containing the LDT/PPT, PnO and the trigeminal motor nucleus (MV). We obtained whole-cell patch clamp recordings from visually (DIC-IR) identified PnO neurons (n=45) that showed input resistances (R_{in}) of 292.17 ± 22.41 MOhm. A 47% of neurons exhibited low-threshold Ca^{2+} spikes (LTS neurons). Both LTS and Non-LTS neurons exhibited electrophysiological responses suggesting the presence of A-type K^+ and I_h-type cationic currents. The existence of a direct functional connection between LDT/PPT and PnO neurons in our slice model was demonstrated using morphological, electrophysiological and pharmacological techniques. A population of ipsilateral LDT/PPT neurons were retrograde labeled after iontophoretic injection of Neurobiotin into the PnO (n=6 slices). Immunodetection of choline acetyltransferase (ChAT) in these cells, revealed the presence of ChAT-positive and ChAT-negative retrograde labeled neurons. Electrical LDT/PPT stimulation elicited mixed (excitatory-inhibitory) short latency synaptic responses in PnO neurons (n=45). Inhibitory and excitatory post-synaptic currents where selectively blocked by 100 μM *picrotoxin* and 50 μM *kynurenic acid*, respectively. Pharmacological activation of LDT/PPT neurons by local application of *Carbachol* (CCh, 1 mM, n=2) and *Glutamate* (10 mM, n=3) resulted in short latency transient increases (2-15 Hz) in the firing rate of PnO neurons. Two out of 30 LDT/PPT neurons exhibited short latency antidromic action potentials in response to electrical stimuli delivered to the PnO. As observed *in vivo*, microvolumes of CCh (1 mM) applied to the PnO resulted in a transient reduction of MV neurons excitability with a decrease in R_{in} as occurs during motor suppression of REM-S. Application of $GABA_A$ agonists (*muscimol*, 10 μM, n=3) had the opposite effect. The above data indicate that connectivity and functionality of the mechanisms that are responsible for the generation and execution of the motor suppression that is typical of REM-S are intact in our mesopontine rat slice preparation.

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Poster

411. Sleep: Molecules Cells and Drugs

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant R01 NS102209

Title: Maternal-placental-fetal disruptions with acute prenatal sleep deprivation: Relating stress and placental immune responses to kynurenine pathway metabolism

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Abstract: The kynurenine pathway (KP) is the dominant pathway for tryptophan degradation in the mammalian body and recent studies suggest that alterations in KP metabolism during pregnancy, including increases in the neuroactive metabolite kynurenic acid (KYNA), may negatively impact fetal neurodevelopment. As levels of KYNA, an endogenous astrocyte-derived antagonist of α7 nicotinic acetylcholine (α7nACh) and NMDA receptors, are elevated in the cerebrospinal fluid and post-mortem brain tissue from patients with schizophrenia, it appears that the KP may play a role in psychiatric illnesses with neurodevelopmental origin. Emerging evidence suggests that acute episodes of sleep deprivation (SD) disrupt KP metabolism (Baratta et al. *Scientific Reports*, 2018). With pregnancy being a critical period during which several factors, such as sleep disruptions, could lead to a higher risk for psychiatric illness in the offspring, we explored the relationship between maternal SD and KP and immune pathways in the maternal, placenta, and fetal tissues. Pregnant Wistar rat dams were sleep deprived by gentle handling for 5 h from zeitgeber time (ZT) 0 to ZT 5. Experimental cohorts included: A) one session of SD on embryonic (ED) 18 or B) three sessions of SD on ED 16 to ED 18. Maternal (plasma, brain), placental and fetal (plasma, brain) tissues were collected immediately after the last session of SD or after 24 h of recovery from SD. Maternal (plasma, brain) and fetal (placenta, plasma, brain) tissues were collected immediately after the last session of SD or after 24 h of recovery from SD. Respective controls were euthanized at ZT 5 on ED 18 and ED 19. Maternal plasma corticosterone, tryptophan and kynurenine levels, as well as fetal brain KYNA, were significantly elevated only after one session of SD on ED 18. Importantly, plasma corticosterone and tryptophan levels correlated significantly with fetal brain KYNA levels. In addition, placental levels of the pro-inflammatory cytokines IL-1beta and IL-6 were altered following maternal SD, suggesting a relationship between placental immune response to SD and fetal brain KYNA accumulation. Collectively, our results demonstrate that sleep loss during pregnancy can adversely impact maternal kynurenine pathway metabolism, placental immune function, and fetal brain KYNA levels. We introduce KYNA as a novel molecular target influenced by sleep loss during pregnancy. Future experiments are designed to unravel the contribution of changes in kynurenine and immune pathways during maternal sleep loss on long-lasting biochemical and behavioral outcomes in developing offspring.

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Poster

411. Sleep: Molecules Cells and Drugs

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Topic: F.08. Biological Rhythms and Sleep

Support: NS091126
NS092652

Title: Ascending projections from parafacial zone to the medial parabrachial neurons

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Abstract: The parafacial zone (PZ) of the rostral medulla is an important region for the generation of non-REM sleep (NREM). It contains GABAergic neurons that are active in NREM sleep and chemogenetic activation of these neurons rapidly induces sustained periods of NREM sleep with high EEG delta power. We previously found that optogenetic stimulation of PZ projections inhibits neurons of the lateral parabrachial (lPB) that project to the basal forebrain (BF). We proposed that the PZ -> lPB -> BF is the circuit through which the PZ neurons promote NREM sleep. Recent studies however have found that while the lPB is important in producing arousals in response to hypercapnia or pain, it might not be involved in promoting spontaneous waking as lesions or deletions of the glutamatergic signal in lPB do not increase NREM sleep or EEG delta power. Conversely, deletion of the glutamatergic signaling in the medial PB (mPB) produces a significant reduction in the total amount of wakefulness suggesting that glutamatergic mPB neurons are necessary for spontaneous arousal. We have found that the PZ heavily innervates the mPB, suggesting that the PZ might promote NREM sleep by inhibiting the mPB. In this study we used an AAV-based channelrhodopsin2 (ChR2)-assisted circuit mapping (CRACM) in brain slices to investigate the functional synaptic connectivity between the PZ and mPB neurons. **Methods.** We stereotaxically injected the PZ region of *Vgat-IRES-cre* mice with a cre-dependent AAV-ChR2-mCherry. We then performed whole-cell recordings in mPB neurons while photostimulating the PZ input that express ChR2. **Results:** Optogenetic-stimulation of the input from PZ Vgat neurons evoked inhibitory postsynaptic currents (oIPSCs) in 21 out of 29 mPB neurons. We have previously found in lPB neurons that optogenetic stimulation of PZ projections evoked oIPSCs that were solely mediated by $GABA_A$ signaling. In this study bath application of the $GABA_A$ antagonist bicuculline only partially reduced the oIPSCs in mPB neurons. The oIPSCs in mPB neurons were abolished only when bicuculine and the glycine antagonist strychnine were co-applied. **Conclusions:** Our results suggest that PZ neurons project to both the lPB and the mPB. They inhibit the lPB neurons by GABA release and they inhibit the mPB neurons through the release of GABA and glycine. Our hypothesis is that the PZ promote NREM sleep by inhibiting the mPB.

Disclosures: R. De Luca: None. **P.M. Fuller:** None. **E. Arrigoni:** None.

Poster

411. Sleep: Molecules Cells and Drugs

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Topic: F.08. Biological Rhythms and Sleep

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Title: Chemogenetic silencing of corticotropin releasing hormone neurons in the paraventricular nucleus attenuates acute stress related sleep disturbance in mice

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Abstract: Introduction: We have previously shown that acute elevation of Corticotropin Releasing Factor (CRF) in rats results in exacerbation of stress-induced sleep disturbance. In the present study in mice we (1) assessed sleep-disturbing effect of an acute stressor, exposure to the dirty cage of a male rat, and (2) examined the effect of chemogenetic silencing of CRF neurons in the hypothalamic paraventricular nucleus (PVN) on sleep occurring after the exposure to this stressor. Methods: First, 9 male mice were implanted with EEG/EMG electrodes and housed individually. Two weeks after surgery, at ZT1, five mice were transferred to the dirty cage of a male rat and recorded continuously for 24-hr. Four control mice were transferred to clean cages. In a second study, 4 male CRF-ires-Cre mice received bilateral injections of AAV-hSyn-DIO-hM4Di-mCherry targeting the PVN and were implanted with EEG/EMG electrodes. Four weeks after surgery, the mice were subjected to intraperitoneal (IP) administration of vehicle or clozapine-n-oxide (CNO; 3 mg/kg) at ZT1, transferred to dirty cages from male rats, and recorded for a 24-hr wake-sleep. Results: In the first study, mice were awake for most of the first 1h period (98.6±1.9%) after being transferred to dirty cages. During the following three hours, mice placed in dirty cage versus clean cage exhibited high percentages of time spent in wakefulness (second hour, 78.6±10.8% vs. 25.9±6.9%; third hour, 47.6±16.2% vs. 35.6±8.3%; fourth hour, 38.8±5.7 vs. 20.7±5.3). In the second study, IP CNO versus saline, resulted in a significant decrease of sleep onset latency, decrease of time spent in wakefulness (first hour, 71.4±17.2 vs. 98.4±1.6, second hour, 35.4±9.5**%** vs. 80.4±35.4**%;** third hour, 44.9±13.6% vs. 66.2±19.6**%;** fourth hour, 61.7±14.6 vs. 66.9±16), and increase in non-rapid eye movement (NREM) sleep time (22.4±12.9% vs. 1.6±1.0%; 62.6**%**±8.1 vs. 15.1 ± 10.9**%;** 54.2±13.7% vs. 29.5±17.0**%;** 39.1±15.4 vs. 31.4±10.1). CNO-treated mice exhibited longer episodes of NREM sleep, compared to saline controls (first hour, 131.3±80.4sec vs. 19±1.9sec; second hour, 437.6±85.7sec vs. 77.8±45.4sec; third hour, 467.5±142.8sec vs. 142±82.7sec; fourth hour,

230.3±86.6sec vs. 194±74.9sec).Conclusion: Chemogenetic silencing of CRF neurons in the PVN attenuates acute stress-induced sleep disturbance in mice.

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Poster

411. Sleep: Molecules Cells and Drugs

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HL095491

Title: CRISPR-cas9 genetic abscission *in vivo* reveals GABA-A receptors of the thalamic reticular nucleus regulate NREM delta oscillations

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Abstract: Understanding the cellular/molecular mechanisms which regulate non-rapid-eye-movement sleep (NREM) delta oscillations (1-4Hz) is of considerable importance due to their association with synaptic homeostasis, cellular energy regulation and clearance of toxic metabolites. Cortical delta-oscillations are generated by intrinsic delta-frequency burst discharge of thalamocortical (TC) neurons whose main inhibitory input is from GABAergic thalamic reticular nucleus (TRN) neurons, the majority of which contain the calcium-binding protein, parvalbumin (PV). TRN-neurons themselves are regulated by extrinsic GABAergic inputs from wake-active neurons acting on alpha3-subunit containing $GABA_A$ receptors ($GABA_AR$). Based on this model, and findings that tonic optogenetic stimulation of TRN-neurons promotes delta-

power, we hypothesize that knockdown of alpha3-GABAAR on TRN-neurons increases NREM-delta.
We used clustered-regularly-interspersed-short-palindromic-repeats (CRISPR) gene-editing to selectively knock-down (KD) alpha-3 GABAARs in a region-specific (TRN) and cell-type specific (GABA-PV) manner in vivo. An adeno-associated viral (AAV) vector encoding three single-guide-RNAs targeting the alpha-3 subunit was injected into TRN of PV-Cre/Cas9 transgenic mice, to disrupt local expression of synaptic $GABA_A$Rs, in TRN-PV neurons. We measured electroencephalography (EEG) and electromyography to determine NREM, REM and wakefulness in freely moving mice before and after AAV induced CRISPR-Cas9 mediated KD of alpha-3 subunits locally in TRN. We validated the effectiveness of the KD by in vitro recordings of inhibitory post synaptic currents.
In the light period, NREM time was increased (9.9±1.7%, p=0.001) with longer bouts (22%±8.7, p=0.02) in mice (N=5) after alpha-3 KD in TRN-PV neurons. Normalized delta-power was higher (light-period:43.9±31.01%, p=0.05, dark-period:38.7±17, p=0.02) after alpha-3 KD in TRN-PV neurons. sIPSCs frequency was reduced in alpha-3 KD TRN-PV-neurons (1.01±0.53, N=5) vs. control PV-tdTomato-neurons (3.47±0.75, N=6, p=0.03).
This is the first use of cell-type and brain-region localized CRISPR-Cas9 technology to study sleep or EEG in vivo. Our data suggests that in the absence of alpha-3-$GABA_A$Rs the TRN-PV neuronal activity is upregulated, leading to the potentiation of NREM sleep and delta activity. This might explain how hypnotics which potentiate alpha-3-$GABA_A$Rs suppress delta-oscillations while promoting sleep.
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Poster

411. Sleep: Molecules Cells and Drugs

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Title: Circuit deconstruction of the arousal-promoting ventral lateral hypothalamic GABA neurons

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Abstract: The inhibitory GABAergic neurons of the ventral lateral hypothalamus (vLH^{GABA} neurons) have recently been characterized as potently wake-promoting. Here, we utilize a battery of molecular-genetic techniques to characterize the inputs to this cellular population, uncover the downstream effector pathways through which these neurons promote wakefulness and determine under what physiological conditions these neurons are activated *in vivo*. We first employed the VGAT-cre mouse line, in combination with stereotaxically injected cre-dependent viral vectors, to gain genetic access to vLH^{GABA} neurons. For conditional retrograde tracing, we injected the helper viruses, AAV8-EF1α-FLEX-TVA-mCherry and AAV8-CAG-FLEX-RG, then 4 weeks later injected the pseudoyped modified rabies virus (EnvA-ΔG-eGFP) into the vLH. Intriguingly, retrogradely labelled eGFP-containing neurons from the vLH were found in brain areas involved in arousal state control (the ventrolateral preoptic nucleus (VLPO), supramammillary nucleus) and stress (paraventricular nucleus of the hypothalamus, amygdala and bed nucleus of the stria terminalis). To determine through what effector pathways vLH^{GABA} neurons elicit arousal we injected AAV10-FLEX-ChR2-eYFP unilaterally into the vLH of VGAT-cre mice and implanted optical fibers over the ipsilateral lateral preoptic area (LPO). During sleep, we delivered 473 nm light via the optical fibre while monitoring electroencephalogram/electromyogram (EEG/EMG) activity to determine arousal state. Optogenetic stimulation of vLH^{GABA} terminals in the LPO induced rapid transitions to wake from NREM sleep, but not from REM sleep. Functional connectivity between vLH GABA neurons and putative sleep-promoting galaninergic neurons in the VLPO was confirmed using ChR2-assisted circuit mapping combined with single cell PCR from recorded neurons. We next expressed the genetically-encoded fluorescent Ca^{2+} indicator, GCaMP6s (AAV10-hSyn-FLEX-GCaMP6s) in vLH^{GABA} neurons and surgically implanted these mice with an optical fibre directed immediately above vLH^{GABA} axons terminals in the LPO. The population Ca^{2+} signal from vLH^{GABA} terminals in the LPO and EEG/EMG activity were acquired simultaneously over multiple sleep-wake cycles. Surprisingly, increases in Ca^{2+} activity did not precede transitions to wake although Ca^{2+} activity during wake was high and progressively decreased as mice transitioned into NREM and REM sleep. Together the data suggest a role for vLH^{GABA} neurons in promoting arousal under behaviorally relevant conditions (for example, stress), through their inhibition of galaninergic neurons in the sleep-promoting VLPO.

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Poster

411. Sleep: Molecules Cells and Drugs

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Topic: F.08. Biological Rhythms and Sleep

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Title: Synaptic regulation of dorsal raphe neurons by prefrontal cortex in narcoleptic mice

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Abstract: Narcolepsy is a sleep disorder that is characterized by excessive daytime sleepiness, fragmented sleep-wake states and bouts of cataplexy. The disorder results from the loss of orexin/hypocretin (OX) neuropeptide neurons in the lateral hypothalamus (LH) and this phenotype is reproduced in animals by the loss of OX signaling. Serotonergic dorsal raphe (5-HT DR) neurons, which receive excitatory input from LH OX neurons, regulate numerous brain functions including sleep-wake states, circadian phase, reward and mood. Recent findings suggest 5-HT DR neurons also play a key role in suppressing cataplexy, since re-expression of OX receptors (OXR) in 5-HT DR neurons of narcoleptic OXR-null mice rescues the cataplexy component of narcolepsy. 5-HT DR neurons project to several regions involved in regulating emotional responses and cataplexy including the amygdala (AMG) and prefrontal cortex (PFC). PFC neurons project to numerous areas, including the AMG, LH, and DR; but it is not known whether PFC synaptic inputs to these areas are altered in narcolepsy or whether these inputs regulate the expression of cataplexy. We have begun investigating PFC inputs to the DR, where we hypothesize that activation of PFC inputs to DR will increase cataplexy in OX-null mice by decreasing 5-HT neuron outflow to the AMG. To test this using an optogenetic approach, we are expressing light-activated ion channels (Chronos) in PFC neurons, which enables optical excitation of their synaptic terminals in the DR. Injections of AAV-Chronos-GFP into PFC produced robust expression in prelimbic, infralimbic and anterior cingulate cortex that resulted in extensive terminal labeling in the DR and other expected areas. In brain slice experiments, we confirmed that optical stimulation of PFC terminals produces monosynaptic EPSCs and di-synaptic IPSCs in both putative 5-HT and GABA DR neurons. Moreover, preliminary findings indicate monosynaptic EPSCs in DR neurons from Ox-null mice are smaller in amplitude compared with EPSCs in WT controls. In behavioral experiments, we have begun examining the effect of PFC terminal stimulation in the DR in WT and OX-null mice on open field locomotion and anxiety-behaviors and reward encoding, using marble burying and conditioned place preference paradigms. We are also studying stimulation effects on bout frequency and duration

of cataplexy-like events. Preliminary findings suggest that PFC terminal stimulation in DR has no effect on mouse locomotion and may increase bouts of immobility in narcoleptic mice. Collectively, these finding suggest that PFC input to DR is an important node regulating emotion-linked behaviors.

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Poster

411. Sleep: Molecules Cells and Drugs

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH R01 #5R01HL129138-03

Title: Estradiol action modulates adenosinergic sleep pressure

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Abstract: Primary sleep disorders are among the most common medical conditions, and clinical data show women are far more likely to experience sleep disorders over their lifespan. This increased risk emerges at puberty and has been associated with fluctuations in ovarian steroids, particularly estrogens such as estradiol (E2), suggesting that gonadal steroids and biological sex are significant risk factors for sleep disruptions. Using adult female Sprague-Dawley rats, studies consistently demonstrate that sleep time is significantly reduced when endogenous ovarian steroids or exogenous E2 are elevated in females. This effect is mediated through the inhibition of sleep-active neurons in the median preoptic nucleus (MnPN). Moreover, E2 is capable of marked suppression of sleep under sleep deprivation, when homeostatic sleep need, also known as sleep pressure, is increased. The nucleoside adenosine is a known mediator of sleep pressure, with established actions at the MnPN and a closely related (but non-E2 sensitive) nucleus, the ventral lateral preoptic (VLPO). We thus employed female rats to test the hypothesis that E2 attenuates the ability of adenosine signaling to stimulate sleep behavior. Our data show that E2 attenuates the action of adenosine signaling at the sleep-promoting A2A receptor, resulting in reduced sleep duration. Female animals were oophorectomized and implanted with a pump containing controlled-release E2 (at a low dose calculated to mimic overall average E2 load, rather than peak proestrus levels) or oil vehicle. One week later, animals received injections of 24nmol of the adenosine 2A receptor agonist CGS21680, a dose shown sufficient to induce additional sleep in male animals, or DMSO vehicle, with animals receiving the opposite

treatment three days later. Sleep behavior was then analyzed for six hours following each injection. In the oil treated animals, the infusion of agonist led to a decrease in wake time as expected. The lower-dose E2 infusion did not decrease wake time on its own. However, in the E2 group, the effect of the agonist on wake time was significantly attenuated ($p<.04$). These data suggest that there is an interplay between estrogen and adenosine which modulates the ability of adenosine to generate sleep pressure, and thus the effect of E2 on wake may be mediated through the adenosinergic system.

Disclosures: P.C. Smith: None. **S. Viechweg:** None. **S. Buck:** None. **A. Pocivavsek:** None. **J.A. Mong:** None.

Poster

411. Sleep: Molecules Cells and Drugs

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 411.20/YY15

Topic: F.08. Biological Rhythms and Sleep

Support: NIH NIAAA AA006059

Title: Effect of suvorexant on sleep in rats exposed to chronic intermittent ethanol vapor and protracted withdrawal

Authors: *M. SANCHEZ-ALAVEZ, J. BENEDICT, D. N. WILLS, C. L. EHLERS
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Abstract: Insomnia is a prominent complaint of alcoholic patients, that can fail to resolve over the course of recovery, and is a leading cause of their relapse to drinking. Despite the importance of sleep in the maintenance of sobriety, treatment options for sleep disturbance in recovering alcoholic patients are currently limited. Literature supports a prominent role of the hypothalamic peptide hypocretin/orexin (Hct/OX) system as a master regulator of the sleep wake cycle. The objective of the present study was to evaluate the effects of suvorexant, a selective, dual Hct/OX receptor antagonist, on chronic ethanol-induced sleep pathology in an animal model. Methods: 44 adult Wistar rats were exposed to 8 weeks of chronic intermittent ethanol [CIE] vapor or control conditions and then withdrawn (blood ethanol [EtOH] concentrations averaged 182.3 ± 6.9 mg/dl). Five hours of sleep electroencephalograms were collected and evaluated at baseline prior to CIE exposure and 24 hours following EtOH withdrawal. Four weeks following EtOH withdrawal, the effects of vehicle and 2 doses of suvorexant (10, 30 mg/kg) on sleep EEGs were evaluated. Results: CIE exposure in the rat was found to reduce the mean duration of SWS episodes 24 hours following EtOH withdrawal as compared to controls ($F=16.7$, $p<0.001$). Band power significantly decreased in delta (1-4 Hz, $F=8.0$, $p=0.008$) and increased in beta (16-32 Hz, $F=4.2$, $p=0.05$) in the parietal cortex and increased in the beta in the frontal cortex ($F=5.9$,

p=0.02) following EtOH withdrawal, as compared to baseline. Four weeks following EtOH withdrawal, during the vehicle treatment, the mean duration of SWS in the EtOH exposed group was found to be no different from the controls. However band power significantly increased in delta in the frontal cortex in the ethanol exposed animals (F=4.1, p=0.05). Administration of suvorexant produced a significant reduction in the latency to, and mean duration of SWS, in both the EtOH exposed and control groups. Additionally, in the EtOH exposed group, the 30 mg/kg dose of suvorexant was found to produce a significant increase in the number of SWS episodes (F=6.2, p=0.018) with no significant effects seen on SWS duration. In addition, both the 10 and 30 mg/kg suvorexant dose were found to reduce delta power in the frontal cortex, when compared to controls, in the EtOH exposed group. Conclusions: Taken together, these studies suggest that an hypocretin/orexin receptor antagonist, suvorexant, has overall sleep-promoting effects, and it can also reverse some aspects of alcohol-induced sleep and EEG pathology.

Disclosures: M. Sanchez-Alavez: None. **J. Benedict:** None. **D.N. Wills:** None. **C.L. Ehlers:** None.

Poster

411. Sleep: Molecules Cells and Drugs

Location: SDCC Halls B-H

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Program #/Poster #: 411.21/YY16

Topic: F.08. Biological Rhythms and Sleep

Support: NIH NINDS Grant 1R01NS107421-01

Title: Intermittent hypoxia induces a fate switch in hippocampal neuroprogenitor cells

Authors: *S. SULLERE[1], T. NALLAMOTHU[3], M. A. KHUU[3], A. J. GARCIA, III[2]
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Abstract: Patients with sleep apnea commonly experience intermittent hypoxia (IH) and exhibit cognitive decline that coincides with changes in the hippocampal formation. However, the basis for these IH-related changes remains poorly defined. The subgranular zone of the hippocampus is a well-established site for adult neurogenesis—a process hypothesized to support the physiology of this brain structure. Our study uses a mouse model to investigate how IH affects hippocampal adult neurogenesis. Immunohistological analysis indicates that IH stimulates the proliferation of $SOX2^+$ progenitor cells. This coincides with an increase in the number of $SOX2^+$ cells residing in hypoxic niches of the subgranular zone. However, IH reduces the proportion of granule neurons produced by adult neurogenesis. Preliminary experiments suggest that the reduction in neuronal number is due to asymmetric division of neuroprogenitor cells favoring the generation of the Type-β cell phenotype ($SOX2^+/S100\beta^+$) over the Type-α cell phenotype

($SOX2^+/S100\beta^-$). We are currently investigating the potential mechanistic role that IH-dependent HIF1α signaling plays in mediating this fate switch. Our findings suggest that IH may have a diminishing effect on the multipotent neuroprogenitor population contributing to the pathophysiology associated with sleep apnea.

Disclosures: S. Sullere: None. **T. Nallamothu:** None. **M.A. Khuu:** None. **A.J. Garcia:** None.

Poster

411. Sleep: Molecules Cells and Drugs

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 411.22/YY17

Topic: F.08. Biological Rhythms and Sleep

Title: changes in human salivary proteome after six hours of sleep deprivation at night

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Abstract: Sleep deprivation (SD) might lead to cellular stress. We subjected humans (n=11) to 6 hours of SD from 22h until 4h in the morning and sampled saliva during a 48h period day and night. Saliva samples was collected after approved protocols and informed consent. A label-free Velos Orbitrap Pro mass spectrometer (LC-MS/MS) was used to analyse the samples. The differently expressed proteins has been analysed using systems biological databases as MetaCore, QuickGO and WebGestalt to discover the changed processes, biological pathways and networks.
Earlier we analysed saliva with a sIgA-kit (Salimetrics) and found a reduction after 3 h of SD also the levels of alpha-amylase (Salimetrics) was found reduced after 6 h of SD.
Out of the 222 identified proteins, 7 proteins were upregulated. These differently expressed proteins after 6 h SD were BPI fold-containing family A member 2 (BPIA2 and Q96DR5, Protein name and SwissProt ID respectively), Carbonic anhydrase 6 (CAH6 and P23280), Cystatin-D (CYTD and P28325), Metalloproteinase inhibitor 1 (TIMP1 and P01033), Cystatin-S (CYTS and P01036), Cystatin-SA (P09228 and CYTT) and SPARC-like protein 1 (SPRL1 and Q14515). These proteins where analysed by QuickGO and classified into three main categories, biological process, molecular function and subcellular localization. «Interactome»-analysis («Interactions by protein function») and enrichment»-analysis was done in MetaCore to look for affected Process Networks and Pathway Maps. The BPIAT2 protein plays a part in hospital infections by inhibitory effect on the growth of *Pseudomonas aeruginosa* also known to play a part in serious hospital infections (Prokopovic et al. 2014 and Schøyen, Josephsen, 2011). Others have also found changes in alpha-amylase after sleep deprivation (Seugnet et al. 2006, Thimgan

et al. 2011, Wilson and Wright 2014).
Preliminary findings of the salivary changed proteome profile indicate compromised immune system and a reduction in cell repair and regeneration after 6 h of SD at night.

Disclosures: A.A. Bjorkum: None. **I. Frich Meling:** None. **B. Kluge:** None. **K. Rosendahl:** None.

Poster

411. Sleep: Molecules Cells and Drugs

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 411.23/YY18

Topic: F.08. Biological Rhythms and Sleep

Support: PRODEP MAMG- DSA/103.5/16/7722

Title: Relation between total sleep duration, mRNA PPAR levels and overweight in a Mexican population

Authors: A. PAVON-ROSADO[1], K. MARCIANO-DIMAS[2], E. SÁNCHEZ-GARCÍA[2], M. BARRADAS-VAJONERO[2], C. PERALTA-VÁZQUEZ[2], S. MARTINEZ-MARTINEZ[2], M. ORGANISTA-ACUA[2], J. SANTIAGO-GARCIA[3], C. BARRIENTOS-SALCEDO[4], *M. A. MELGAREJO-GUTIERREZ[5]
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Abstract: Overweight and obesity has become one of the most serious global health issues due to the high rates of prevalence that presents in different populations. It has been described that the unbalanced diet with high caloric content, genetic factors and decreased physical activity are the main associated factors. However, it has been shown that sleep plays a fundamental role in the endocrine regulation of the hormones involved in lipid and glucose metabolism. Several studies have proposed that a reduction in the total sleep time (TST) is a risk factor for the development of overweight and obesity. However, little is known about the physiological and molecular mechanisms that could be involved in this alteration. It has been suggested that a Peroxisome Proliferator Active Receptors (PPARs) is involved with circadian clock control, manly in the regulation of homeostasis and sleep architecture such as metabolism of lipids and glucose. The aim of the present study was to investigate associations between TST and mRNA expression levels of PPAR alpha and gamma, in the population with the normal weight, overweight and with obesity. For the analysis of molecular parameters (qPCR), a blood sample was taken. For these purpose, 90 participants of Universidad Veracruzana (18-25 years) participated. The study was conducted in according to standards established by the declaration of

Helsinki as well as their applied the informed consent. All subjects completed a questionnaire Pittsburgh scale for measuring the quality and quantity of sleep. Results showed that subjects who have a reduced sleep time (less 7 hours) increase body mass index (Pearson's correlation r = -.222 P = .162) compared to those who have adequate sleep time (more 7 hours). On the other hand, the analysis of body mass index showed a positive and statistically significant association with the parameters of body fat percentage (r = .377, P = .015), hip waist ratio (r = .771, P = .000) and visceral fat level (r = 709, *P* = .0001) with TST. Gene expression no showed differences in the levels of both genes in the groups with decreased sleep. In conclusion It is necessary more studies to analyze the relation between TST and circadian gene expression with overweight and obesity.

Disclosures: A. Pavon-Rosado: None. **K. Marciano-Dimas:** None. **E. Sánchez-García:** None. **M. Barradas-Vajonero:** None. **C. Peralta-Vázquez:** None. **S. Martinez-Martinez:** None. **M. Organista-Acua:** None. **J. Santiago-Garcia:** None. **C. Barrientos-Salcedo:** None. **M.A. Melgarejo-Gutierrez:** None.

Poster

412. Appetitive and Incentive Learning and Memory I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 412.01/YY19

Topic: G.01. Appetitive and Aversive Learning

Support: China Scholarship Council

Title: Redundant control of water intake by type-1 cannabinoid receptors

Authors: *Z. ZHAO[1,2], E. SORIA[1,2], M. VARILH[1,2], A. DUVEAU[1,2], A. CASTIGLIONE[1,2], A. CANNICH[1,2], L. BELLOCCHIO[1,2], A. BUSQUETS-GARCIA[1,2], G. MARSICANO[1,2]
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Abstract: The cannabinoid receptor type-1 (CB1) is a G protein-coupled receptor abundantly expressed in different cell types of the central and peripheral nervous system. It is mainly a presynaptic receptor, which is activated by exogenous cannabinoids and endogenous lipid-based retrograde neurotransmitters. Stimulation of CB1 causes the inhibition of synaptic transmission and contributes to the regulation of a variety of brain functions (e.g. memory, learning, analgesia, feeding etc.). However, the role of CB1 in the control of water intake and the underlying mechanisms are scantly known. Several brain regions known to be involved in the regulation of drinking behavior contain CB1 mRNA expression. Therefore, we hypothesize that CB1 is involved in drinking behavior by regulating specific brain circuits. We found that constitutive genetic deletion and systemic pharmacological blockade of CB1 decreases water intake in different experimental conditions. Interestingly, using genetic deletion and rescue approaches,

we observed that conditional deletion of CB1 in several brain cell types does not impact water intake. However, specific genetic re-expression of CB1 in cortical glutamatergic neurons or in the anterior cingulate cortex fully rescues drinking behavior in mice lacking the CB1 protein elsewhere. These data indicate that general CB1 signaling is necessary and sufficient to promote water intake. CB1 expression in cortical glutamatergic neurons plays a sufficient but not necessary role in controlling water intake. These results strongly indicate that CB1 receptor signaling is redundantly involved in water intake in mice. Currently performed experiments aim at identifying additional brain regions and cell types where CB1 receptors contribute to the central regulation of water intake.

Disclosures: Z. Zhao: None. **E. Soria:** None. **M. Varilh:** None. **A. Duveau:** None. **A. Castiglione:** None. **A. Cannich:** None. **L. Bellocchio:** None. **A. Busquets-Garcia:** None. **G. Marsicano:** None.

Poster

412. Appetitive and Incentive Learning and Memory I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 412.02/YY20

Topic: G.01. Appetitive and Aversive Learning

Support: FRM, DRM20101220445
ORUPS ANR-16-CE37-0010-01
CannaPreg, ERC-2014-PoC-640923
FP7-PEOPLE-2013-IEF-623638
ANR-10-IDEX-03-02

Title: Cannabinoids, astroglial mitochondria and social behaviour

Authors: *A. BUSQUETS-GARCIA[1], D. JIMENEZ-BLASCO[2], E. HEBERT-CHATELAIN[3], R. SERRAT[4], C. VICENTE-GUTIERREZ[2], I. LOPEZ-FABUEL[2], M. RESCH[2], E. RESEL[5], D. SARASWAT[1], M. VARILH[1], L. BELLOCCHIO[1], A. CANNICH[1], I. BONILLA-DEL RIO[6], A. ALMEIDA[2], N. PUENTE[6], M.-L. LOPEZ-RODRIGUEZ[5], B. LUTZ[7], P.-V. PIAZZA[1], M. GUZMAN[5], A.-K. BOUZIER-SORE[8], P. GRANDES[6], J. BOLANOS[2], G. MARSICANO[1]
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Abstract: Astrocytes uptake glucose from brain blood circulation to produce usable energetic substrates, thereby determining neuronal activity and neurotransmitter signaling. In turn, astrocytes respond to neurotransmitters through specific receptors. However, whether activation

of astroglial neurotransmitter receptors can directly regulate cellular glucose metabolism to eventually modulate behavioral responses is not known. Here, we show that specific activation of type-1 cannabinoid receptors located in astroglial mitochondria ($mtCB_1$) impairs social behavior by altering brain glucose and lactate metabolism. By modulation of the phosphorylation of specific Complex I subunits, activation of astroglial $mtCB_1$ receptors inhibits stability and activity of complex I to attenuate reactive oxygen species levels, leading to reduced glycolytic lactate production. Conditional deletion of astroglial CB_1 receptors, genetic inhibition of cannabinoid effects on phosphorylation of Complex I subunits and brain lactate supplementation reversed cannabinoid-induced behavioral deficits in mice. These results reveal that mitochondrial neurotransmitter receptor signaling can directly regulate brain glucose metabolism to modulate high order behavioral responses.

Disclosures: A. Busquets-Garcia: None. **D. Jimenez-Blasco:** None. **E. Hebert-Chatelain:** None. **R. Serrat:** None. **C. Vicente-Gutierrez:** None. **I. Lopez-Fabuel:** None. **M. Resch:** None. **E. Resel:** None. **D. Saraswat:** None. **M. Varilh:** None. **L. Bellocchio:** None. **A. Cannich:** None. **I. Bonilla-Del rio:** None. **A. Almeida:** None. **N. Puente:** None. **M. Lopez-Rodriguez:** None. **B. Lutz:** None. **P. Piazza:** None. **M. Guzman:** None. **A. Bouzier-Sore:** None. **P. Grandes:** None. **J. Bolanos:** None. **G. Marsicano:** None.

Poster

412. Appetitive and Incentive Learning and Memory I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 412.03/YY21

Topic: G.01. Appetitive and Aversive Learning

Support: FRM

Title: The endocannabinoid system controls olfactory processes in the anterior Piriform Cortex

Authors: *G. TERRAL[1], M. VARILH[1], A. CANNICH[1], L. BELLOCCHIO[1], A. PANATIER[1], F. MASSA[1], E. SORIA-GOMEZ[1], A. BUSQUETS-GARCIA[1], G. FERREIRA[2], G. MARSICANO[1]
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Abstract: The ability to perceive and retrieve sensory information is crucial for survival and appropriate behavioral responses. In particular, olfactory perception regulates many behavioral functions in animals and human.
However, the mechanisms behind the modulation of integrative olfactory processing such as odor perception and odor memory are not fully understood. Here, we show that type-1 cannabinoid receptors (CB1) in the anterior Piriform Cortex (aPC) regulate olfactory processes

by modulating synaptic transmission and plasticity. By combining immunostaining and double fluorescent *in situ* hybridation, we observed that CB1 receptors are mainly expressed in GABAergic interneurons projecting to the principal neurons layer of the aPC. Local injections of the CB1 receptor antagonist AM251 into the aPC improved odor perception by increasing odor detection but impaired odor retrieval of odor-dependent memory. *Ex vivo* whole-cell patch clamp experiments of principal neurons revealed that CB1 receptor agonists reduce GABAergic transmission and that a layer-dependent endocannabinoid-ediated long-term plasticity exists in the aPC. These findings show that the endocannabinoid system is present in the aPC, where its physiological activation is involved in the control of inhibitory synaptic transmission and plasticity, eventually modulating olfactory sensory processing and memory.

Disclosures: G. Terral: None. **M. Varilh:** None. **A. Cannich:** None. **L. Bellocchio:** None. **A. Panatier:** None. **F. Massa:** None. **E. Soria-Gomez:** None. **A. Busquets-Garcia:** None. **G. Ferreira:** None. **G. Marsicano:** None.

Poster

412. Appetitive and Incentive Learning and Memory I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 412.04/YY22

Topic: G.01. Appetitive and Aversive Learning

Support: University scholarship

Title: Role of the endocannabinoid system in wheel-running preference/motivation

Authors: *B. REDON[1,2], C. MUGURUZA[1,2,3], G. MARSICANO[1,2], F. CHAOULOFF[1,2]
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Abstract: Sedentarity and physical inactivity rest mainly on the lack of intrinsic motivation to engage and persist in physical exercise. The identification of the neurobiological bases of exercise motivation is thus mandatory to improve health. Based on wheel-running performance and/or after-running conditioned preference tests, animal studies have suggested candidate systems, among which the opiate, the leptin and the endocannabinoid system. However, none of these studies has been able (i) to provide direct evidence for their role in running motivation, and (ii) to discriminate between the different dimensions of such a process, i.e. appetitive motivation and consummatory motivation. The goal of this study was to examine the role of the main cannabinoid receptor in the brain, namely type-1 (CB1) receptor, on wheel-running motivation. We thus used CB1 receptor mouse mutants and CB1 receptor antagonist/agonist and examined their respective influences on the behaviors of mice tested in a T-maze while choosing between

one free running wheel and one locked running wheel located at two extremities of the maze. Pretreatment with the CB1 receptor antagonist/inverse agonist SR141716 decreased the preference for the free wheel over the locked wheel, the running duration per sequence and the number of entries into the free wheels, doing so without affecting total arm entries, an index of locomotion. Pretreatment with the main psychoactive component of cannabis, namely delta9-tetrahydrocannabinol, a CB1/CB2 receptor agonist, did not alter the preference for the free wheel. Full deletion of CB1 receptors promoted behavioral changes similar to those measured after SR141716 administration, including an increase in the initial latency to run. These results show that CB1 receptors exert a tonic control on the preference/motivation for wheel-running. In order to further define the role of CB1 receptors on running motivation, on-going experiments test constitutive and conditional CB1 receptor mutants on the behaviors of mice undergoing an operant conditioning procedure where wheel-running is used as a positive reinforcer.

Disclosures: B. Redon: None. **C. Muguruza:** None. **G. Marsicano:** None. **F. Chaouloff:** None.

Poster

412. Appetitive and Incentive Learning and Memory I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 412.05/YY23

Topic: G.01. Appetitive and Aversive Learning

Support: NIH MH114026

Title: Exercise-induced activation of the hypothalamic-pituitary-adrenal axis in male and female rats

Authors: *J. DAVIS, J. JAIME, M. K. TANNER, E. C. LOETZ, R. M. FORIGHT, B. N. GREENWOOD
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Abstract: Chronic exercise can produce resistance against the deleterious effects of future non-exercise stressors on mental and physical health, including attenuation of the hypothalamic-pituitary-adrenal-axis (HPA) response to mild stressors. Exercise can constrain the HPA response to future stressors despite itself recruiting neuroendocrine components of the stress response. In male rats, the HPA response to acute bouts of voluntary exercise quickly habituates during repeated daily exercise. This habituation of the HPA response to chronic exercise in males could generalize to non-exercise stressors, a phenomenon known as cross-stressor habituation. Female rats typically have exaggerated HPA responses to non-exercise stressors, but the HPA response to chronic voluntary exercise has not been characterized in females. Adult, male and female, Long-Evans rats were housed with either locked or mobile running wheels for

4 days or 4 weeks. Estrus phase was monitored daily by vaginal lavage and males were handled for an equivalent period of time. After either 4 days or 4 weeks, thymus, adrenals, spleen and trunk blood were collected 30 min after the start of the active (dark) cycle, during which time rats with mobile wheels were allowed voluntarily access to their wheels. Running distance was observed to be sexually dimorphic. Females ran more than males regardless of estrus phase, but females ran the greatest distance during pro-estrus compared to other phases of the estrus cycle. There was no effect of exercise on organ weights in either males or females. Corticosterone is currently being analyzed with ELISA. Results will indicate whether the HPA response to acute and chronic exercise differs between the sexes.

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Poster

412. Appetitive and Incentive Learning and Memory I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 412.06/YY24

Topic: G.01. Appetitive and Aversive Learning

Support: NIH MH114026

Title: Sex- and duration- dependent neural circuit control of voluntary exercise behavior

Authors: *M. K. TANNER[1], N. HADDAD[3], J. JAIME[2], J. K. P. DAVIS[2], E. C. LOETZ[2], N. A. MOYA[2], B. N. GREENWOOD[2]
[2]Dept. of Psychology, [1]Univ. of Colorado Denver, Denver, CO; [3]Dept. of Integrative Biol., Univ. Of Colorado Denver, Denver, CO

Abstract: Despite the ability of exercise to increase resistance against stress-related psychiatric disorders, participation in regular exercise is decreasing. Identifying the neural circuits that control exercise could lead to novel strategies to promote exercise participation, as well as reveal mechanisms underlying exercise-induced stress resistance. Rats given running wheels demonstrate robust voluntary exercise behavior that follows distinct phases of acquisition, during which wheel running escalates, and maintenance, during which nightly running reaches a steady state. Although dopamine (DA) and the dorsal striatum are critical for movement, the specific DA-striatal circuits used to control the phases of voluntary exercise are unknown. The goal of the current studies was to identify the DA-striatal circuits controlling exercise in adult, male and female, Long-Evans rats. In males, exercise during the acquisition phase recruits midbrain DA neurons projecting to the dorsomedial striatum (DMS), a region important for goal-directed learning. Once in the maintenance phase; however, an acute bout of running no longer recruits DMS-projecting midbrain DA neurons. Even so, chronic exercise produces neural adaptations in

the DMS consistent with stress-resistance and a hyperdopaminergic state. These changes are not observed in the dorsolateral striatum (DLS), a region supporting habitual behavior. Temporary inactivation of the DMS reduces voluntary exercise during the acquisition phase, but not the maintenance phase. In contrast, whereas temporary inactivation of the DLS has no effect on running during the acquisition phase, DLS inactivation reduces running during the maintenance phase, suggesting that wheel running becomes habitual during the maintenance phase. Together, these data suggest that "goal-directed" DA-DMS circuits control the acquisition of voluntary exercise and plasticity in this circuit could contribute to exercise-induced stress resistance, which is robust in males. In contrast, females acquire wheel running behavior faster than males and run more than males, regardless of estrous stage. This rapid acquisition of exercise resembles the more rapid development of other habitual behaviors, such as drug taking, in females relative to males. Although adaptations in response to exercise in DMS and DLS circuits have not been characterized in females, these data suggest that females rely on the habit DA-DLS circuit to control exercise. We are currently assessing the roles of the DMS and DLS to control the acquisition and maintenance of exercise in females.

Disclosures: M.K. Tanner: None. **N. Haddad:** None. **J. Jaime:** None. **J.K.P. Davis:** None. **E.C. Loetz:** None. **N.A. Moya:** None. **B.N. Greenwood:** None.

Poster

412. Appetitive and Incentive Learning and Memory I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 412.07/ZZ1

Topic: G.01. Appetitive and Aversive Learning

Support: NSERC CREATE grant

Title: Lesions of ventrolateral striatum eliminates lose-shift but not win-stay behaviour in rats

Authors: *R. THAPA[1], A. J. GRUBER[2]
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Abstract: Animals tend to repeat actions that are associated with reward delivery, whereas they tend to shift responses to alternate choices following reward omission. These so-called win-stay and lose-shift responses are employed by a wide range of animals in a variety of decision-making scenarios, and these proximal effects of reinforcement often overshadow optimal actions based on the utility of choice options. These response strategies depend on dissociated regions of the striatum. Specifically, lose-shift responding is impaired by extensive excitotoxic lesions of the lateral striatum. Here we used focal lesions to assess whether dorsal and ventral regions of the lateral striatum contribute differently to this effect. We found that damage to ventrolateral striatum reduced lose-shift responding without impairing win-stay, motoric, or motivational

aspects of behaviour in the task, whereas lesions confined to the dorsolateral striatum significantly impaired the ability of rats to complete trials of the task. Moreover, lesions to the dorsomedial striatum had no effect on either lose-shift or win-stay responding. Together, these data suggest a novel role of the ventral portion of the lateral striatum in driving lose-shift decisions.

Disclosures: R. Thapa: None. **A.J. Gruber:** None.

Poster

412. Appetitive and Incentive Learning and Memory I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 412.08/ZZ2

Topic: G.01. Appetitive and Aversive Learning

Title: Identifying the threshold of habitual responding in female long evans rats

Authors: *H. SCHOENBERG, E. X. SOLA, C. WINN, D. J. TOUFEXIS
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Abstract: Our previous results show that although male rats are sensitive to lithium chloride-induced reward-devaluation after 240 reinforcer-outcome pairings, devalued female rats continue to respond like non-devalued control females at this same level of training. Thus, females exhibit an earlier onset of habitual responding than do males. In order to further investigate the mechanisms controlling habit formation in both sexes, it is first necessary to identify the narrow range of training within which male and female rats transition from goal-directed to habitual behavior. Identifying this threshold range will allow us to test a variety of manipulations that might accelerate or attenuate the progression from goal-directed to habitual behavior and how these may differ due to sex. To begin this endeavor, we trained female rats on a variable interval 30-s schedule to nose-poke for sucrose with decreasing response-reinforcer pairings starting at 200 reinforcer exposures. We found that female rats show habitual behavior down to 140 response-reinforcer pairings, but remain goal-directed at 120 reinforcer pairings. Thus, the habit-threshold range in females is between these two levels of reinforcer exposure. Based on this information we are currently using viral vectors to selectively enhance activation of particular pathways within the habit-mediating portion of the basal ganglia, the dorsolateral striatum (DLS), in female rats. Data is as of yet inconclusive, however we hypothesize that enhanced activation of the direct pathway at the habit sub-threshold range of 120 reinforcer pairings will accelerate habit formation, and enhanced indirect pathway activation will offset the development of habitual behavior at the habit super-threshold level of 150 reinforcers.

Disclosures: H. Schoenberg: None. **E.X. Sola:** None. **C. Winn:** None. **D.J. Toufexis:** None.

Poster

412. Appetitive and Incentive Learning and Memory I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 412.09/ZZ3

Topic: G.01. Appetitive and Aversive Learning

Title: Stress recruits dopamine in the ventrolateral striatum to promote goal-directed behaviors

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Abstract: The mesolimbic dopamine system is a critical locus for controlling motivated behavior. Behavioral studies examining dopamine's role in the ventral striatum have largely focused on dopamine's action in the ventral medial striatum (VMS), while neglecting the contribution of dopamine transmission in the ventral lateral striatum (VLS). The dopamine neurons projecting to the VLS undergo rapid changes in excitatory synaptic strength after exposure to stress, suggesting the VLS dopamine projection could mediate the influence of stress on motivated behavior. Here, we utilized fast-scanning cyclic voltammetry to monitor dopamine release in the VMS and VLS. We found that a single exposure to acute stress elicited a persistent enhancement in conditioned responding toward food-paired cues, which was mediated by an elevated cue-evoked dopamine response in the VLS. Using a negative reinforcement behavioral paradigm, our preliminary data suggests the VMS dopamine response reflects the negative valence of stress-predictive cues, whereas the VLS dopamine response reflects the salience of these cues. These findings suggest that dopamine in the VLS confers enhanced cue reactivity during and after stressful circumstances. Therefore, the dopamine projection to the VLS could be a putative site of dysfunction in stress-associated pathologies such as post-traumatic stress disorder and depression.

Disclosures: C. Stelly: None. **G. Haug:** None. **Y. Rafati:** None. **M. Wanat:** None.

Poster

412. Appetitive and Incentive Learning and Memory I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 412.10/ZZ4

Topic: G.01. Appetitive and Aversive Learning

Support: NIH R00-MH105549
Rising STARs Award from University of Texas System

Title: Neuronal correlates of reward vs. fear memory discrimination in the prelimbic cortex

Authors: *J. A. FERNANDEZ-LEON, D. S. ENGELKE, M. NAIM-RASHEED, A. B. TERZIAN, F. H. DO MONTE
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Abstract: The brain ability to identify and discriminate cues associated with reward and aversive stimuli allows an organism to select the most appropriate response. Neurons in the prelimbic prefrontal cortex (PL) can discriminate between cues that anticipate imminent rewards and threats, implicating this region in decision-making under states of certainty. Because PL neurons are critical for the retrieval of reward and fear-associated memories, we speculated that these neurons would also discriminate reward and fear cues under states of uncertainty, when decision-making depends entirely on the associated memories. To address this question, male Long-Evans rats previously implanted with single-unit recording electrodes in PL were trained to learn that each lever press during a cue tone delivered a sucrose pellet in a nearby dish. After training, animals were fear conditioned by pairing a neutral odor with electrical foot shocks. Rats were then tested in a rectangular arena (60cm x 26cm x 40cm) comprising two different zones: a hidden zone and a foraging zone where the lever and the dish were located. The test session was separated in three different phases: only tone cues (reward), only odor cues (fear), or both at the same time (decision-making). To search for food during the decision-making phase, animals had to leave the hidden zone and confront the conditioned odor presented in the foraging zone. During the reward phase, animals pressed the lever in 95% of the tone cue presentations with an average latency to press of 5.3s. During the fear phase, animals showed stronger defensive behaviors characterized by a reduction in time exploring the foraging zone (17% vs. 62% in the reward phase, $p<0.001$) and an increase in time spent in the hidden zone (77% vs. 35% in the reward phase, $p=0.001$). During the decision-making phase, animals showed 66% of reduction in lever presses, with an increase of 49% in the average latency to press. Recordings from PL neurons during the reward phase revealed two distinct populations of responsive neurons that changed their firing rates during the tone cues (Z-score > 2.54 for excitatory and < -1.96 for inhibitory responses, first bin of 300ms). Neurons showing excitatory tone responses during the reward phase continued to respond during the decision-making phase. Interestingly, neurons showing inhibitory tone responses during the reward phase did not respond to the cues during the decision-making phase. Together, our findings suggest that PL inhibitory responses to reward cues are involved in action selection during competing behavioral alternatives (searching for food vs. avoiding potential threats) that depend on previously associated memories.

Disclosures: J.A. Fernandez-Leon: None. **D.S. Engelke:** None. **M. Naim-Rasheed:** None. **A.B. Terzian:** None. **F.H. Do Monte:** None.

Poster

412. Appetitive and Incentive Learning and Memory I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 412.11/ZZ5

Topic: G.01. Appetitive and Aversive Learning

Support: NIH R00-MH105549
Rising STARs Award from University of Texas System

Title: Balancing food seeking with the risk of predation recruits the paraventricular thalamus

Authors: *D. S. ENGELKE, J. A. FERNANDEZ-LEON, A. L. TERZIAN, M. NAIM-RASHEED, F. H. DO MONTE
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Abstract: The ability to survive in nature depends on a balance between foraging and the risk of being attacked by a predator. Although the brain mechanisms modulating food seeking and fear have been extensively studied apart, it remains unknown which neural circuits integrate both behaviors. Neurons in the paraventricular thalamus (PVT) change their activities during the presentation of both food and fear-associated cues, making this region a strong candidate to regulate reward and fear responses. To investigate whether PVT is involved in the competition between fear and food-seeking behavior, male and female Long-Evans adult rats were initially trained to press a bar for sucrose in the presence of cues (reward cues). Cat saliva was used to induce innate fear responses (predator odor). Rats exposed to predator odor alone showed stronger defensive behaviors characterized by increased freezing and avoidance responses, when compared to neutral odor controls (Freezing, neutral= 1.8% vs. predator odor= 38.1%, $p<0.001$; Avoidance, neutral= 36.5% vs. predator odor= 75.7%, p= 0.007, Unpaired *Student's* t-test). Predator odor exposure also increased the expression of the neural activity marker cFos in the anterior portion of PVT (aPVT), when compared to neutral odor (neutral= 59.7 cells/mm^2 vs. predator odor= 102cells/mm^2, p= 0.039). Single-unit recordings from aPVT neurons revealed two distinct populations of neurons that changed their firing rate in response to either predator odor or reward cues, compared to baseline (Z-score > 2.54 for excitatory and < -1.96 for inhibitory responses). During a competition test, when predator odor and reward cues were simultaneously presented, excitatory responses observed during the reward cues were abolished. This neuronal change was associated with a dramatic reduction in food-seeking behavior (presses/min: neutral= 19.1 vs. predator odor= 1.5, $p<0.001$). Inactivation of aPVT with the GABA(A) agonist muscimol increased time approaching the food area (Vehicle= 11.36%, Muscimol= 35.19%, p= 0.038) and reduced the percentage of time avoiding the predator odor area during the competition test (Vehicle= 75.6%, Muscimol= 48%, p= 0.04). Notably, inactivation of aPVT had no effect when the predator odor or the food-seeking tasks were carried

out independently. We have previously reported that photoactivation of aPVT induces aversion and food-seeking suppression. Together, the present results suggest that aPVT activity is necessary to balance food seeking in face of an aversive stimulus. We are currently investigating which aPVT projections and cellular subtypes are involved in the integration of reward and fear responses.

Disclosures: D.S. Engelke: None. **J.A. Fernandez-Leon:** None. **A.L. Terzian:** None. **M. Naim-Rasheed:** None. **F.H. Do Monte:** None.

Poster

412. Appetitive and Incentive Learning and Memory I

Location: SDCC Halls B-H

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Program #/Poster #: 412.12/ZZ6

Topic: G.01. Appetitive and Aversive Learning

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Title: CRF neurons in the paraventricular thalamus reduce food-seeking behavior

Authors: *A. B. TERZIAN[1,2], D. S. ENGELKE[2], M. NAIM-RASHEED[2], S. LI[3], J. J. O'MALLEY[2], R. DASGUPTA[2], J. A. FERNANDEZ-LEON[2], N. J. JUSTICE[4], M. BEIERLEIN[2], G. J. KIROAC[3], F. H. DO-MONTE[2]
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Abstract: The paraventricular nucleus of the thalamus (PVT) regulates behavioral responses under emotionally arousing conditions. Photoactivation of anterior PVT (aPVT) neurons abolishes sucrose seeking and induces aversive behaviors in rodents. However, the specific aPVT neuronal subpopulation regulating these functions remains unknown. The stress neuropeptide corticotropin-releasing factor (CRF) has been shown to reduce food intake and induce anxiety-like behavior in different species. Interestingly, a recent neuroanatomical study demonstrated that CRF neurons are present in the aPVT, but their physiological functions have never been explored. To assess the role of aPVT-CRF neurons during sucrose seeking, adult male Long-Evans rats were infused with a mixture of viral vectors (AAV-CRF-Cre and AAV-

ChR2-DIO-eYFP) to express channelrhodopsin in aPVT-CRF neurons. Animals were trained in a reward conditioning task, where each bar press during a 30s cue tone delivered a sugar pellet in a nearby dish. High-frequency photoactivation of aPVT CRF neurons (20 Hz, 5ms pulse width, 10 mW) during the cue tone reduced bar presses when compared to the eYFP-Control group (aPVT-CRF-ChR2, presses/min: Laser OFF: 14.5±1.4, Laser ON: 4.2±1.1; eYFP-Control, presses/min: Laser OFF: 17.5±1.1, Laser ON: 18.8±1.2, $p<0.05$). In contrast, 5Hz low-frequency photoactivation of aPVT-CRF neurons had no effect (aPVT-CRF-ChR2, presses/min: Laser OFF: 17.2±0.9, Laser ON: 14.88±1.6, $p=0.20$). Photoactivation of aPVT-CRF neurons also reduced the time spent on the side of the chamber paired with 20Hz laser stimulation in a real-time place preference task, indicating that stimulation of aPVT-CRF neurons is aversive (Laser OFF side: 80.7%, Laser ON side: 19.2%, $p<0.05$). Neuroanatomical investigation of aPVT-CRF efferents revealed dense projections to the nucleus accumbens shell (NAc-shell); moderate projections to the nucleus accumbens core, lateral region of the bed nucleus of the stria terminalis, lateral subnuclei of the central nucleus of the amygdala and suprachiasmatic nucleus; and relatively weak projections to the infralimbic/prelimbic cortex, basolateral nucleus of the amygdala, and medial regions of the hypothalamus. Slice recordings from NAc-shell neurons demonstrated that photoactivation of aPVT-CRF fibers in the NAc-shell elicits large excitatory postsynaptic responses, which are blocked by AMPA and NMDA receptor antagonists. Our results demonstrate the existence of a defined glutamatergic-CRF-expressing subpopulation of neurons in aPVT that is sufficient to mediate anorexigenic and aversive effects in rats.

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Poster

412. Appetitive and Incentive Learning and Memory I

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 412.13/ZZ7

Topic: G.01. Appetitive and Aversive Learning

Support: NSF IOS 0094377
NSF SMA 1041755 Temporal Dynamics of Learning Center

Title: Predictive coding by neural ensembles in the amygdala

Authors: L. K. QUINN*[1], V. H. MINCES*[1], I. M. RUSSELL[3], *A. A. CHIBA[2]
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Abstract: Animals rely on the associative history of stimuli in their environments to guide their choices and to develop a behavioral repertoire that is adaptive to survival. Learning theory asserts that a given stimulus in the environment holds the value of its prior associations, indicating a shared code for the stimulus and its reinforcers. Given its rich interconnectivity with sensory and associative areas of the brain, the amygdala is anatomically situated to play a role in building such associations. To date, a large body of literature supports this view, including studies demonstrating that the same neurons respond to a stimulus and its associated punishment (Baroh et al., 2008; Grewe et al., 2017). We devised a novel associative learning task for rats, in order to assess the neurophysiology of the basolateral amygdala complex (BLA) in associating visual stimuli with specific food punishments or reinforcers, ranging from aversive to highly appetitive. The resultant data demonstrated that, as expected, individual amygdala neurons differentially responded to relevant stimuli (Lego objects) and their outcomes (aversive and appetitive). Additionally, a population-based neural decoder revealed that the network activity in the amygdala can accurately encode sensory stimuli (objects) and outcomes (aversive and appetitive food pellets). Finally, the neural decoder revealed that the population activity during the object presentation accurately predicted the identity of the subsequent outcome. This provides evidence for neurophysiological populations in the amygdala that hold a shared code for a stimulus and its outcome, a theoretical necessity of evaluative conditioning. These findings solidify a role for network properties of the amygdala in coordinating a salience map of the environment, allowing an animal to advantageously interact with the environment.

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Poster

412. Appetitive and Incentive Learning and Memory I

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant DA035443
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NIH Grant DA044734

Title: Amygdala-cortical circuitry in reward-expectation guided behavior

Authors: *N. T. LICHTENBERG[1], L. SEPE-FORREST[1], Z. T. PENNINGTON[1], S. M. HOLLEY[2], V. Y. GREENFIELD[1], C. CEPEDA[2], M. S. LEVINE[2,3], K. M. WASSUM[1,3]
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Abstract: Reward-seeking behaviors are heavily influenced by expectations about future potential rewards and their value. These expectations are often generated by using stimuli in the environment to retrieve detailed memories of associated rewards. Little is known about the neural circuits supporting such processes. Using tract tracing, we identified distinct reciprocal connections between the basolateral amygdala (BLA) and both the lateral (lOFC) and medial (mOFC) orbitofrontal cortex subdivisions. Using chemogenetics, we evaluated the unique function of each pathway in expectation-guided behavior. Inactivation of BLA to lOFC projections was found to disrupt the influence of cue-triggered reward expectations over both reward-seeking decisions and adaptive conditional goal-approach responding following a value shift. Inactivation of BL to mOFC disrupted only the latter, leaving cue-directed decision making intact. lOF to BLA projections were found to be unnecessary for either behavior, whereas inactivation of mOFC to BLA projections attenuated the influence of cue-triggered expectations over decisions and adaptive conditional responding. Our working hypothesis, which is undergoing further investigation, is that both lOFC and mOFC to BLA projections enable the retrieval of detailed stimulus-reward memories, whereas BLA to mOFC projections mediate access to the current value of specific cue-predicted rewards. The cognitive symptoms underlying many psychiatric diseases, such as addiction, result from a failure to appropriately anticipate potential future events. These data, therefore, provide insight into potential dysfunction that might underlie these conditions.

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Poster

412. Appetitive and Incentive Learning and Memory I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 412.15/ZZ9

Topic: G.01. Appetitive and Aversive Learning

Title: An intersectional approach to investigate the roles of central amygdala Htr2a-positive neurons in appetitive behaviors

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Abstract: The amygdala is an integrative brain center for emotions and motivation and plays an essential role in processing both fearful and rewarding environmental stimuli. Recent studies have focused on the role of different amygdala neuron subpopulations in appetitive behaviors. Central amygdala (CeA) protein kinase C delta (PKCδ)-positive neurons were shown to mediate

the anorexigenic effects of malaise and satiety signals, in part via local inhibitory connections within the CeA (Cai et al., Nat. Neurosci., 2014). Other subpopulations within the lateral (CeL) and medial (CeM) subdivisions of the CeA were shown to drive appetitive behaviors in self-stimulation experiments (Kim*, Zhang* et al., Neuron, 2017).
Recently in the lab, a subpopulation of CeA PKCδ-negative neurons that express the serotonin receptor 2a (Htr2a) (Isosaka et al., 2015) was shown to promote feeding and reward-related behaviors (Douglass*, Kucukdereli*, Ponserre*, et al., Nat. Neurosci., 2017). These findings suggest that within the CeA there are genetically-defined GABAergic cell types with functional antagonism that process signals of opposite valence.Taking advantage of intersectional genetic tools we are investigating the specific roles of CeA^{Htr2a} neurons located in either the CeL or CeM subdivisions, in a series of rewarding behaviors.
To do so, we have generated a novel transgenic mouse line that expresses on optimized and Tamoxifen-inducible version of the Flp recombinase under regulatory elements of the Wfs1 gene (*Wfs1-FlpoER* mouse line). In this mouse line FlpoER is expressed specifically in the majority of neurons of the CeL (not CeM). The crossing between *Htr2a-Cre* and *Wfs1-FlpoER* mice generates double transgenic intersectional mice (*Wfs1-FlpoER;Htr2a-Cre*) in which CeL^{Htr2a} cells are positive for both Cre and Flp, while CeM^{Htr2a} cells are positive for Cre only. We are currently independently and functionally manipulating the Htr2a subpopulations of the different CeA subdivisions with the help of specific intersectional viruses, addressing the question of how they independently contribute to the different rewarding behaviors.

Disclosures: F. Fermani: None. **P.L. Alcala Morales:** None. **R. Klein:** None.

Poster

412. Appetitive and Incentive Learning and Memory I

Location: SDCC Halls B-H

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Program #/Poster #: 412.16/ZZ10

Topic: F.03. Neuroendocrine Processes

Support: JSPS KAKENHI Grant Number JP17H06049
JSPS KAKENHI Grant Number JP16K08541

Title: A synthetic orexin agonist improves inflammation-induced immobility through activating the medullary raphe nucleus

Authors: *S. UCHIDA, Y. IRUKAYAMA-TOMOBE, Y. OGAWA, T. YAMAGUCHI, Y. ISHIKAWA, K. SAKURAI, S. SOYA, T. SAITO, H. NAGASE, M. YANAGISAWA, T. SAKURAI
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Abstract: Hospitalization in an intensive care unit (ICU) can be associated with a perilous functional decline. Patients can develop impairments in mobility, cognition, and ability to perform daily living activity. Immobility due to prolonged bed rest in the ICU play a major role in the development of ICU-acquired weakness. However, there has been no means to evoke early mobilization of patients. Recently, we found peripherally administered orexin rescues mice with endotoxin shock. Furthermore, our group developed a non-peptide orexin type-2 receptor selective agonist, YNT-185, which penetrates through the blood-brain barrier. The purpose of this study is to examine whether an orexin agonist could improve inflammation-induced immobility state in mice. We found that intraperitoneal administration of YNT-185 accelerates horizontal activity and wheel running after LPS challenge in wild type mice, but not in in orexin type-2 receptor knockout mice. This effect is mimicked by ICV injection of orexin, or transgene-driven overexpression of orexin in mice. Immunohistochemistry using anti-Fos antibody suggested that serotonergic neurons in the medullary raphe nucleus are activated after administration of YNT-185 in LPS-challenged mice. By anterograde tracing study, we found that these neurons send projections to the dorsal raphe nuclei, the ventral tegmental area, nucleus of the solitary tract, and the rostral ventrolateral medulla, regions involved in the regulation of sleep/wakefulness states and autonomic function. Furthermore, chemogenetic activation of the raphe nuclei also promotes recovery from immobility after LPS challenge. These findings demonstrate that orexin accelerate recovery from inflammation through the raphe nuclei and this effect may be due to activation of the serotonergic neurons that regulate the autonomic nervous system.

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Poster

413. Fear and Aversive Learning and Memory: Acquisition

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 413.01/ZZ11

Topic: G.01. Appetitive and Aversive Learning

Support: DGAPA-PAPIIT 201018
Technical assistants Gabriela Vera
Technical assistants Alejandro Rangel-Hernández
English revision Shaun Harris

Title: Activation of NMDA receptors in the insular cortex alters aversive-appetitive stimuli association

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Abstract: During conditioned taste aversion or inhibitory avoidance (IA), an inherently appetitive or pleasant stimulus carries positive valence that changes during association with another stimulus, which is inherently aversive and has negative valence. In these tasks, taste or context is learned under negative or aversive consequences and the insular cortex (IC) is significantly involved in the association. However, few studies have evaluated the behavior and cortical function when stimuli, with opposite valence, compete during taste and context association. Thus, the purpose of this research was to assess, in male Wistar rats, the involvement of NMDA receptors (NMDAr) in the IC, when two stimuli with opposite valences, e.g., sweet taste and aversive context, were associated simultaneously. Accordingly, a modified IA task was used; at the end of the dark compartment (DC), a graduated bottle with sugar was placed (positive valence) and then competed with a mild foot shock (negative valence) administered before rats drank. Three parameters were obtained during acquisition and test retrieval: 1) the entry latency to DC to evaluate the response to the context; 2) the latency of sugar consumption (hedonic response); 3) ml liquid consumption, to evaluate the appetitive or aversive response. Five minutes before the IA acquisition, rats received NMDA (1 µg/µl) bilateral injection in the IC. The results showed that IC-NMDAr activation disrupted IA, since entry latency to DC decreased, regardless of the presence of water or sugar at the end of DC. However, NMDAr activation significantly increased sugar consumption, even though no difference was observed in liquid consumption latency between groups. These results indicate the increment in NMDA receptors activity in the IC during association of novel stimuli with opposite valence, significantly strengthened the appetitive memory formation. These results suggest that glutamatergic activity in the IC might be modulating the appetitive valence when two opposite stimuli are competing for an association.

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Poster

413. Fear and Aversive Learning and Memory: Acquisition

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 413.02/ZZ12

Topic: G.01. Appetitive and Aversive Learning

Support: NARSAD YI

Title: Chemogenetic evidence for the protein synthesis requirement during memory consolidation

Authors: *P. SHRESTHA[1], P. AYATA[2], P. M. H. VIDAL[1], A. GASTONE[1], N. HEINTZ[3], E. KLANN[4]
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Abstract: Studies over the last several decades have established that *de novo* protein synthesis is a requirement for the conversion of short to long term memories. However the widespread use of broad antibiotics, such as anisomycin, in neurobehavioral studies for blocking protein synthesis has been considered problematic due to their lack of specificity including activation of cellular stress pathways aside from their intended action on translation. We have developed a novel chemogenetic strategy, cre-conditional drug-inducible protein synthesis inhibitor (ciPSI)- to address the need for a highly selective and inducible translation inhibiting agent. The ciPSI knock-in mouse line comprises transgenic cre-dependent expression of a drug activatable kinase, iPKRkd, for eukaryotic translation initiation factor eIF2α that has been rendered inducible by an NS3/4 protease site tailored within the kinase. NS3/4 protease, also expressed transgenically, binds to and inhibits iPKRkd at steady state but upon administration of NS3/4 protease inhibitor, we are able to disinhibit iPKRkd to mediate its action on protein synthesis. Phosphorylation of eIF2α is a physiologically relevant molecular event for consolidation of long term threat memories. While phosphorylation of eIF2α at Serine 51 blocks translation initiation by limiting the abundance of eIF2 ternary complex, dephosphorylation of eIF2α at the same residue has been shown to be a crucial molecular event for the switch from short to long term plasticity and memory in classical fear conditioning paradigms. We have applied the ciPSI strategy in Nestin.iPKRkd animals to interrogate whether *de novo* translation is necessary for consolidation of long term threat memories. Our data indicates that there is a sensitive time period following threat conditioning for *de novo* translation initiation during which consolidation of long term threat memories, lasting over 24h, is vulnerable to disruption. Further, we have found that bidirectionally switching the phosphorylation status of eIF2α in CamK2α principal neurons in the lateral amygdala causes a robust change in the defensive state exhibited by animals 24h following threat conditioning. The ciPSI strategy is also engineered to express EGFP-tagged ribosomal protein L10, making the system amenable to translating ribosome affinity purification (TRAP)-seq. Overall, we have generated a tripartite chemogenetic protein synthesis inhibitor that allows us to interrogate various memory systems in healthy and diseased brain states.

Disclosures: P. Shrestha: None. **P. Ayata:** None. **P.M.H. Vidal:** None. **A. Gastone:** None. **N. Heintz:** None. **E. Klann:** None.

Poster

413. Fear and Aversive Learning and Memory: Acquisition

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 413.03/ZZ13

Topic: G.01. Appetitive and Aversive Learning

Title: Sex differences and neural correlates of safety learning

Authors: *A. R. FOILB, G. SANSARICQ, K. FERNANDO, J. P. CHRISTIANSON
Psychology, Boston Col., Chestnut Hill, MA

Abstract: Distinguishing between safety and danger is critical to survival and is impaired in individuals with post-traumatic stress disorder (PTSD). More females are diagnosed with PTSD than males and women with PTSD display difficulty inhibiting fear responses. To investigate sex differences in the neural correlates of safety learning, intact male and normally cycling female adult Sprague-Dawley rats received discrimination conditioning with a danger signal (CS+) co-terminating with a mild footshock and a safety signal (CS-) indicating the absence of shock and were perfused one hour later for brain wide c-Fos quantification. Females displayed less freezing, an index of fear, to the CS- and greater discrimination between the CS+ and CS- compared to males. This pattern suggests sex differences exist within the neural circuits that encode and recall safety information, which is the focus of ongoing study. In addition to comparing regions activated in discrimination in males and females, we are also comparing animals that underwent discrimination training to rats exposed to danger conditioning only (CS+ only), or to a control treatment (CS only). The activation of prefrontal cortex, amygdala nuclei, and plasma levels of stress-related hormones are under current investigation. Together, these components will lead to a better understanding of the neural mechanisms that underlie improved safety learning and discrimination in females compared to males.

Disclosures: A.R. Foilb: None. **G. Sansaricq:** None. **K. Fernando:** None. **J.P. Christianson:** None.

Poster

413. Fear and Aversive Learning and Memory: Acquisition

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 413.04/ZZ14

Topic: G.01. Appetitive and Aversive Learning

Title: Vitamin D attenuates retention of conditioned fear in low startle rats

Authors: L. W. AYERS[1], J. SCHULKIN[2], *J. B. ROSEN[3]
[1]Psychology Dept., Widener Univ., Chester, PA; [2]Dept. of Neurosci., Georgetown Univ., San Rafael, CA; [3]Psychological and Brain Sci., Univ. Delaware, Newark, DE

Abstract: Calcitriol (1,25-dihydroxycholecalciferol or 1α,25-$(OH)_2D_3$) is the biologically active form of vitamin D3 best known for its role in calcium regulation. The Vitamin D Receptor (VDR) is found in notable concentrations within brain areas associated with autonomic-

neuroendocrine function and fear/anxiety. Namely, it is found in high densities within the paraventricular nucleus of the hypothalamus (PVN), the intermedolateral nucleus of the spinal cord, the bed nucleus of the stria terminalis (BNST), the central nucleus of the amygdala, and the hippocampus (Stumpf & Privette, 1991). Recent evidence from Ithier et al, (2018) also suggests that Calcitriol activity may suppress corticotrophin-releasing hormone (CRH), a key signal used in the neuroendocrine stress response and a mediator of fear/anxiety behavior.
We hypothesize that Calcitriol may be an important mediator of fear and anxiety, possibly through its regulation of CRH in amygdala, BNST and/or PVN. To test this, a 1, 10 or 100 µg/kg dose of Calcitriol was given subcutaneously to male Sprague-Dawley rats 6 hours prior to contextual fear conditioning. Subjects were then tested for fear retention 24 hours later. Calcitriol had no effect on conditioned fear acquisition, but fear retention was reduced by the 10 µg/kg dose in a subset of subjects classified as having low baseline startle. Subjects with high baseline startle were unaffected by the compound. This finding is important given the previously described associations between baseline startle responding and the expression of learned fear in both rodents and humans (Bush, Sotres-Bayon, & LeDoux, 2007; Holmes & Singewald, 2013; Russo & Parsons, 2017), and suggests trait-anxiety might be a factor in vitamin D's ability to reduce the retention of conditioned fear. Follow-up studies are planned which will examine the effects of Calcitriol on CRH gene expression within the amygdala, BNST, and PVN.

Disclosures: L.W. Ayers: None. **J. Schulkin:** None. **J.B. Rosen:** None.

Poster

413. Fear and Aversive Learning and Memory: Acquisition

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 413.05/ZZ15

Topic: G.01. Appetitive and Aversive Learning

Support: MOST 106-2410-H-006-037

Title: Amygdala lesions impaired fear conditioning under dexmedetomidine-induced anesthesia in rats

Authors: *H.-Y. HSIAO, D.-Y. CHEN
Dept. of Psychology, Natl. Cheng Kung Univ., Tainan, Taiwan

Abstract: Anesthesia may interfere with brain and cognitive function, but animals and human still be able to perform certain learning and memory under general anesthesia. These evidences indicate that fully conscious state may be not necessary for all cognitive functions. Previously, we have demonstrated that rats could learn a modified inhibitory avoidance (IA) task when they were anesthetized by dexmedetomidine (DEX). This paradigm provides an animal model to study the neural mechanism of fear conditioning under anesthesia. The present study examined

whether the amygdala play an important role in this learning task. In Experiment 1, rats received bilateral ibotenic acid lesions of the amygdala or a sham surgical procedure about one week before the training sessions. On Day 1, rats were allowed to shortly explore the entire apparatus. On Day 2~4, rats were anesthetized by DEX (60 μg/kg, s.c.) and placed into the light component of the apparatus for 5 minutes (light exposure phase). Then, they would be waked up by atipamezole (0.84 mg/kg, s.c.) that reversed the effect of DEX, and returned their home cage. After 6 hours, rats were anesthetized again. They received ten foot-shocks (1.2 mA, 2 s, ITI: 30 s) in the dark component under anesthesia (dark-shock pairing phase). Rats were wake up after the training sessions. On Day 5, they were placed into light component for a memory retention test. In contrast to the sham control group, rats with lesions in the amygdala had significant shorter latencies stepping into the dark component. This result indicated that the amygdala play an important role in the learning of IA task under anesthesia. In Experiment 2, we further examined the importance of the basolateral amygdala (BLA) in learning under anesthesia. Rats received bilateral ibotenic acid lesions of the BLA. After one week of recovery, they received the same training procedure in Experiment 1. In the retention test, the BLA-lesioned group also showed impaired memory. In summary, the present findings suggested that learning of fear conditioning under anesthesia required the amygdala, and the BLA might be critical. Further experiments are required to examine whether other nuclei in the amygdala are also important in learning under anesthesia.

Disclosures: H. Hsiao: None. **D. Chen:** None.

Poster

413. Fear and Aversive Learning and Memory: Acquisition

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 413.06/ZZ16

Topic: G.01. Appetitive and Aversive Learning

Support: NSC 101-2410-H-002-082-MY3
MOST105-2420-H-006-004-MY2
MOST 105-2410-H-002-051

Title: Functional connectivity between the mediodorsal thalamic nucleus and frontal cortex accounts for the variability in fear memory acquired under dexmedetomidine

Authors: *K.-H. CHEN[1,2], D.-Y. CHEN[3], K. LIANG[2]
[1]Ministry of Sci. and Technol., Res. Inst. for the Humanities and Social S, Taipei, Taiwan; [2]Psychology, Natl. Taiwan Univ., Taipei, Taiwan; [3]Dept Psychology, Natl. Cheng Kung Univ., Tainan, Taiwan

Abstract: In search of the neural change altered by learning, we trained male Wistar rats under dexmedetomidine (0.1 mg/kg/hr, s.c) to associate a visual CS with a nociceptive US and concurrently obtained functional images with a 7T Bruker Biospec scanner. A within-subject paradigm was adopted by arranging the functional scans of CS-only, US-only, and CS-US association trials in separate blocks interleaved with resting state fMRI scans. The memory was probed by a fear potentiated startle (FPS) task on awake and drug-free rats in the next day. As huge variability in memory performance was found, rats were categorized into the good and poor learners for further analyses based on their FPS scores. The diversity in the memory scores could be attributed to variability of the subject in several aspects, such as sensitivity of detecting external stimulus or state of the brain when learning was initiated. The first possibility was excluded as no difference was found in the sensory stimulus-evoked BOLD signal. Concerning the initial state during learning, we focused on the signal synchronization between the thalamus and cortices as the former is involved in arousal and acts as a hub to regulate cortical connectivity. Before any sensory stimulation or learning experiences, the functional connectivity between the mediodorsal thalamic nucleus (MDN) and the frontal cortex positively correlated with the FPS scores (MDN-S1, r = .598; MDN-ACC, r= .497), but that between the MDN and the posterior parietal cortex (V1 and V2/RSC) did not. This indicated that a certain kind of thalamo-cortical activity coherence may play a critical role for learning to occur under sedation, and the initial state of the MDN-frontal cortex connectivity is critical. Additionally, the good and poor learners showed an opposite pattern in the MDN-S1 connectivity, either after the sensory stimulation or after the CS-US association session, and the changes in both phases significantly correlated with the behavioral outcome. Findings of this connectivity analysis could be viewed as interaction between the general brain state and task-evoked specific activity contributing to the individual difference in fear learning under sedation.

Disclosures: K. Chen: None. **D. Chen:** None. **K. Liang:** None.

Poster

413. Fear and Aversive Learning and Memory: Acquisition

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 413.07/ZZ17

Topic: G.01. Appetitive and Aversive Learning

Support: Whitehall Foundation Research Grant 2014-08-67
National Science Foundation IOS:1558121

Title: Bidirectional communication between the prelimbic cortex and the basolateral amygdala modulates the acquisition of trace fear memory

Authors: *A. J. KIRRY, M. R. HERBST, K. LEPAK, 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) in fear conditioning critically depends on the amygdala. However, if the CS and UCS are separated by several seconds as in trace fear conditioning, additional brain areas are needed, including the prelimbic area (PL) of the medial prefrontal cortex. A subset of PL cells exhibit sustained firing in response to the CS and prefrontal activity during the trace interval between the cue and shock is necessary for learning (Gilmartin & McEchron, 2005; Gilmartin et al., 2013). This suggests that the PL may provide a bridging signal to link the CS and UCS in memory, but it is unclear whether and how this activity is directly integrated in the basolateral amygdala (BLA) to support fear learning. Here we selectively manipulated PL inputs to the amygdala using projection-targeting optogenetics during training. The PL-BLA connection was either silenced or stimulated during the trace interval by delivering laser light to opsin-expressing terminals in the BLA during training. Memory retention was tested the following day in the absence of laser illumination. Memory formation was largely unaffected by inhibition or stimulation restricted to the trace interval. However, analysis of fiber placement revealed a correlation between impaired cued fear and anterior fiber placement, suggesting a rostral-caudal gradient in the function of PL inputs to the BLA. A similar gradient was observed in response to PL-BLA stimulation during fear extinction. ChR2-mediated stimulation of PL terminals in the BLA increased the expression of freezing to the CS, which was mediated by PL inputs to the anterior, but not posterior, BLA. These results suggest a functional rostral-caudal organization of PL input to the BLA in the acquisition and expression of cued fear. In a separate set of studies, an intersectional chemogenetic approach was used to silence communication between the PL and BLA in both directions and during the entire training session. Inhibition of PL to BLA slowed the acquisition of cued but not contextual fear memory and inhibition of BLA to PL impaired memory formation, leading to reduced fear to the CS at test. Together, these studies support a role for direct communication between the PL and BLA in the formation of cued, but not contextual, fear memories. To determine whether BLA input to the PL is necessary for CS or trace interval encoding, we are currently testing the consequence of BLA inactivation on PL neuronal firing during trace fear conditioning.

Disclosures: A.J. Kirry: None. **M.R. Herbst:** None. **K. Lepak:** None. **R.C. Twining:** None. **M.R. Gilmartin:** None.

Poster

413. Fear and Aversive Learning and Memory: Acquisition

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 413.08/ZZ18

Topic: G.01. Appetitive and Aversive Learning

Support: NIH-NINDS NS48156

Title: Conditioned inhibition training results in reduced intracellular calcium signals in Hermissenda type B photoreceptors

Authors: *J. FARLEY[1], J. CAVALLO[2], J. B. ANDERSON[2]
[2]Neurosci., [1]Indiana Univ. Bloomington, Bloomington, IN

Abstract: Previous research has demonstrated that induction of bidirectional learning-produced changes in photoresponses, excitability, and somatic K^+ currents of *Hermissenda* Type B photoreceptors, by either paired or explicitly-unpaired (EU) presentations of light and rotation, are calcium-dependent phenomena. The changes are prevented if B cells are first injected with a Ca^{2+}-chelator prior to either training protocol (paired vs EU). Several Ca^{2+}-dependent signaling pathways have been implicated in these changes: PKC and PTKs for pairings, and PP1/PP2B and AA/12-LOX metabolites for EU training. Enhanced light-evoked $[Ca^{2+}]_i$ -transients have also been reported for B cells on days following paired training, suggesting that learning may also alter the rules of $[Ca^{2+}]_i$ -signaling in B cells. To determine if EU training also produces persistent alterations in B cell $[Ca^{2+}]_i$-signaling, we undertook measurements of both resting and light-evoked $[Ca^{2+}]$i in B cells from animals exposed to either EU or control training conditions. Following ionophoresis of fura-2 into single B cells, measurement of light-evoked generator potentials and spike frequencies and input resistances (which confirmed previously described EU-produced reductions in photoresponses and excitability), ratiometric dye fluorescence was measured using dual-wavelength (340/380 nm) microphotometry. B cells were repetitively illuminated for 30 sec periods (9 min ISIs) using an incandescent epi-illumination source (~300 $uW \cdot cm^{-2}$) similar to that used in behavioral/neurophysiology experiments. In standard (10 mM Ca^{2+}) ASW, baseline $[Ca^{2+}]_i$ of dark-adapted control B cells (n=13) averaged 91.1 ± 14 nM. Light evoked large increases in sustained $[Ca^{2+}]_i$ (209 nM) that remained elevated for ~ 3-4 min following light offset and then dipped below the pre-light level for 2-3 min before recovering to the original baseline. In contrast, dark-adapted B cells (n=3) from EU-animals had significantly lower baseline $[Ca^{2+}]_i$,levels of 43.0 ± 8 nM. Light-evoked sustained $[Ca^{2+}]_i$ levels (104.7 nM) in these cells were ~50% of control cell values, but showed similar kinetics as control cells and exhibited the same dip below baseline ~ 4 min post light. Thus, paired and EU-training produce opposing metaplasticity within the Ca^{2+}-signaling system(s) of B cells. We suggest that the enhanced $[Ca^{2+}]_i$ in paired B cells contributes to extinction-produced reversal of original pairing-produced excitability, while reduced light-evoked $[Ca^{2+}]_i$ levels in EU-trained B cells are insufficient to support normal PKC activation by pairings, and thus contribute to retarded acquisition of paired (excitatory) conditioning.

Disclosures: J. Farley: None. **J. Cavallo:** None. **J.B. Anderson:** None.

Poster

413. Fear and Aversive Learning and Memory: Acquisition

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 413.09/ZZ19

Topic: G.01. Appetitive and Aversive Learning

Support: NSF SBE Postdoctoral Fellowship
Klingenstein-Simons Fellowship Award in Neuroscience

Title: Aversive learning strengthens episodic memory in adolescents and adults

Authors: *A. O. COHEN, N. G. MATESE, A. FILIMONTSEVA, C. A. HARTLEY
New York Univ., New York, NY

Abstract: Adolescence is often filled with emotional experiences that may change how individuals remember and respond to stimuli in their environment. Recent studies in adults have shown that learned aversive associations can generalize across a category of stimuli and lead to enhanced memory for the reinforced category of trial-unique exemplars. The present study tests whether learned aversive associations similarly lead to better memory and generalization across a category of stimuli in adolescents. Participants complete a pavlovian category conditioning task where one of two categories is partially reinforced with an aversive odor, and return 24 hours later to complete a surprise recognition memory test. Preliminary data analyses including 30 individuals ages 13 to 25 (half of our planned sample) show better corrected recognition memory for the reinforced than the unreinforced category of exemplars in both adults and adolescents. Further analysis reveals that enhanced recognition memory is driven by better memory for the reinforced exemplars in both age groups. Thus, while we do not presently observe category conditioning effects in either adolescents or adults, we do see enhanced memory for the items with an acquired aversive association in both adults and adolescents. These findings build on previous work in adolescent and adult humans and rodents showing similar acquisition of aversive pavlovian conditioning using simple stimuli across age groups. Analyses in the full sample will examine age continuously to test for age-related changes in memory across adolescence.

Disclosures: A.O. Cohen: None. **N.G. Matese:** None. **A. Filimontseva:** None. **C.A. Hartley:** None.

Poster

413. Fear and Aversive Learning and Memory: Acquisition

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 413.10/ZZ20

Topic: G.01. Appetitive and Aversive Learning

Title: Phosphorylation of mitogen-activated protein kinase in the medial prefrontal cortex is associated with individual variation in extinction recall in rats

Authors: *A. S. RUSSO[1], R. G. PARSONS[2]
[1]Psychology, [2]Stony Brook Univ., Stony Brook, NY

Abstract: Although most individuals are exposed to a traumatic event at some point, only a small portion of individuals develops posttraumatic stress disorder (PTSD), suggesting there are individual differences rendering some people more susceptible to PTSD-development than others. Pavlovian fear conditioning involves the pairing of an aversive, unconditioned stimulus (US) with a neutral stimulus (CS) such that presentation of the CS alone comes to yield a conditioned fear response (CR). Decrease in the CR after repeated presentations of the CS alone is referred to as extinction. It has been shown that individuals with PTSD have a poor ability to extinguish CRs, suggesting that studying individual variation in extinction learning using rodent models may inform our understanding of the neurobiological basis of PTSD. However, the neural mechanisms underlying variation in extinction have been investigated sparsely. To understand whether altered activity in brain areas known to be involved in extinction corresponds to expression of extinction learning and recall, we assessed levels of phosphorylated mitogen-activated protein kinase (p-MAPK) in the amygdala and medial prefrontal cortex (mPFC) of rats with good and poor extinction learning and recall. Phosphorylation of MAPK is a key event underlying many forms of neural plasticity and is known to be crucial for formation of long-term fear and extinction memories. Rats were exposed to a Pavlovian fear conditioning paradigm with conditioning consisting of two combinations of tone and foot-shock, extinction training consisting of 20 presentations of the tone alone, and extinction recall testing consisting of 8 presentations of the tone alone. Rats were sacrificed either after extinction training or extinction recall sessions, and all brains were saved for western blotting. We found that there was significantly less p-MAPK in the mPFC of rats which had poor extinction recall than rats which had good extinction recall following an extinction recall session. There was no significant difference in p-MAPK between rats with poor and good extinction recall in the amygdala, nor were there significant differences in p-MAPK between rats with good and poor extinction learning in the mPFC or amygdala. These data suggest that the inability to extinguish learned fear is the result of aberrant neural plasticity in the mPFC. Future investigation will seek to delineate the role of specific projections from areas like the basolateral amygdala (BLA) and paraventricular nucleus of the thalamus (PVT) to the mPFC.

Disclosures: A.S. Russo: None. **R.G. Parsons:** None.

Poster

413. Fear and Aversive Learning and Memory: Acquisition

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 413.11/ZZ21

Topic: G.01. Appetitive and Aversive Learning

Title: Manipulating the strength of initial fear learning does not affect the extent to which subsequent fear learning is facilitated

Authors: *K. COLE[1], J. LEE[1], R. G. PARSONS[2]
[1]Psychology, [2]Stony Brook Univ., Stony Brook, NY

Abstract: Prior experience can have a profound effect on memory formation. Therefore, it is important to study the conditions whereby an initial learning event can affect subsequent learning. A recent study from our laboratory showed that learning of a weak fear conditioning trial was enhanced by prior auditory fear conditioning when the two training events were spaced 24 hours apart. Interestingly, a correlation between fear levels for the two training events was observed such that the rats that learned the initial auditory fear conditioning well, showed poor facilitation to the second single light-shock trial. Conversely, rats that showed weaker learning of the initial auditory fear conditioning showed good facilitation to the subsequent light-shock trial. These data suggest that the strong learning to the initial learning event somehow occludes subsequent learning. If that is the case, then altering the strength of the initial learning by changing the number of trials should dictate whether or not the subsequent learning is facilitated. Here, we manipulated the strength of the auditory fear conditioning event by varying the number of tone-shock pairings. Rats received either one, two, six, eight tone-shock pairings followed by a single light-shock pairing 24 hours later. Long-term memory for both cues was assessed in separate test sessions. We report that there was facilitation of learning to the subsequent event in all groups. Therefore, while weak learning is facilitated by prior auditory fear conditioning, manipulating the amount of shocks during auditory conditioning does not affect the extent of facilitation to subsequent learning. These findings suggest that the mechanism that primes future learning is engaged regardless of the strength of initial learning.

Disclosures: K. Cole: None. **J. Lee:** None. **R.G. Parsons:** None.

Poster

413. Fear and Aversive Learning and Memory: Acquisition

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 413.12/ZZ22

Topic: G.01. Appetitive and Aversive Learning

Title: Activation of the Mitogen-Activated Protein Kinase in female rats during the expression and extinction of fear

Authors: *M. VOULO[1], R. G. PARSONS[2]
[1]Psychology, [2]Stony Brook Univ., Stony Brook, NY

Abstract: Activation of the Mitogen-Activated Protein Kinase in female rats during the expression and extinction of fear.
Women are more than twice as likely as men to develop post-traumatic stress disorder (PTSD), which is associated with impaired retention of fear extinction. Despite this, the majority of basic research regarding fear extinction has been conducted using males as subjects. Studies of fear conditioning and extinction learning have begun to include female animals, but results have been mixed with respect to sex differences. Most studies only use one measure of fear – freezing behavior. However, it has been suggested that females show more active fear responses than males, and therefore measures of fear susceptible to differences in locomotor activity, such as freezing, may not be ideal. Our lab recently found a response-specific sex difference in fear extinction such that females show impaired extinction when measuring fear-potentiated startle (FPS), but not when measuring freezing behavior, however the neural basis of this difference between males and females is unknown. To begin to address this question, we examined phosphorylation of the mitogen activated protein kinase (pMAPK) in female rats following a test for the expression of fear memory and following an extinction retention test. There were two test groups, one that underwent fear conditioning and the next day was exposed to a test session to reactivate the memory for acquisition. The second group underwent fear conditioning, followed by extinction training the next day, and the final day received an extinction retention test. Rats in both groups were sacrificed 30 minutes following the final test session, allowing us to look at pMAPK activity during fear expression after acquisition and extinction memory expression. We compared MAPK activity in these two groups to that in naive controls. Our results show that rats that underwent only fear conditioning before the test showed enhanced activity in both the infralimbic (IL) and prelimbic (PL) areas of the prefrontal cortex compared to the other two groups. Surprisingly, there was no increase in PL or IL activity in the group that underwent extinction training prior to the test day. We are currently analyzing pMAPK in the periaqueductal grey and the amygdala to determine if there are other differences between the groups. In addition, we are beginning to compare brain activity in males and females undergoing

FPS extinction to understand any sex differences in the brain mechanisms underlying this behavioral effect.

Disclosures: M. Voulo: None. **R.G. Parsons:** None.

Poster

413. Fear and Aversive Learning and Memory: Acquisition

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 413.13/ZZ23

Topic: G.01. Appetitive and Aversive Learning

Support: Stony Brook University

Title: Chemogenetic inhibition of excitatory neurons in the basolateral amygdala disrupts the facilitation of subsequent fear learning

Authors: *R. G. PARSONS, J. LEE
Stony Brook Univ., Stony Brook, NY

Abstract: Memory permits adaptation to future experience. In the laboratory we can study this essential property of memory by assessing how an initial learning experience can alter subsequent learning. We have previously shown that while a single weak fear conditioning trial is insufficient to support long-term memory, it alters the capacity for future learning such that another trial delivered within a protracted time window results in robust memory. In this set of experiments, we sought to determine whether or not manipulating neural activity in the basolateral amygdala (BLA) using designer receptors exclusively activated by designer drugs (DREADDs) during the initial learning trial affected the facilitation of subsequent learning. Male rats were either given injections of AAV8-hM4Di under the control of the neuron specific promoter (hSYN), AAV8-hM4Di under the control of the CaMKII promoter, or AAV8-eGFP. Rats were then trained with two fear conditioning trials spaced 24 hours apart. One hour prior to the first trial rats received systemic injections of the DREADD ligand, clozapine N-oxide (CNO; 5mg/kg). An additional control group of rats was included that were given the CaMKII expressing inhibitory DREADD into the BLA, but given vehicle injections prior to training. During the initial training trial, there were no differences between groups in activity levels or in reactivity to the shock. Memory was tested 48 hours later by presenting rats with the cue that had predicted shock and fear-potentiated startle was measured. Preliminary results indicate a promoter specific effect on learning, such that rats infected AAV-hM4Di-CAMKII exhibited a deficit in fear-potentiated startle compared to rats expressing AAV8-hSYN-hM4Di, rats infected with AAV-eGFP, or controls given vehicle injections. We are currently testing if different doses of CNO produce the same pattern of results. These findings indicate that 1) the blocking neural activity in the BLA during an initial fear conditioning trial prevents the mechanism responsible

for facilitating subsequent learning and 2) that this effect requires that expression of the inhibitory DREADD receptor be restricted to excitatory neurons in the BLA.

Disclosures: R.G. Parsons: None. **J. Lee:** None.

Poster

413. Fear and Aversive Learning and Memory: Acquisition

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 413.14/ZZ24

Topic: G.01. Appetitive and Aversive Learning

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UCLA Brain Injury Research Center
1R01NS27544: DH, CG
Centre for NeuroSkills: DH
UCLA Steve Tisch BrainSPORT: CG

Title: Projection specific mechanisms of auditory sensitivity that contribute to enhanced fear after traumatic brain injury

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Abstract: Traumatic brain injury (TBI) increases the risk for post traumatic stress disorder (PTSD), however the underlying neurobiology of this comorbidity is unknown. Sensory sensitivity is common following TBI, and changes in sensory processing might influence the encoding of traumatic events in the wake of injury. The lateral amygdala (LA) receives direct sensory cortical and thalamic inputs necessary for the formation of auditory fear memories, and may be vulnerable to TBI. We have shown enhanced contextual fear after lateral fluid percussion injury (FPI) in rats following fear conditioning when white noise, but not when low frequency tones signal mild aversive footshocks. Altered sensitivity to white noise might reflect phonophobia after FPI and underlie increased fear after injury when used in fear conditioning. We hypothesized that FPI enhances contextual fear to white noise-signaled conditioning due to altered sensory-emotional network processing. In this study we show that when rats are exposed to 75dB white noise alone prior to fear conditioning, FPI rats demonstrate robust defensive

behavior and show increased context fear after white noise-signaled fear conditioning relative to sham. We also show increased neuronal activity within the ipsilateral LA in FPI rats during white noise exposure relative to noise-exposed shams and quiet FPI controls with Arc immunohistochemistry. Finally, to determine functional activity in sensory projections to LA, we bilaterally infused retrograde tracer cholera toxin B (CTB) three weeks before FPI and measured c-Fos expression in CTB+ cells during white noise exposure. In this experiment we demonstrate that the increased activity in the ipsilateral LA is driven by increased activity in neurons projecting from ipsilateral auditory thalamus (medial geniculate nucleus, MGN) to LA as measured by more c-Fos in CTB+ MGN neurons during white noise exposure. This effect was specific to the MGN and not secondary auditory cortex (Te3)-LA projections, indicating the specificity of the inputs that drive increased plasticity and corresponding defensive behavior. These data provide implications for the vulnerability of the thalamo-amygdala pathway underlying phonophobia and altered sensory processing after TBI, where otherwise neutral stimuli may adopt aversive properties and impact encoding of traumatic memories contributing to psychiatric comorbidities.

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Poster

413. Fear and Aversive Learning and Memory: Acquisition

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 413.15/ZZ25

Topic: G.01. Appetitive and Aversive Learning

Support: Staglin Center for Brain and Behavioral Health
NIH Grant R01AA026530

Title: Dissociation in effective treatment and behavioral phenotype between stress-enhanced fear learning and learned helplessness

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Abstract: An acute traumatic event can lead to life-long changes in stress susceptibility and result in psychiatric disease, such as Post-Traumatic Stress Disorder (PTSD). Despite the relatively high prevalence of PTSD, limited progress has been made in identifying practical interventions to stress-induced psychopathology. Historically, two animal models of PTSD that use unpredictable and uncontrollable shock have been used to examine the underlying mechanisms of stress-induced maladaptive behavior: learned helplessness (LH) and stress-enhanced fear learning (SEFL). An interesting disparity between LH and SEFL behavior is the relatively brief window of time in which LH persists compared to SEFL's apparent persistence. Despite many differences, the two models share one physiological mechanism. Post-stress elevations in glucocorticoid concentrations are necessary for phenotypic induction in both LH and SEFL. We have previously shown that access to a concentrated glucose solution after trauma decreased stress-related pathology in LH. Furthermore, it appears that post-stress glucose consumption decreases peripheral concentrations of free corticosterone, suggesting that the treatment may also work in eliminating SEFL. Our first experiment tested the hypothesis that exposing rats to glucose following exposure to the standard SEFL protocol of 15 footshocks would inhibit expression of the SEFL phenotype. We exposed 32 Long-Evans rats to 15, 1mA footshocks, or context exposure without shock. Rats in each condition had 18-hours access to a 40% glucose solution or water immediately following termination of the session. 24-hours later, we measured freezing in a novel context that shared the same olfactory cues as the previous context. The following day, all rats were exposed to 1, 1mA footshock in a novel context and were tested for freezing in that same context 24-hours later. Our second experiment examined the effect of shock number on the induction of SEFL behavior. We exposed 24 male Sprague-Dawley rats to 100 tail shocks (1 mA, an LH protocol), 15 tail shocks (1 mA, SEFL protocol), or simple restraint. One week later, rats received 1, .5 mA footshock in a novel environment and were tested for freezing in that same context 24-hours later. We found that glucose ingestion did not eliminate SEFL, but did decrease freezing in the context that shared olfactory cues with the acute-stress context. Number of shocks appeared to have a small, but significant effect on SEFL. These data suggest many elements of dissociation between the two models of PTSD. Future studies examining shared mechanisms between LH and SEFL may open the door for the development of potent PTSD interventions.

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Poster

413. Fear and Aversive Learning and Memory: Acquisition

Location: SDCC Halls B-H

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Topic: G.01. Appetitive and Aversive Learning

Support: NARSAD 26612
NRSA F32 MH10721201A1
Staglin Center for Brain and Behavioral Health
RO1MH62122

Title: Differential modulation of fear behaviors in female mice with deletion of PAC1 receptors from the ventral intercalated cells and the basolateral amygdala

Authors: *A. K. RAJBHANDARI[1,2], J. CHAVEZ[1], L. NGUYEN[1], N. KECES[1], J. A. WASCHEK[3], M. S. FANSELOW[1,2,3]
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Abstract: Fear responses are critical for survival. However, exposure to extremely traumatic stimuli can cause fear regulation via certain brain regions to go awry leading some individuals to develop anxiety-related disorders like post-traumatic stress disorder (PTSD). Various brain regions, particularly the amygdala, govern fear. The amygdala microcircuitry containing the intercalated cells (ICCs) that lie in the interface of basolateral (BLA) and central amygdala are important modulators of fear behavior. Several neuropeptide systems are part of the amygdala microcircuitry including the neuropeptide PACAP (pituitary adenylyl cyclase-activating peptide) and its G-protein coupled receptor PAC1. In this regard, it was reported that the PACAP and PAC1 receptors are linked to PTSD symptom severity at both genetic and epigenetic levels, and this link was stronger in females with PTSD. We investigated whether deletion of PAC1 receptors from the ventral ICCs or BLA of female mice alters fear acquisition, generalization, retention or extinction. We infused AAV-driven Cre-recombinase or a GFP control into the vICCs or BLA of female mice with a floxed PAC1 gene. After viral expression, mice went through a contextual fear acquisition protocol (1 trial/day for 5 days) in which 4-minute after being placed into a conditioning context they received a 0.65 mA, 1-second shock. We found that mice with PAC1 deletion in the vICCs showed decreased fear acquisition, but deletion in the BLA led to normal fear acquisition. No differences were found in fear generalization, which was measured by placing the mice in a novel context for four minutes. Mice with PAC1 receptor deletion in the BLA showed decreased fear retention, which was measured in the acquisition context for four minutes. Lastly, deletion of PAC1 receptors from the BLA, but not vICCs, led to decreased fear extinction, which was carried out by placing them in the acquisition context for 30 minutes every day for five days and freezing measured during the first 4 minutes of the session. Our previous results showed that deletion of PAC1 receptors from the vICCs of male mice enhances fear generalization, but deletion from the BLA decreases acquisition of fear. Overall, the current behavioral findings in female mice with PAC1 deletion, when combined with the results from male mice with the same manipulation, indicate that PACAP/PAC1 system is poised to modulate fear in a dynamic manner via the amygdala microciruitry. This dynamic balance is significantly different in males and females. It will be interesting to determine the physiological properties of these PACAP/PAC1 neural networks within the amygdala sub-regions.

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Poster

413. Fear and Aversive Learning and Memory: Acquisition

Location: SDCC Halls B-H

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Support: NIMH Grant RO1MH62122
Staglin Center for Brain and Behavioral Health
NRSA-F32 MH10721201A1
NARSAD 26612
NSF DGE-1650604

Title: Blockade of corticotropin-releasing factor type 1 receptors in the basolateral amygdala decreases post-shock freezing but does not prevent stress-enhanced fear learning

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Abstract: Post-traumatic stress disorder (PTSD) is an anxiety disorder that develops after exposure to traumatic events and can involve an exaggerated fear response to stimuli that are reminiscent of the original stressor. Our laboratory has developed a rodent model of PTSD that captures this symptom termed stress-enhanced fear learning (SEFL). In this model, exposure to a traumatic stressor (15 unsignalled footshocks) in one context sensitizes fear learning to a mild stressor (1 unsignalled footshock) in a second context. However, the stress-induced changes in the fear learning circuitry that support this enhancement are not fully understood. The neuropeptide corticotropin-releasing factor (CRF) plays a key role in the stress response and blockade of CRF activity has been shown to impair fear learning. In this study we investigated whether CRF activity in the basolateral amygdala (BLA) during the traumatic stressor is necessary for the SEFL phenotype. Rats received an infusion of either a CRF1 receptor antagonist (CP-376395) or vehicle into the BLA immediately prior to the traumatic stressor. We found that blockade of CRF1 receptors decreased post-shock freezing during the traumatic stressor, but did not prevent stress-enhanced fear learning to the mild stressor. These findings suggest that while CRF activity in the BLA does play an important role in the fear response, blockade of these receptors is insufficient to prevent development of the SEFL phenotype.

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Poster

413. Fear and Aversive Learning and Memory: Acquisition

Location: SDCC Halls B-H

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German Research Foundation (Deutsche Forschungsgemeinschaft, DFG) as part of the DFG Collaborative Research Centre SFB 1280

Title: Brain structure and function relationships underlying differential fear conditioning in healthy women and men

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Abstract: The ability to respond to biologically relevant stimuli is critical for adaptive behavior. These responses are likely shaped by associative learning that provides initially neutral cues with predictive value signaling impending threat or safety. Disturbances in fear and safety learning are of high clinical relevance in anxiety and pain-related disorders, both with increased prevalence in women. Brain imaging studies support distinct processing of learned emotional responses in networks involving amygdala, hippocampus and prefrontal cortex (PFC). However, evidence is missing on sex differences in the activity and connectivity within networks supporting threat versus safety learning, particularly in visceral pain. We therefore aimed to discern the structural and functional mechanisms underlying learned emotional responses in healthy women and men. For this re-analysis, data of 75 volunteers (38 women, 37 men; mean age 28.9 y) from two 3T MRI studies were pooled. Participants underwent differential fear conditioning during which an experimental visceral pain stimulus was repeatedly paired with a visual cue (CS^+) while a second cue (CS^-) was presented unpaired. CS valence was assessed on visual analogue scales before and after conditioning. Group differences were assessed on the behavioral and neural level in response to threat and safety cues and analyzed regarding brain structure-function associations. Overall, participants rated the CS^+ as significantly more unpleasant and the CS^- as more pleasant

after conditioning without evidence of sex differences. On a neural level, an amygdala-hippocampus network was found for both cues that further encompassed dorsal anterior cingulate for CS^+ only. This network correlated negatively with parahippocampal volume in men. For women, a network including nucleus accumbens correlated positively with CS^- valence. Women also revealed higher functional connectivity between amygdala, dorsolateral PFC, mid-cingulum and precuneus in response to the CS^+. In conclusion, the amygdala-hippocampus network seems of pivotal relevance in both, visceral, pain-related fear and safety learning, yet with highly diverging mechanisms. While for men, fear learning was associated with regional volume change, women showed a stronger discrimination between fear and safety learning as evidenced by increased processing of the rewarding safety cue and stronger amygdala coupling in response to threat. Together, these results shed light on the neural circuitry possibly underlying the high female prevalence in anxiety and pain-related disorders and call for future studies extending sex-specific effects in fear conditioning.

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Poster

413. Fear and Aversive Learning and Memory: Acquisition

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Program #/Poster #: 413.19/AAA3

Topic: G.01. Appetitive and Aversive Learning

Support: Beijing Municipal Science and Technology Commission (Z141110001814068)

Title: Theta oscillations synchronize ventral/dorsal medial prefrontal cortex and amygdala during conditioned fear acquisition using human intracranial EEG

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Abstract: Fear expression is one of the fundamental emotions of human beings and is crucial for living organisms and adapting to the environment. The understanding of the formation and extinction of fear emotions can promote the non-drug treatment of mental disorders, such as post-traumatic stress disorder. Numerous animal studies have demonstrated that fear expression and extinction relies on the coordinated activity of dorsal medial prefrontal cortex (dmPFC) and amygdala and 4Hz oscillations specific to fear expression supports this long-range synchronization. However, it remains unclear whether this circuit and brain rhythm can be

generalized to human fear expression. In addition to dmPFC, few studies have addressed the roles of ventral medial prefrontal cortex in fear acquisition. This study aims to investigate the roles of dorsal/ventral prefrontal cortex and amygdala in conditioned fear acquisition using human intracranial EEG recordings.Seventeen patients with pharmacologically refractory epilepsy (five female and twelve male) participated in this study. SEEG electrodes were surgically implanted in the medial prefrontal cortex and medial temporal lobe, including amygdala and hippocampus, under a clinical protocol. Implantation sites were chosen solely on the basis of clinical criteria. In the experiment, each patient performed a classical fear-conditioning task. This task consisted of two different color-coded squares, each of which was assigned randomly to CS+ or CS− across patients. The US was electric shock (0.15s duration before the termination of 4s CS+, 50% of CS+ trials were paired with US). The shock level was manipulated according to the individual pain rating. The interval between CS trials varied from 8 to10s.Skin conductor response recorded from eight patient confirmed successful acquisition of fear conditioning. Patients who failed to show fear acquisition were eliminated from further analysis. We found that during fear acquisition, amygdala and ventral/dorsal medial PFC cortex showed an increased power of theta-band (2-8Hz) activity, and the theta synchronization between amygdala and medial prefrontal cortex were significantly enhanced for CS+ condition. Single-trial dynamic analysis on power and coherence revealed neural mechanisms of fear acquisition processing. The preliminary results indicated that the formation of human fear acquisition invoked medial prefrontal cortex-amygdala circuit that communicated through theta-band oscillation. Notably, the ventral medial prefrontal cortex also played critical roles in fear acquisition, which challenged the findings from previous animal and human studies.

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Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 414.01/AAA4

Topic: G.01. Appetitive and Aversive Learning

Support: DFG

Title: Contribution of CRF and 5-HT in the anterodorsal BNST to phasic and sustained fear in freely behaving mice

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Abstract: Sustained fear paradigms in rodents have been developed to model clinical situations in patients suffering from long-lasting anxiety disorders. Rodent data suggest that short-lasting (phasic) fear responses rely on the central amygdala, whereas more long-lasting (sustained) fear responses critically depend on the bed nucleus of the stria terminalis (BNST). Conditioned fear can be mediated by the amygdala via corticotropin-releasing factor (CRF), a stress hormone that acts on receptors in the BNST. CRF-containing cell bodies and CRF receptors were found in high concentrations in the BNST and CRF neurons co-localize with 5-HT (Serotonin) terminals in this brain region. Therefore, in this study we used a pharmacological approach combined with fear behavioral protocols in an established phasic/sustained fear mouse model to reveal the critical involvement of CRF and 5-HT in the anterodorsal (ad)BNST to modulate phasic and sustained fear.
Bilateral local application of a CRF1-receptor agonist (Stressin I) before fear memory retrieval, 24h after predictable CS-US training, induced sustained fear (maintained freezing) indicating the critical contribution of the CRF1-receptor during sustained states of conditioned fear. Application of saline (control) revealed only phasic fear 24 hours after predictable CS-US pairing as expected. Bilateral local application of a 5-HT2A-receptor antagonist (R-96544) either before unpredictable CS-US training or before fear memory retrieval, 24 hours after unpredictable conditioning, blocked the sustained component of fear while phasic fear component was not affected, indicating the critical contribution of serotonergic transmission mediated by the 5-HT2A-receptor during sustained states of conditioned fear. Saline application as control revealed sustained fear 24 hours after unpredictable CS-US pairing. Further, data also revealed that 5-HT1A– and 5-HT1B-receptor in the adBNST critically contribute to the modulation of phasic and sustained fear.
In summary, here we show the critical contribution of CRF and 5-HT in the adBNST to phasic and sustained fear in freely behaving mice. This study will advance the understanding of clinical anxiety and its treatment strategies and will thus provide a putative perspective for pharmacological treatments that specifically target the BNST.
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Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 414.02/AAA5

Topic: G.01. Appetitive and Aversive Learning

Support: ANPCyT PICT 2014-3155
CONICET
SECyT - Universidad Nacional de Cordoba

Title: Connection from granular to dysgranular retrosplenial cortex is required for contextual fear memory retrieval

Authors: S. DE OLMOS[1], E. L. SGWALD[1], N. E. PONCE[1], E. A. BIGNANTE[1], *A. G. LORENZO[2]
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Abstract: The retrosplenial cortex (RSC) is divided in dysgranular (A30) and granular (A29) subregions. However, the functional role of each RSC subdivision in episodic memory processing remains poorly understood. In recent years, the participation of the RSC in contextual fear memory (CFM) has gained increased attention; nevertheless these studies have regarded the RSC as a uniform structure. The aim of this work was to reveal functional differences of RSC subdivisions in CFM processing. We used male rats and assessment of c-Fos and Egr-1 expression to infer neuronal activity during contextual fear conditioning. Data showed that during training and test, expression of Egr-1 dropped in A29 while Egr-1 and c-Fos rose in A30. Repeated measures ANOVA ($F(5,37)= 83.967$, $p=0.0000$) confirmed significant difference between RSC subdivisions, suggesting distinct or complementary roles for each area. Further analysis and statistical comparisons showed that similar to A29, reduced Egr-1 expression during training and test also occurred in caudomedial entorhinal cortex (CEnt). Contrarily, increases in c-Fos and Egr-1 in A30 during training and test were coincidental with medial entorhinal cortex (MEnt), dorsal CA1 field of hippocampus (CA1), central (CeA) and basolateral (BLA) nucleus of the amygdala (ANOVA ($F(12,108)=75.006$, $p=0.0000$), suggesting that A30 and these limbic structures are coupled into a distributed network supporting CFM encoding and retrieval. We later used systemic applications of MK801 to induce a non-invasive and selective elimination of neurons in layers IV-Va of A29 ($A29^{MK801}$ neurons). Loss of $A29^{MK801}$ neurons after CFM consolidation did not affect activation of limbic structures during test, but significantly impaired activation of A30 (ANOVA ($F(18,144)=5.7599$, $p=0.0000$) and the expression of freezing during test (ANOVA $F(2,12)=34.168$, $p=0.00001$). This observation indicates that during test, $A29^{MK801}$ neurons are critically required for coupling activity of A30 with limbic structures and the retrieval of CFM. By using silver staining, immunolabeling and track-tracing methods we showed that $A29^{MK801}$ are typical pyramidal neurons (likely excitatory) projecting to superficial layers of A30, and confirmed that GABAergic neurons in A29 were not affected by MK801. Collectively, our results indicate that $A29^{MK801}$ neurons play a critical role coupling activation of A30 with limbic structures during fear memory recall, which is required for CFM retrieval and expression of adaptive freezing behavior.

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Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

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Program #/Poster #: 414.03/AAA6

Topic: G.01. Appetitive and Aversive Learning

Title: Effects on fear versus safety discrimination by the partial NMDAR agonist D-cycloserine

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Abstract: Learning to inhibit fear and maintain reduced fear levels to non-threatening environmental cues is a constant challenge for individuals suffering from posttraumatic stress disorder (PTSD). PTSD individuals have difficulty maintaining reduced fear levels after extinction-based therapies, and are also impaired in learning to inhibit fear to explicit safety cues trained under conditioned inhibition paradigms. Prior research has shown the beneficial effects of exposure therapy in reducing fear can be facilitated by the partial NMDA receptor agonist, D-Cycloserine (DCS). However, it is unclear if DCS is facilitating fear versus safety discrimination or if it is promoting non-specific fear reduction. Thus, the present study examined the effects of DCS in adult male Long Evans rats during a fear, safety, and reward cue discrimination conditioning task (DC) that is well-established in our laboratory. In this DC task, rats are exposed to pairings of a) a fear cue with shock, b) a safety cue with no shock, c) a reward cue with sucrose, and most importantly, d) a compound fear+safety cue with no shock. Adult male rats typically show significant inhibition of freezing to the compound fear+safety cue compared to the fear cue during the 3rd discrimination session. In this study, we hypothesized that DCS would facilitate discrimination learning between the fear cue and the fear+safety cue during earlier discrimination sessions, and that this improved discrimination may facilitate subsequent fear extinction under drug-free conditions. DCS (30.0 mg/kg i.p) was administered during the first three of four DC sessions. Saline was administered during the last DC session, to assess behavior under drug-free conditions, and subsequent extinction. As is typical, the saline group showed significant inhibition of fear during DC3, but preliminary data suggests the level of inhibition to the fear+safety cue versus the fear cue may be greater in DCS group. Based on our preliminary data it does not appear this improved fear versus fear+safety cue discrimination has any beneficial effects on subsequent fear extinction, as both saline and DCS groups showed similar fear extinction curves. This may suggest extinction and conditioned inhibition are mediated by different mechanisms.

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Poster

414. Fear and Aversive Learning and Memory: Modulation

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Program #/Poster #: 414.04/AAA7

Topic: G.01. Appetitive and Aversive Learning

Support: NHMRC Grant

Title: Oxytocin receptor activation in the basolateral complex of the amygdala facilitates discrimination between stimuli and promotes configural processing of stimuli

Authors: *J. FAM[1], N. M. HOLMES[1], A. J. DELANEY[2], J. W. CRANE[3], R. F. WESTBROOK[1]
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Abstract: Oxytocin (OT) is a neuropeptide which influences the expression of social behaviour and regulates its distribution according to the social context - OT is associated with increased pro-social effects in the absence of social threat and defensive aggression when threats are present. However, this bi-directional effect of OT has not been demonstrated with stimuli outside the domain of social behaviour, and studies have yet to examine the learning mechanisms that underlie the effects of OT manipulations. The present experiments investigated the effects of OT beyond that of social behaviour by training rats in a Pavlovian fear conditioning protocol. In Experiment 1, an OT receptor agonist (TGOT) microinjected into the basolateral amygdala enhanced the discrimination between an auditory cue that was predictive of shock and another auditory cue that signalled the absence of shock. Experiment 2 replicated the TGOT-facilitated discrimination using compound cues that consisted of auditory and visual elements, and additionally demonstrated that the enhanced discrimination following TGOT administration was accompanied by a qualitative shift in the learning mechanisms underlying the discrimination. Specifically, rats given TGOT microinjections into the BLA engaged in configural (Pearce, 1987) processing of stimuli while rats given vehicle microinjections used an elemental strategy (Rescorla & Wagner, 1972).

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Poster

414. Fear and Aversive Learning and Memory: Modulation

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant DA034010

Title: Fear and reward intersect in the ventral pallidum

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Abstract: The ventral pallidum (VP) is integral to reward seeking and a variety of reinforcement-driven behaviors. However, little is known about the contribution of the VP to adaptive behavioral responses to threating stimuli. Here, we examined the role of the VP in multi-cue fear discrimination. To induce neurotoxic lesions, male Long Evans rats were injected with N-Methyl-D-aspartic acid (NMDA; 20 mg/ml; 0.3µl per side) into the VP (n = 8). For controls (n = 7), the needle was lowered to the VP, but no injection was made. Following recovery, rats were food deprived to 85% of their body weight and trained to nose poke in order to receive food pellets. In 16 sessions of fear discrimination, rats were presented with three 10-s auditory cues, each associated with a unique probability of foot shock; danger, p = 1.00; uncertainty, p = 0.25; and safety, p = 0.00. The schedule for rewarded nose poking was completely independent from auditory cue presentation and foot shock. Fear was measured using suppression of rewarded nose poking. Consistent with the demonstrated role for the VP in reward seeking, VP lesioned rats showed lower rewarded nose poking rates than their control counterparts. Controls acquired excellent discrimination: showing high fear to danger, intermediate to uncertainty and low to danger. VP lesioned rats readily acquired fear but were markedly impaired at reducing fear to uncertainty and safety. Elevated and excessive fear was demonstrated to these cues for the entirety of the 16 sessions of testing. These results reiterate that the VP is required for reward seeking but reveal a crucial role for VP in reducing fear in the face of uncertainty and safety. Studies are now underway to determine if information about reward, danger and safety are processed in overlapping or distinct neurons, revealing the nature of the intersection between fear and reward in the ventral pallidum.

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Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 414.06/AAA9

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant MH113053

Title: Optogenetic inhibition of caudal substantia nigra globally inflates fear in multi-cue Pavlovian discrimination

Authors: *K. M. WRIGHT, S. CIESLEWSKI, M. A. MCDANNALD
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Abstract: Appropriate modulation of fear behavior in situations of potential threat is vital. Disruption in neural circuits that support this process are thought to underlie maladaptive fear responses observed in psychiatric disorders of stress and anxiety. The goal of the experiment was to demonstrate a causal role for the caudal substantia nigra (cSN), a region beyond currently identified fear circuitry,in fear modulation. Adult male, Long Evans rats received bilateral intra-cSNinfusions of either enhanced halorhodopsin (eNpHR: AAV-hSyn-eNpHR3.0-EYFP) or a control fluorophore (YFP: AAV-hSyn-EYFP), and bilateral optical ferrules dorsal to the cSN. Following recovery, all rats were trained to nose poke for a food reward, exposed to the to-be-conditioned cues, then given 8 sessions of Pavlovian fear discrimination. Discrimination consisted of three auditory cues, each associated with a unique foot shock probability: danger, p = 1.00; uncertainty, p = 0.25; and safety, p = 0.00. eNpHR and YFP rats achieved discrimination during these eight sessions: demonstrating high fear to danger, intermediate fear to uncertainty and low fear to safety. The remaining 10 sessions were broken down into 5, 2-session blocks. All rats were habituated to the optogenetic cables in the first 2-session block. For one group of rats [(eNpHR (n = 3), YFP (n = 4)], the next 8 sessions consisted of 2-session blocks of CUE illumination, NO illumination, ITI illumination and NO illumination. During CUE illumination sessions, 12.5 or 25 mW - 532 nm light was delivered bilaterally for the entirety of the 10-s cues. During ITI illumination sessions, light was delivered for 10-s ITI periods between cue trials. The NO illumination sessions provided measures of pre- and post-illumination fear behavior. A second group of rats [(eNpHR (n=6), YFP (n=6)] received the exact same procedure, only ITI stimulation was given first to counterbalance for potential order effects of CUE/ITI light stimulation. Optogenetic inhibition of the cSN during the cue inflated fear to danger, uncertainty and safety. That is, eNpHR - but not YFP - rats receiving CUE illumination showed inflated fear to all cues. ITI illumination had no effect on nose poking in either eNpHR or YFP rats, demonstrating that cSN optogenetic inhibition did not simply suppress nose poking. The cSN then normally acts to moderate overall fear levels, ensuring they are appropriate to the level of

threat. The results uncover a novel node in the fear circuit, and a possible contributor to dysfunction in psychiatric disorders of stress and anxiety that are characterized by excessive fear.

Disclosures: K.M. Wright: None. **S. Cieslewski:** None. **M.A. McDannald:** None.

Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 414.07/AAA10

Topic: G.01. Appetitive and Aversive Learning

Support: VA Merit Award 1I01BX001374 to MAW

Title: Cholinergic regulation of individual differences in fear extinction in rats

Authors: *D. M. KELLIS[1], K. F. KAIGLER[1,2], E. J. WITHERSPOON[1], J. R. FADEL[1], M. A. WILSON[1,2]
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Abstract: It has been demonstrated previously that outbred Long-Evans rats display individual differences in the ability to extinguish fear memories, an observation that mirrors human individual differences in resilience to traumatic stress and the development of post-traumatic stress disorder. Due to the cholinergic system's involvement in attending to environmental cues and heavy innervation to brain regions involved in conditioned fear behaviors in rodents, such as freezing and ultrasonic vocalizations (USVs), the role of cholinergic neurotransmission in cued fear extinction was investigated via numerous methods. We found that rats showing resistance to extinction in freezing behavior also vocalized more during fear acquisition and extinction trials. We administered the muscarinic cholinergic antagonist scopolamine (1 mg/kg, i.p.) before extinction learning and extinction recall to investigate the role of muscarinic receptors in individual differences in extinction learning and recall. Results suggested that muscarinic receptor activation was involved in some of the individual differences in freezing behavior and USVs during extinction, but that freezing and USVs were differentially regulated by muscarinic receptor activation. Further, we investigated plasma and brain acetylcholinesterase (ACHE) in relation to freezing behavior during fear conditioning and extinction. ACHE levels in plasma and the basolateral amygdala (BLA) assessed after extinction recall were negatively correlated with freezing behavior during fear extinction, such that extinction resistant rats showed lower ACHE levels compared to rats with normal extinction patterns; current studies are investigating if ACHE may serve as a biomarker for the extinction resistant phenotype. Finally, we utilized *in-vivo* microdialysis to examine acetylcholine (ACh) and glutamate (GLU) release in the BLA in relation to freezing behavior during fear recall and extinction. Preliminary results show increased

ACh efflux in the BLA during cue-conditioned fear recall and extinction learning. Overall, our data indicate that cholinergic neurotransmission plays a role in individual differences in cued fear extinction and may include differences in neurotransmitter release, degradation, and/or muscarinic receptor binding.

Disclosures: D.M. Kellis: None. **K.F. Kaigler:** None. **E.J. Witherspoon:** None. **J.R. Fadel:** None. **M.A. Wilson:** None.

Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 414.08/AAA11

Topic: G.01. Appetitive and Aversive Learning

Support: Vassar College Start Up Operating Funds
Undergraduate Research Summer Institute
Morton and Ruth Berger Foundation

Title: Chronic alcohol intensifies fear memory generalization: A role for the medial prefrontal cortex

Authors: *M. SCARLATA, S. LEE, S. KANDIGIAN, K. LAWSON, I. SOLER, A. NG, J. BEZEK, H. BERGSTROM
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Abstract: Post-traumatic stress disorder (PTSD) and alcohol use disorder (AUD) often co-occur. Drinking tends to increase following exposure to a traumatic event and has been associated with more severe trauma-related symptoms. This raises the question of whether alcohol changes how traumatic fear memories are expressed. The objective of this study is to determine the neurobehavioral impact of chronic alcohol (ethanol; EtOH) on the retrieval of fear conditioning. Male adult C57BL/6N mice underwent auditory cued fear conditioning and were then administered 2.5 g/kg EtOH (i.p.) once daily over 5 days. Following three EtOH-free days, the fear memory was reactivated using either the “target” conditioned stimulus (CS; 5-kHz) or a “non-target” novel tone frequency (3-kHz) to test cued discrimination and generalization. Results revealed greater freezing behavior in response to the novel tone in the EtOH group, indicating enhanced cued fear memory generalization. Importantly, there were no effects of alcohol on the retrieval of the “target” CS, a memory of weaker strength, or contextual fear memory, and the effects on generalization reversed with the passage of time. Further, no effects were uncovered with a lower EtOH dose (1.0 g/kg). Together, these results identify a highly selective effect of chronic alcohol on cued fear memory generalization. Next, using immunohistochemistry we visualized the activity-regulated cytoskeletal arg/Arc3.1 (*Arc*) protein in the medial prefrontal

cortex (mPFC) following retrieval of the novel stimulus (generalization test). Results revealed a significant reduction in *Arc* expression in the shallow layers of the infralimbic cortex (IL) that was associated with fear memory generalization. No effects were observed in IL deep or prelimbic cortical layers. Considering a role for the IL in inhibitory control over conditioned fear expression, these data lead to a model whereby EtOH-induced hypoactivity of the IL drives fear memory overgeneralization. Chemogenetic manipulations (excitatory DREADDs) are currently underway to determine a causal role for EtOH-induced mPFC neuroadaptation in fear memory overgeneralization.

Disclosures: M. Scarlata: None. **S. Lee:** None. **S. Kandigian:** None. **K. Lawson:** None. **I. Soler:** None. **A. Ng:** None. **J. Bezek:** None. **H. Bergstrom:** None.

Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 414.09/AAA12

Topic: G.01. Appetitive and Aversive Learning

Title: Increased feedforward inhibition to engram neurons required for precision of fear memory

Authors: *S. LEE, H.-Y. LEE, J.-H. KIM
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Abstract: Fear memory would be stored mainly in a specific population of neurons (engram neurons) that had been activated during fear learning. Excitatory inputs to fear engram neurons in the lateral amygdala is enhanced after fear learning, but it remains unknown whether or not inhibitory microcircuits for engram neurons are also altered. Here, we report that feedforward inhibition to fear engram neurons in the lateral amygdala was significantly increased after fear conditioning. Importantly, the increase in feedforward inhibition correlates with precision for expression of fear memory. The feedforward inhibition onto fear engram neurons in the lateral amygdala is likely to be a critical factor for keeping appropriate fidelity of fear memory.

Disclosures: S. Lee: None. **H. Lee:** None. **J. Kim:** None.

Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 414.10/AAA13

Topic: G.01. Appetitive and Aversive Learning

Support: National Natural Science Foundation of China (No. 81571888)
Foundational and Cutting-edge Research Plan of Chongqing (cstc2014jcyjA10072)

Title: Involvement of adenosine A_{2A} receptors in enhanced fear learning following traumatic brain injury

Authors: Y.-L. NING[1], N. YANG[1], X. CHEN[1], F. LUO[1], Y.-W. XU[1], Y.-L. JIANG[1], H.-K. TIAN[2], *Y. ZHOU[1]
[1]Res. Inst. Surg and Daping Hospital, Army Med. Univ., Chongqing City, China; [2]Dept. of Trauma and Microsurgery, the PLA No. 324 Hosp., Chongqing, China

Abstract: Traumatic brain injury (TBI) usually causes lasting neuropsychological abnormalities such as cognitive and emotional dysfunction. However, the regulatory mechanisms are poorly understood and there is no treatment available. Adenosine A_{2A} ($A_{2A}R$) is one of the key modulators of neuropsychical function and brain injury in the central nervous system (CNS). We have demonstrated previously that inactivation of $A_{2A}R$ exerted neuroprotective effects against TBI-induced spatial memory impairment. Here we investigated whether TBI causes augmented fear and the roles of adenosine $A_{2A}R$ in contextual fear learning following TBI. Moderate controlled cortical impact (CCI) was used to perform TBI in adult male c57BL/6 mice. At 12h post TBI, mice were submitted to a contextual fear conditioning (FC) paradigm (n=12 per group). Fear learning was assessed at 24h post FC by quantifying freezing behavior. The results showed that the percentage of freezing time in TBI+FC group was significantly higher than that in FC group, indicating an enhanced contextual fear following TBI. Meanwhile, the immunofluorescent staining showed that $A_{2A}R$ level increased remarkably in CA1, CA3 and DG regions of dorsal hippocampus (dHP), as well as the basolateral amygdala (BLA). The effect of dHP- or BLA- specific $A_{2A}R$ deletion by local injection of AAV5-Cre into floxed-$A_{2A}R$ mice was also examined. The results demonstrated that both dHP and BLA $A_{2A}R$ deletion attenuated contextual fear conditioning, comparing with TBI+FC mice. Together, this study provides the evidence that TBI facilitates contextual fear conditioning and that inactivation of $A_{2A}R$ in dHP or BLA inhibits fear memory following TBI, suggesting a therapeutic rationale of brain region-specific $A_{2A}R$ for the treatment of TBI-induced cognitive and emotional impairment. Since these injuries enhances the risk for the development of neuropsychiatric disorders including post-traumatic stress disorder (PTSD), $A_{2A}R$ may also be a promising therapeutic target for PTSD.

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Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 414.11/AAA14

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant DA034010

Title: Roles for the nucleus accumbens core, and its Gad1 subpopulation, in adaptive scaling of fear

Authors: *M. H. RAY, A. N. RUSS, E. K. ENABULELE, E. LEE, M. A. MCDANNALD
Psychology, Boston Col., Chestnut Hill, MA

Abstract: Environmental threats exist on a continuum from unlikely to certain. Adaptive behavior requires fear to scale to the level of threat. Such scaling would be maximally adaptive if observed rapidly following threatening encounters. In two experiments, we examined roles for the nucleus accumbens core (NAcc), and its Gad1 subpopulation, in adaptive scaling of fear. In experiment 1, male Long Evans rats received bilateral neurotoxic NAcc lesions, deleting all neuron types, or sham surgery, leaving NAcc intact. In experiment 2, male and female Gad1-cre rats received bilateral NAcc viral infusions of pAAV-flex-taCasp3-TEVp (Casp3), selectively deleting NAcc Gad1 neuron types, or AAV-EF1a-DIO-EYFP (YFP), leaving Gad1 neurons intact. Following recovery, rats received 16 days of fear discrimination in which three auditory cues were associated with unique foot shock probabilities: danger (p = 1.00), uncertainty (p = 0.25), and safety (p = 0.00). Fear was measured over the entire 10-s cue, or in 100-ms intervals during the first 2-s, using suppression of rewarded nose poking. In experiment 1, sham rats acquired excellent fear discrimination, showing high fear to danger, intermediate fear to uncertainty, and low fear to safety. NAcc lesioned rats failed to show the same degree of discrimination, exhibiting decreased fear to danger and increased fear to safety. This pattern was most evident when assessing fear in the first 2-s of cue onset. While shams showed evidence of discrimination in ~600 ms, such discrimination was not observed in NAcc rats until nearly 2-s. The NAcc is then necessary for adaptive scaling of fear on multiple timescales. In experiment 2, YFP and Casp3 rats acquired excellent fear discrimination. In YFP rats, discrimination emerged rapidly and adaptively scaled to shock probability. Casp3 rats demonstrated a similar rapid discrimination, however, fear was heightened to all three cues. The results demonstrate an integral role for the NAcc in adaptive scaling of fear. Further, the results suggest that distinct NAcc neuron types may underlie specific components of adaptive scaling. In particular, NAcc GAD1 neurons may be tuned to onset of threatening stimuli, permitting fear to rapidly scale to the level of threat.

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Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 414.12/AAA15

Topic: G.01. Appetitive and Aversive Learning

Support: R21 MH104018
R01 MH052619
R01 MH065702

Title: Network dynamics of PSD95 and nNOS interactions in acquisition of conditioned fear

Authors: *E. T. DUSTRUDE[1,2], L. LI[2,3], S. FITZ[1,2], A. I. MOLOSH[1,2], Y. Y. LAI[5,6,4], A. SHEKHAR[4,1,6,2,3]
[1]Dept. of Psychiatry, [2]Stark Neurosci. Res. Institue, [3]Med. Neurosci. Program, [4]Indiana Clin. and Translational Sci. Inst., Indiana Univ. Sch. of Med., Indianapolis, IN; [5]Dept. of Psychological and Brain Sci., Indiana Univ. Bloomington, Bloomington, IN; [6]Anagin Inc., Indianapolis, IN

Abstract: Formation of fear memories involves stimulation of N-methyl-D-aspartic acid receptors (NMDARs) that results in greater interaction between the post-synaptic density 95 (PSD95) and nitric oxide synthase (nNOS) which increases production of nitric oxide (NO) and facilitates synaptic plasticity and fear conditioning. We have demonstrated that disrupting this protein-protein interaction within the basolateral amygdala (BLA) reduces conditioned fear, but little is known about the dynamics of PSD95 and nNOS interactions within conditioned fear network or the effect of this protein-protein interaction on neural network properties during acquisition of conditioned fear. Utilizing co-immunoprecipitation, electrophysiology, behavioral paradigms, and RNA sequencing, we investigated the dynamics of as well as the synaptic and molecular effects of PSD95/nNOS binding in various brain regions critical for conditioned fear, utilizing a small molecule disruptor of PSD95-nNOS interaction, ZL006. Immediately following fear conditioning, association of PSD95 and nNOS is enhanced in the basolateral amygdala (BLA) and ventral hippocampus (vHP) but not in the medial prefrontal cortex (mPFC). Systemic and intra-BLA treatment with ZL006 significantly attenuated cue-induced fear consolidation. Utilizing whole tissue RNAseq, we found that fear conditioning altered expression of 81 genes in the BLA, with the only a small group of gene changes reversed by ZL006 treatment. Further studies are underway to elucidate the molecular and synaptic mechanisms underlying the role of PSD95-nNOS-NO in conditioned fear in both the BLA and vHP. These data suggest that PSD95-nNOS interaction at specific sites within the fear network is a key step in the acquisition of

conditioned fears and disrupting this protein-protein interaction is a viable strategy for treating fear related disorders.

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Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 414.13/AAA16

Topic: G.01. Appetitive and Aversive Learning

Support: US Army W81XWH-17-1-0001

Title: Chronic intermittent nicotine exposure attenuates conditioned fear: A therapeutic model of the nicotine patch

Authors: *W. A. CARLEZON, Jr[1], I. HOLLOWAY[2], M. A. ROBBLE[2], E. MELONI[2], R. DESAI[2]
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Abstract: The use of nicotine products such as cigarettes and smokeless (chewing) tobacco in the military is highly prevalent, but it is not known how nicotine affects vulnerability to stress and stress-related conditions including post-traumatic stress disorder (PTSD). Previous work has demonstrated that nicotine can relieve stress while also enhancing cognitive function. These two broad actions may have opposing effects on vulnerability to stress-related illness such as PTSD, which is thought to involve learning and memory components. Preliminary studies from our lab suggest that intravenous self-administration (IVSA) of nicotine in rats can reduce the impact of a traumatic event, as reflected by decreased responsivity to a context previously associated with footshock in the fear-potentiated startle (FPS) paradigm. These findings suggest that nicotine use in soldiers might reduce pathological responses that occur in contexts that have similarities with those in which a trauma was experienced, whether in combat settings or after returning home. Here we examined if the putative beneficial effects of nicotine IVSA on contextual fear learning are retained when the drug is given by a different (safer) route of administration, using a model of the nicotine patch. Male Long-Evans rats were implanted with a subcutaneous iPRECIO$_{TM}$ programmable minipump. After 7 days of recovery, rats received 1, 10, or 21-day exposure to either 0.3 mg/kg (low) or 1.0 mg/kg (high) of nicotine or saline for a 12-hr on/12-hr off period;

doses were based on previous work showing that rats self-administer ~1.0 mg/kg in 12-hr sessions. Following exposure, rats were trained in the FPS paradigm, which provides an index trauma and enables quantification of exaggerated startle response, a characteristic observed in humans with PTSD. Context-potentiated startle (CPS) and FPS were determined in each subject in a test session 10 days after training. We found that a 10-day exposure to the high dose of nicotine leads to decreased responsiveness to trauma (footshock)-associated cues and contexts, similar to findings after nicotine IVSA. Notably, a 1-day exposure produces the opposite effect on CPS, suggesting that chronic exposure is an important factor for the potential therapeutic benefits of nicotine. We are currently evaluating the effects on fear conditioning when nicotine exposure is maintained during the 10 days between training and testing. Our findings suggest that passive administration of nicotine can impact physiological responses trauma-associated stimuli and contexts, raising the possibility that medical application of nicotine could reduce the psychological impact of trauma.

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Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant R15 MH085280-01
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Ramapo College Foundation Grant to CGR.

Title: Cannabinoid and chronic mild stress modulation of hippocampal fear conditioning in adolescent female rats

Authors: *L. J. BARON[1], C. G. REICH[2]
[1]Ramapo Col., Paramus, NJ; [2]SSHS/Psychology, Ramapo Col. of New Jersey, Mahwah, NJ

Abstract: Chronic-Mild-Unpredictable-Stress (CMUS), an animal model of depression, selectively enhances hippocampal-dependent trace fear conditioning in male adolescent rats. The CMUS-induced fear enhancement is relieved by administration of a cannabinoid type 1 (CB1) receptor agonist (Reich et al., 2013). CMUS also decreases CB1 in male rats while increasing receptor levels in females. Furthermore, hippocampal CB1 levels are lower in control female rats compared to male rats (Reich et al., 2009). These findings suggest that hippocampal CB1 receptors are organized to differentially respond to stress depending on sex. However, it is unclear how CB1 sex differences translate behaviorally; particularly there is a lack of female

data. We therefore investigated the effects of CMUS and CB1 activation on the acquisition, recall, and extinction of both hippocampal-dependent trace and contextual fear conditioning. Trace (auditory-cued) or contextual fear conditioning were performed in female adolescent (40-60 days old) Sprague-Dawley rats. The CB1 agonist, ACEA (1 µM), was administered to both stress and non-stress rats prior to acquisition or prior to a 24 hr recall/extinction session. This was done in a factorial fashion with sample sizes were greater than n=6, the size needed for 80% statistical power. Our findings indicate that the CMUS enhances fear behavior in trace (cued) but not contextual paradigms. This is similar to our previous results in male rats. CMUS did not affect extinction; however, exogenous CB1 activation prior to acquisition impaired both short-term and long-term extinction in non-stress animals. In stressed females, CB1 activation prior to acquisition and memory recall decreased trace fear memory recall compared to controls; although ACEA impaired short and long-term extinction in stressed rats. Our results demonstrate that chronic stress can enhance trace fear conditioning (a form of episodic memory) in both male and female rats. However, the observed CB1-mediated extinction impairments in both non-stressed and stressed females (CB1 activation in males reliably facilitates fear extinction) reveal how sex differences in CB1 signalling manifests behaviorally.
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Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 414.15/AAA18

Topic: G.01. Appetitive and Aversive Learning

Support:
NIH Grant MH048404
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ARCS scholar award

Title: A novel rodent model to assess resistance to risk of punishment during reward seeking suggests dopamine related sex differences

Authors: *D. S. JACOBS[1], M. C. ALLEN[2], B. MOGHADDAM[3]
[1]Oregon Hlth. and Sci. Univ. Sch. of Med., Portland, OR; [2]Oregon Hlth. and Sci. Univ., Portland, OR; [3]Behavioral Neurosci., OHSU, Portland, OR

Abstract: The ability to adapt to the complex relationship between reward and risk of punishment is critical for optimal action selection. While there have been some recent advances

in assessing this relationship (e.g. Park & Moghaddam, 2017) the neuronal basis of how punishment risk probability influences reward processing remains poorly understood. Here we designed and characterized a novel behavioral model to assess how varying probabilities of punishment modifies reward seeking actions in rats. Male and female rats were trained to perform instrumental responses in a chained seek-take procedure for food reinforcement. After behavioral stability was achieved in the seek-take stage, a punishment contingency was introduced during the seek link where reward seeking responses were punished by mild foot shock with increasing probability. Both male and female rats reliably decreased total responding and increased their latency to perform the punished (reward seeking) response as the probability of punishment increased. Results indicate that males were more resistant to punishment than females. Both non-punished (reward taking) responding and reward retrieval were unaffected, suggesting that changes in reward seeking were not due to satiety or the general suppression of behavior. Furthermore, task performance remained stable over experimental sessions, indicating robust and reliable patterns of behavioral reinforcement and suppression for males and females. Pharmacological D2 receptor manipulation generally decreased resistance to punishment, though females qualitatively appear to be less sensitive to D2 agonism-induced changes on punishment resistance than males. Circuit-level manipulation and electrophysiological studies that assess the mechanistic underpinnings of these observations are currently underway.

Disclosures: D.S. Jacobs: None. **M.C. Allen:** None. **B. Moghaddam:** None.

Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 414.16/AAA19

Topic: G.01. Appetitive and Aversive Learning

Title: Deletion of the prion-like domain of CPEB2 impairs long-term memory of one-trial inhibitory avoidance

Authors: *M. L. HUFF[1], B. A. EBNER[3,5], K. SI[2,4]
[1]Si Lab., [2]Stowers Inst. for Med. Res., Kansas City, MO; [3]Univ. of Kansas Med. Ctr., Kansas City, KS; [4]Dept. of Mol. & Integrative Physiol., Univ. of Kansas Med. Ctr., Kansas City, MO; [5]Stowers Inst. for Med. Reserach, Kansas City, MO

Abstract: Memories can last for hours or even years in humans, yet the molecular basis of this memory stabilization is still not fully understood. Previous work in our lab has revealed that the neuronal RNA binding protein cytoplasmic polyadenylation element binding protein (CPEB) is required specifically for the persistence of memory in a drosophila and this stability relies on self-sustaining amyloidogenic (prion-like) properties. Structural maintenance is also proposed to lead to memory stabilization in mammals and the CPEB2 isoform has been linked to synaptic

plasticity and long-term memory. Therefore, we hypothesized that removal of CEPB2 in a mouse model would impair long-term memory and synaptic plasticity. Deletion of the first exon and the prion-like domain led to viable homozygous CPEB2 knockouts (KO) with no detectable physical defects. Tissue isolated from KOs show no detectable full length CPEB2 as either a monomer or oligomer. To assess the role of CPEB2 in learning and memory, wildtype (B6/J) and KO littermates were trained on one-trial inhibitory avoidance. On training day, mice received a single, inescapable footshock (0.5mA, 1s) upon fully crossing from the illuminated to the darkened compartment. Retention was tested 48h later and latency to cross from the illuminated to darkened "shock" compartment was measured. Long-term potentiation was measured in hippocampal slice preparations from animals at 1 and 6-months of age following 4 trains of 100 Hz high frequency stimulation. An open field test (30 min session) was used to examine any general anxiety deficits or locomotor changes. Sessions were recorded and analyzed using EthoVision software. During inhibitory avoidance testing, KO animals show reduced retention latencies compared to matched wild type when trained at 3 or 6 months of age. This impairment persisted when retention was tested up to 2 months after initial training. Long-term potentiation and paired-pulse facilitation was impaired in KO animals at one-month but not 6 months of age. KO animals travel significantly less during a 30 min open field test but show no preference in center vs. border regions. Together, these findings suggest that lack of the CPEB2 prion-like domain disrupts normal learning and memory. Future work will investigate the role of CPEB2 in short term memory across development as well as long term memory at earlier timepoints (i.e. 1 month) when synaptic potentiation is impaired.

Disclosures: M.L. Huff: None. **B.A. Ebner:** None. **K. Si:** None.

Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

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Program #/Poster #: 414.17/AAA20

Topic: G.01. Appetitive and Aversive Learning

Support: CNPQ/Universal 14/2014

Title: Shifting from fear to safety through reconsolidation memory updating: Deconditioning as a novel approach to eliminate traumatic memories

Authors: *L. O. ALVARES[1], B. POPIK[2]
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Abstract: Traumatic memories are at the heart of psychiatric disorders such as post-traumatic stress disorder (PTSD) and lead to severe economic and social burden. Pharmacological or psychological treatments such as exposure therapy have limited efficacy and are transient,

because the traumatic memories reemerge with the passage of time. Here, we show a new approach that eliminate aversive memories in an effective and permanent way, transforming them into an innocuous memory. This method consists of "deconditioning" rats which have previously been trained to associate a sound with a strong foot-shock where the shock information is updated and replaced by an extremely low aversive/neutral stimulus during the plastic state induced by memory retrieval, resulting in a permanent reduction of fear responses in the presence of the sound. Our results indicate that deconditioning-update is effective in eliminating up to 80% of fear responses. Moreover, such effects are long-lasting, and insensitive to renewal and spontaneous recovery, suggesting a permanent update in fear memory. Remarkably, this new strategy overcame important boundary conditions, as it was effective in eliminating either a remote or a very strong traumatic memory. Furthermore, the same beneficial effect was also found in other type of fear-related memories (contextual memory and inhibitory avoidance.

Disclosures: L.O. Alvares: None. **B. Popik:** None.

Poster

414. Fear and Aversive Learning and Memory: Modulation

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Program #/Poster #: 414.18/AAA21

Topic: G.01. Appetitive and Aversive Learning

Support: NIMH K01MH105731
NARSAD Young Investigator Award
Undergraduate Research Fellowship

Title: Infralimbic inputs to the basal forebrain regulate consolidation of extinction

Authors: *S. LEI[1], M. B. HARNOIS[2], E. LIKHTIK[3]
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Abstract: The medial prefrontal cortex (mPFC) is an important region for regulating emotion, and its infralimbic (IL) subregion plays a crucial role in consolidation of fear extinction learning. The IL suppresses defensive responding after extinction by inhibiting amygdala output. However, it is not known whether this outcome is achieved only via direct IL-amygdala connectivity or with additional, indirect modulation of the amygdala by the IL. The basal forebrain (BF) may be one node on the indirect pathway from the IL-to-amygdala that plays a role in extinction. The BF receives IL input, sends GABAergic and cholinergic afferents to the amygdala, and is known to regulate attention to task relevant cues. We tested the contribution of IL-to-BF projections to consolidation of extinction by optogenetically inhibiting IL inputs to the

BF during Extinction Training and recording physiology across the IL-BF-basolateral amygdala (BLA) circuit. Male mice (C57B6J) were injected with either the inhibitory opsin archaerhodopsin (eArch3.0) or with eYFP into the IL, and optic fibers were placed above the BF to inhibit IL terminals. Mice underwent fear conditioning and then Extinction Training, when IL inputs to the BF were either inhibited (eArch3.0, n=13) or left undisturbed (eYFP, n=9). Extinction Recall was tested 24 hours later without the presence of light. Whereas same day acquisition of extinction in the eArch group was undisturbed, eArch animals showed significantly impaired recall the next day, suggesting that IL inputs to the BF during training are important for consolidation of extinction learning. Immunohistochemical analyses of cFos across the BF revealed that mice in the eArch group showed significantly more neural activity during Extinction Recall than the eYFP group. Quantification of cFos in choline acetyltransferase (ChAT+) expressing cells of the BF show that increased freezing during recall significantly correlates with cholinergic (ChAT+) cell activity in the ventral pallidum (VP) of the BF. Moreover, analyses of cFos in parvalbumin expressing (PV+) GABAergic cells and ChAT+ cholinergic cells after recall suggest that activity in these two cell groups is reciprocally modulated in the VP. Impaired Extinction Recall is associated with higher activation of cholinergic cells and dampened activity in PV+ cells, whereas successful Extinction Recall shows increased activity of PV+ cells and a concomitant decrease in cholinergic activation. We suggest that the IL projections we recently demonstrated in PV+ cells of the BF contribute to extinction consolidation by inhibiting cholinergic activity that is normally associated with recall of fearful cues.

Disclosures: S. Lei: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Undergraduate Research Initiative. **M.B. Harnois:** None. **E. Likhtik:** None.

Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 414.19/AAA22

Topic: G.01. Appetitive and Aversive Learning

Support: NIMH K01MH105731
NARSAD Young Investigator Award
Undergraduate Research Fellowship
Jonas E. Salk Scholarship

Title: Differential connectivity between the infralimbic and prelimbic cortices with the basal forebrain - amygdala circuit

Authors: *R. ZHANG[1], M. LABKOVICH[1], N. BURNEY[1], D. GOLDER[1], E. LIKHTIK[1,2]
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Abstract: To develop targeted treatments for anxiety disorders, we need a comprehensive understanding of the connectivity between regions involved in aversive memory formation and extinction in both sexes. Direct projections from the medial prefrontal cortex (mPFC) to the basolateral amygdala (BLA) are important for modulating aversive and extinction learning. However, we know much less about the indirect pathways from the mPFC to the BLA that may also be involved in modulating behavior. Here, we examine how the prelimbic (PL) and infralimbic (IL) sub-regions of the mPFC connect with the BLA via the basal forebrain (BF), a structure that sends cholinergic and GABAergic projections to the BLA, and modulates the signal-to-noise ratio in the BLA during aversive learning. To investigate this pathway, we injected retrograde tracers (Retrobeads, Lumafluor and Cholera Toxin B) in the BLA, and an AAV anterograde synaptic labeling vector (AAV8.2-hEF1α-DIO-synaptophysin-mCherry, Rachel Neve, MIT Viral Core) in either the IL or PL of the mPFC in male and female mice (C57BL6J/129SvEv F1 generation). We then examined the overlap in synaptophysin-labeled axon terminals and retrograde- labeled cell bodies across three nuclei of the BF: the ventral pallidum, the substantia innominata, and the horizontal limb of the diagonal band. In order to identify BF cholinergic and GABAergic cells, we counterstained the tissue with choline acetyl transferase (ChAT+), parvalbumin (PV+), and somatostatin (SOM+). Our analyses confirm previous findings in the rat, showing that whereas cholinergic and PV+ cells project to the BLA from all examined nuclei in the BF, projections from SOM+ cells are very rare. We then show that the IL contacts a significantly larger proportion of PV+ than cholinergic BLA projectors, whereas the PL contacts more cholinergic than PV+ BLA projectors. Our preliminary analyses also show that this dichotomy is even more pronounced in female mice. These findings suggest that the PL may promote BLA activation during fear expression by driving BF cholinergic inputs to the BLA. Conversely, the IL may contribute to consolidation of extinction learning by activating BF PV+ cells that would disfacilitate cholinergic input to the BLA on the one hand, and inhibit cells in the BLA directly on the other.

Disclosures: R. Zhang: None. **M. Labkovich:** None. **N. Burney:** None. **D. Golder:** None. **E. Likhtik:** None.

Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 414.20/AAA23

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant 5R25NS080686-08
NIMH R56MH111700
PSC-CUNY Research Award, City University of New York

Title: Developing a safety learning paradigm as a therapeutic tool for high anxiety

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[1]Chem. Dept., [2]Biol. Dept., Hunter Col., New York, NY; [4]Biol. Dept., [3]CUNY Grad. Ctr., New York, NY

Abstract: Post-Traumatic Stress Disorder (PTSD) is characterized by generalized fear, such as indiscriminate autonomic and defensive behavioral responses to cues that are unrelated to threat. Extinction has been the most successful behavioral therapy for these disorders. However, extinction is cue- and context- specific, and for a significant proportion of patients this approach doesn't alleviate generalized fear. Therefore, an expanded repertoire of behavioral therapeutic approaches is desirable to address the needs of a larger patient population. Previous work in wild type C57BL6J mice has shown that Safety Conditioning, where a conditioned stimulus (tone CS) explicitly signals the absence of an aversive unconditioned stimulus (shock US), also known as "Unpaired" (US//CS_T) training, the CS can become a conditioned inhibitor of fear in and outside of the training context. However, we show that in a high anxiety strain of mice (129SvEv, Taconic) the "Unpaired" Safety Conditioning doesn't result in conditioned inhibition of fear to the CS. To improve safety learning in high anxiety mice, we enhanced Safety Conditioning in two ways: (1) via increasing the salience of the CS ("Salient CS") where the CS signaling the absence of shock consisted of a tone and a houselight (CS_{TL}//US), and (2) by making the CS a "Feature Positive" stimulus that is paired with the onset of a neutral house light, whereas the US remained unpaired (US//CS_T-L). A fourth group of mice underwent paired fear conditioning training ("Paired", CS_T-US). We then assessed the degree of defensive freezing during CS presentation in the training context, and whether CS meaning can transfer to a new context by presenting the CS in a novel open field. We also tested whether the initial Safety or Fear Conditioning affects acquisition of discrimination learning on a new differential fear conditioning task learned 7 days later. Initial analyses show that Enhanced Safety Conditioning increases percent center time in the open field and improves discrimination learning in high anxiety mice. We propose that Enhanced Safety Conditioning increases attention to the "Salient" and the "Feature Positive" CS during training making it a better predictor of safety for high anxiety mice. Likewise, increased attention allocated to the "Salient" and the "Feature Positive" CS may teach animals to pay attention to "Feature Negative" (or unpaired) stimuli during subsequent learning, leading to better discrimination. These findings point to Enhanced Safety Conditioning as a potentially useful therapeutic approach to improve generalized anxiety and adaptive learning in patients with PTSD.

Disclosures: I. Nahmoud: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); P-ENDURE & the UnderGraduate Research Initiative (UGRI) at Hunter College, CUNY. **H. Cho:** None. **S. Talbot:** None. **T. Dennis-Tiwary:** None. **E. Likhtik:** A. Employment/Salary (full or part-time):; Full-time.

Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 414.21/AAA24

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant R01MH065961
Brain & Behavior Research Foundation
McKnight Endowment for Neuroscience

Title: Inhibition of protein synthesis in the dorsal hippocampus prevents reconsolidation of a covertly retrieved fear memory

Authors: *R. RESSLER, T. D. GOODE, S. KIM, S. MAREN
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Abstract: Memories enter a labile state after retrieval, and administration of protein synthesis inhibitors interferes with the reconsolidation of reactivated memories. After fear conditioning, infusion of protein synthesis inhibitors into the amygdala impairs the reconsolidation of fear to an auditory conditioned stimulus. However, it has been reported that indirectly reactivated memories (e.g., memories to a second-order conditioned stimulus, CS) are not sensitive to protein synthesis inhibition in the amygdala. Because the hippocampus is thought to play a role in the S-S associations that underlie higher order conditioning phenomena, we explored whether an indirectly retrieved context memory would be sensitive to protein synthesis inhibition in the hippocampus. It has previously been reported that backward conditioning, a procedure in which the unconditioned stimulus (US) directly precedes the CS, is mediated by contextual fear association (Chang et al. 2003). We tested whether the extinction of fear to the conditioning context selectively attenuates fear to a backward--conditioned CS. Accordingly, rats were conditioned using either forward (FW; CS-US) or backward (BW; US-CS) trials (context A). The following day animals underwent context extinction (context A) or novel context exposure (context B). Animals were then tested for fear to the CS in a third context (C). Results revealed that extinction of the conditioning context selectively reduced freezing in BW-conditioned but not FW-conditioned animals. In Exp. 2, rats were implanted with bilateral cannulae aimed at the DH. After recovery, FW or BW conditioning was conducted as in Exp. 1. Twenty-four hrs later, all rats received a single CS exposure in a familiar context (B), which we hypothesized would reactivate the memory of the conditioning context in BW-conditioned but not FW-conditioned rats. To target reconsolidation of the memory retrieved by the CS, we infused the protein synthesis inhibitor rapamycin (or vehicle) into the DH immediately followed the reactivation session. Two days later, we assessed freezing behavior in the original conditioning context (A)

during a 20-min test. In support of our hypothesis, intra-DH rapamycin infusion reduced contextual freezing in only rats conditioned with the BW CS. These results provide evidence that the backward, but not forward, 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. **S. Kim:** None. **S. Maren:** None.

Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 414.22/AAA25

Topic: G.01. Appetitive and Aversive Learning

Support: R01MH065961
F31MH107113
McKnight Foundation Memory and Cognitive Disorders Award
Brain & Behavior Research Foundation NARSAD Distinguished Investigator Grant

Title: NMDA receptors in the BNST are necessary for learning to fear ambiguous threats

Authors: *T. D. GOODE, R. RESSLER, C. EVEMY, K. FRENCH, S. MAREN
Inst. for Neurosci. and Dept. of Psychology, Texas A&M Univ., College Station, TX

Abstract: We have recently shown that the bed nucleus of the stria terminalis (BNST) is critical for the expression of conditioned freezing to ambiguous threat signals in rats. In particular, BNST inactivation impairs freezing to a conditioned stimulus (CS) arranged to follow an aversive unconditioned stimulus (US) during conditioning (i.e., backward conditioning), but does not impair freezing to a CS that precedes the US (i.e., forward conditioning). Here we explored whether *N*-methyl-D-aspartate (NMDA) receptors in the BNST and the amygdala are necessary for backward fear conditioning. We hypothesized that NMDA receptor activity in the BNST is required for backward conditioning, whereas central amygdala (CeA) NMDA receptors are important for forward fear conditioning; basolateral amygdala (BLA) NMDA receptors were expected to mediate fear conditioning in both cases. Adult male and female Long-Evans rats were implanted with cannula aimed at the CeA, BLA, or BNST. Intracranial microinfusions (0.275 μl/hemisphere) of APV (10 μg/μl) or vehicle occurred immediately before fear conditioning in which an auditory CS (10 sec, 2 kHz, 80 dB tone) was presented with an aversive US (2 sec, 1 mA footshock). For half of the rats, the CS immediately preceded the US (forward conditioning), while the other rats experienced the US immediately before the CS (backward conditioning). Training consisted of twelve forward or backward trials in a distinct context.

Defensive immobility (freezing) served as the dependent variable. Forty-eight hrs after training, animals (drug-free) were placed in a novel context and tested to the CS (five trials). Twenty-four hrs later, animals were returned to the training context to assess levels of context fear. Interestingly, APV blocked the acquisition of conditioned freezing to the backward CS when infused into the BNST or CeA (but not BLA), while conditioned freezing to the forward CS was blocked by infusions of APV into CeA and BLA (but not BNST). Additionally, APV infusions in the BNST and BLA completely blocked fear acquisition to the conditioning context, whereas CeA infusions produced a partial impairment in contextual freezing. All animals acquired fear when retrained drug-free and similar effects were observed in males and females in all cases. Overall, these data reveal dissociable roles for the BLA, CeA, and BNST in fear conditioning to ambiguous and certain threats.

Disclosures: T.D. Goode: None. **R. Ressler:** None. **C. Evemy:** None. **K. French:** None. **S. Maren:** None.

Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 414.23/AAA26

Topic: G.01. Appetitive and Aversive Learning

Support: R01MH065961
F31MH112208
McKnight Foundation Memory and Cognitive Disorders Award
Brain & Behavior Research Foundation NARSAD Distinguished Investigator Grant

Title: Propranolol stabilizes shock-induced increases in spike firing in the basolateral amygdala: Implications for the immediate extinction deficit

Authors: *T. F. GIUSTINO[1], M. TOTTY[2], S. MAREN[2]
[2]Psychological and Brain Sci. and Inst. for Neurosci., [1]Texas A&M Univ., College Station, TX

Abstract: Early interventions, such as psychological debriefing and exposure therapy, after trauma exposure aim to reduce the development of trauma- and stressor-related disorders such as posttraumatic stress disorder (PTSD). Our lab and others have shown that when extinction learning occurs soon (i.e., minutes to hours) after fear conditioning that animals and humans show impaired extinction learning, a phenomenon termed the immediate extinction deficit (IED). We have recently shown that both systemic ß-adrenergic blockade with propranolol and local propranolol infusions into the basolateral amygdala (BLA) attenuate the IED. This suggests that stress-induced deficits in extinction learning might operate through noradrenergic signaling in the BLA. As a first step to testing this hypothesis, we recorded single-unit activity in the BLA

immediately after fear conditioning during a post-conditioning window that is associated with the IED, and examined the effects of systemic propranolol on shock-induced changes in BLA activity. Animals were implanted with a 16-channel microelectrode array targeting the BLA and after a 3 min stimulus free baseline period were injected with either vehicle or propranolol (10 mg/kg, i.p.). Twenty min after the injection animals underwent standard auditory fear conditioning procedures as previously described (Fitzgerald et al., 2015) and remained in the chamber for 60 min following the last shock. Twenty-four hours later, animals were placed in a novel context and were tested for fear memory retention. Neural and behavioral data were recorded continuously throughout the sessions. We found that fear conditioning resulted in a rapid and sustained increase in BLA spontaneous firing rates and this corresponded with high levels of post-shock freezing. Interestingly, propranolol treatment reduced both post-shock freezing and the shock-induced increases in BLA spike firing. During the fear recall test, controls animals showed high levels of tone-evoked freezing as well CS-induced increases in BLA spike firing. Prior propranolol treatment reduced both CS-evoked freezing and BLA spike firing. Overall, these data suggest that footshock produces rapid and sustained excitation in amygdala single-unit activity that are dependent, at least in part, on ß -adrenoceptors. Stress-induced increases in BLA noradrenergic activity may be a therapeutic target for trauma- and stressor-related disorders.

Disclosures: M. Totty: None. **S. Maren:** None.

Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

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Program #/Poster #: 414.24/BBB1

Topic: G.01. Appetitive and Aversive Learning

Support: NIH R01 MH065961
McKnight Foundation Memory and Cognitive Disorders Award

Title: Hormonal basis for state-dependent conditioned fear in naturally cycling female rats

Authors: N. WARREN[1], G. M. ACCA[2], B. TSAO[1], A. MATHEW[2], A. PHAN[2], N. CAYARD[1], J. JULIETTE[2], S. MAREN[2], *N. NAGAYA[2]
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Abstract: Women are more susceptible than men to stress- and trauma-related disorders, suggesting that ovarian steroid hormones play a modulatory role in fear and anxiety. Indeed, in both humans and rodents, estrogen and progesterone (PROG) have been shown to influence various aspects of fear learning. We have recently found that allopregnanolone (ALLO), a neuroactive metabolite of PROG, can confer state dependence to contextual fear in adult male

rats when infused into the bed nucleus of the stria terminalis. Given that circulating ALLO levels mirror fluctuations in PROG across the estrous cycle in female rats, we hypothesized that PROG confers state dependence to contextual fear in naturally cycling females. To this end, we performed Pavlovian fear conditioning on gonadally intact, cycling female rats in high (late proestrus) or low (late diestrus) PROG states. Animals were conditioned with 5 tone (2 kHz, 10 s, 80 dB) - footshock (2 s, 1 mA) pairings and tested 3 to 6 days later for context-induced freezing behavior in the conditioning chamber (10 min). Conditioning and testing were timed such that rats were in either the same or different estrous cycle phase in a factorial design. Animals that were conditioned in diestrus and tested in proestrus displayed significantly lower levels of contextual freezing compared to those that were conditioned and tested in the same estrous cycle phase (either diestrus or proestrus), suggesting a state-dependent effect of estrous cycle phase and PROG level on fear learning. This state dependence was asymmetric, insofar as animals conditioned in proestrus and tested in diestrus displayed levels of freezing that were similar to those for which estrous cycle phase was matched during conditioning and testing. As a first step in exploring the role for ovarian steroids in this effect, we examined whether exogenously administered PROG would regulate fear expression in ovariectomized (OVX) animals. Following 1 week of recovery, OVX females were conditioned and tested in either the same or different hormonal state (induced by acute injection of either 4 mg/kg PROG or 1 ml/kg sesame oil vehicle, VEH, 1 hr prior to behavior) in a factorial design. PROG administration did not influence the acquisition or expression of contextual fear. However, we did observe lower levels of conditioned freezing to the tone in the VEH-PROG group compared to the VEH-VEH and PROG-PROG groups, indicating that some state dependence could be generated in this model. Together, these results suggest that natural fluctuations in PROG (and perhaps ALLO) levels can confer state-dependence such that hormonal state serves as an interoceptive contextual cue in conditioned fear.

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Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 414.25/BBB2

Topic: G.01. Appetitive and Aversive Learning

Support: R01MH065961
McKnight Endowment for Neuroscience (Memory and Cognitive Disorders Award)
Brain & Behavior Research Foundation (Distinguished Investigator Grant)

Title: Does stress or event segmentation account for the immediate extinction deficit?

Authors: *M. TOTTY, S. MAREN
Psychological and Brain Sci. and Inst. for Neurosci., Texas A&M Univ., College Station, TX

Abstract: Research in both rodents and humans has demonstrated that when extinction training occurs soon after (15 min) fear conditioning it results in impaired long-term extinction memory, a phenomenon called the "immediate extinction deficit" (IED). Considerable data suggest that IED is caused by a stress-induced dysregulation of neural circuits, including the medial prefrontal cortex and amygdala, that are essential for extinction learning (Fitzgerald et al., 2015). However, a recent study in humans (Dunsmoor et al., 2018) suggests that a short break between conditioning and extinction produces an "event boundary" that segments these events, prioritizes consolidation of the fear memory, and interferes with extinction. By this view, a "continuous" (CONT) extinction procedure [in which extinction trials follow conditioning trials at identical inter-trial intervals (ITIs)] would not yield an IED relative to a procedure in which there is a 15-min break (BREAK) between conditioning and extinction. To test this hypothesis, animals underwent auditory fear conditioning consisting of five tone conditioned stimulus (CS; 10 sec, 80 dB, 2kHz)-shock unconditioned stimulus (US; 2 sec, 1.0 mA) pairings in a distinct context. Conditioning was followed by extinction training (in the same context) after either a 60-sec (CONT; extinction trials delivered at the same ITI used during conditioning) or a 15-min break (BREAK); a third group of animals did not receive extinction but remained in the chambers an equivalent period of time (NO-EXT). Time in the training context was equated for all animals and freezing served as the index of fear. During extinction training, animals in the CONT group exhibited greater within-session extinction of freezing relative to BREAK or NO-EXT animals. However, this difference did not persist to the retention test conducted 48 hrs later in the same context. As we have previously reported, animals receiving extinction trials within 15-min of conditioning (CONT and BREAK) exhibited levels of freezing to the CS during the test and were identical to the high levels of freezing exhibited by animals that were not extinguished; animals in the CONT and BREAK conditions did not differ from one another. Therefore, eliminating the event boundary with a continuous extinction procedure did not eliminate the IED. These results support the view that the the IED is caused by the stress associated with fear conditioning, which disrupts the brain circuits and neural mechanisms involved in extinction learning.

Disclosures: M. Totty: None. **S. Maren:** None.

Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

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Program #/Poster #: 414.26/BBB3

Topic: G.01. Appetitive and Aversive Learning

Support: NIH R01 MH065961
McKnight memory and cognitive research award

Title: Nucleus reuniens inactivation impairs the acquisition and expression of contextual fear conditioning in rats

Authors: *K. R. RAMANATHAN[1], R. L. RESSLER[2], J. JIN[2], S. MAREN[2]
[2]Inst. for neurosciences and Dept. of Psychological and brain sciences,, [1]Texas A&M Univ., College Station, TX

Abstract: The nucleus reuniens (RE) is a ventral midline thalamic nucleus that interconnects the medial prefrontal cortex (mPFC) and hippocampus (HPC). Recent data suggest that RE lesions or inactivation produce deficits in spatial and working memory, which depend on HPC-mPFC interactions. Contextual fear conditioning also requires HPC-mPFC circuitry, but it is unclear whether RE manipulations affect the acquisition or retrieval of these memories and, importantly, whether RE inactivation affects performance in a state-dependent manner. To test this hypothesis, we inactivated the RE with muscimol (MUS, 0.3 µl at 0.1µg/µl concentration) either before the acquisition or expression (or both) of fear conditioning in a 2 X 2 factorial design; saline (SAL) infusions served as a control. The design yielded four groups of animals: SAL-SAL, SAL-MUS, MUS-SAL, and MUS-MUS (where drug conditions during conditioning and retrieval testing are indicated). Fear conditioning was conducted with either signaled (10 sec, 80 dB, 2 kHz tone) or unsignaled footshock (2 sec, 1 mA) and freezing served as the index of fear. Twenty-four hours after conditioning, rats were again infused with MUS or SAL and contextual freezing was assessed in either the conditioning context or a novel context in counterbalanced tests. Inactivation of the RE produced a robust impairment in the acquisition of contextual freezing in MUS-SAL rats, however, this impairment was absent in animals that were both trained and tested after RE inactivation (MUS-MUS). Interestingly, RE inactivation alone (SAL-MUS) did not cause a state-dependent generalization decrements, but increased freezing in a novel context as has previously been reported; this effect was not simply due to a nonspecific increase in freezing. Inactivation of RE did not affect the acquisition or expression of auditory fear conditioning. Together, these data suggest that RE inactivation impairs contextual processing and reveal that alternate, elemental representations of context support conditioning when the RE is offline.

Disclosures: K.R. Ramanathan: None. **R.L. Ressler:** None. **J. Jin:** None. **S. Maren:** None.

Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 414.27/BBB4

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant R56MH 114193

Title: Sex-specific effects of endocannabinoid action on cued fear conditioning and extinction

Authors: M. MEJDELL[1], A. LI[1], J. LAPETINA-COLOM[1], B. BROWN[1], I. SHURNAYTE[1], A. WINTER[1], S. BEGLEY[1], J. ABETTAN[1], *R. SHANSKY[2]
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Abstract: Experiencing a traumatic event is twice as likely to cause post-traumatic stress disorder in women as it is in men. Despite this imbalance, most of what we know about the neural mechanisms that underlie PTSD comes from research in male animals. One especially under-studied area is the endocannabinoid (eCB) system, whose role in modulating the stress response is just beginning to be uncovered. A better understanding of sex differences in these processes is critical to progress in developing more personalized therapies for PTSD patients of both sexes. To explore the influence of eCB signaling on aversive learning and memory processes, we tested male and female rats in a standard cued fear conditioning and extinction paradigm after systemic administration of FAAH inhibitor URB597, CB1 receptor antagonist AM251, MAGL inhibitor MJN110, or vehicle. We measured both cue-elicited freezing and darting in all animals, finding that as we have previously reported, females are more likely than males to engage in darting behavior during fear conditioning. In addition, AM251 administered during fear conditioning increased context generalization in females, but not males. These data suggest that endocannabinoid systems may differentially affect learning and memory processes in males and females.

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Poster

414. Fear and Aversive Learning and Memory: Modulation

Location: SDCC Halls B-H

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Program #/Poster #: 414.28/BBB5

Topic: G.01. Appetitive and Aversive Learning

Support: Brain Research Foundation BRFSG-2016-06

Title: A multifaceted approach for investigating sex-specific behavioral profiles during associative fear learning in rats

Authors: *A. LI, J. COLOM-LAPETINA, A. KIRUNDA, L. MILLER, R. SHANSKY
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Abstract: Classical Pavlovian fear conditioning has been widely used to study stress learning mechanisms. An animal's level of fear is typically evaluated by "freezing", but recent studies have identified a wide array of behaviors, suggesting that freezing is an incomplete measure of fear. Our previous work identified and characterized "darting", a sexually dimorphic active fear response predictive of improved extinction retention. In this study, we hypothesized that previous stress exposure biases fear behavior toward darting. One week before fear conditioning, male and female Sprague-Dawley rats underwent either a single sham surgery (n = 35) or five days of restraint stress (n = 30) to induce physiological stress or psychological stress, respectively. An additional control cohort (n = 31) was briefly handled. Five days after initial stress exposure, we used the tail flick test (TFT) to evaluate pain sensitivity. Then, the rats underwent fear conditioning on Day 1 via exposure to five unpaired tone presentations (CS) followed by seven footshock-paired tone presentations (CS-US). On Day 2, the animals underwent extinction with 20 unpaired CS presentations. Day 3 tested extinction retention with five unpaired CS presentations. Across the fear conditioning paradigm, velocity data were collected using EthoVision and analyzed with a set of custom Python scripts. Our high-throughput analysis method allows for efficient and accurate evaluation of a variety of behaviors, including darting, freezing, and general locomotion. Importantly, visualization of each animal's velocity across the entire paradigm allows us to dissect individual differences and predictive factors for these divergent behavior profiles.

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Poster

415. Fear and Aversive Learning and Memory: Extinction

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Program #/Poster #: 415.01/BBB6

Topic: G.01. Appetitive and Aversive Learning

Support: Grant National Science Centre Poland number 02910

Title: Social buffering in fear memory extinction

Authors: *T. GORKIEWICZ, E. KNAPSKA
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Abstract: Social support during the exposure-based psychotherapy has been suggested to have an important influence on the course of exposure treatment; however the mechanisms of such influence remain unknown. The literature review indicates that group treatment for posttraumatic stress disorder (PTSD) is efficacious compared with no treatment; however, some randomized clinical trials show that individual therapy may be more effective than group therapy. To

compare neuronal correlates of individual and group exposure therapy, we have developed a rat model of socially modulated fear extinction. In this model pair-housed rats are separately fear conditioned to the tones and then subjected either to fear extinction or merely exposure to the experimental cage. Next, the rats are placed in the experimental cage and presented to conditioned tones in pairs or separately. We observed much lower freezing response in rats tested with a partner compared to rats tested separately. The magnitude of social buffering effect depended on familiarity. However, the effect was transient, when fear memory was measured on the next day there was little difference between rats exposed to the conditioned tones together or separately. Lower freezing level during exposure with a partner was associated with lower activation of the prefrontal cortex; with the dominant input to active neurons from the hippocampus, whereas the retrieval of fear memory was associated with greater amygdalar input. Taken together, these results suggest that the social buffering effects may partially rely on the same neuronal circuits as individual fear extinction but the social fear memory suppression is transient.

Disclosures: T. Gorkiewicz: None. **E. Knapska:** None.

Poster

415. Fear and Aversive Learning and Memory: Extinction

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 415.02/BBB7

Topic: G.01. Appetitive and Aversive Learning

Support: Rockefeller Center Grant

Title: Exposure to elevated levels of kynurenic acid impairs the extinction of conditioned fear: Implications for anxiety and stress disorders

Authors: C. H. PUSKAS, M. C. EDDY, N. E. DEANGELI, K. B. COCHRAN, *D. J. BUCCI
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Abstract: Kynurenic acid (KYNA), a metabolite of tryptophan degradation, acts as an antagonist of both NMDA glutamate and alpha-7 nicotinic acetylcholine receptors. Prior work has shown that in rats with a compromised stress response system (e.g., adrenalectomy, ADX), subsequent exposure to a mild stressor increases the concentration of KYNA in the prefrontal cortex. Moreover, the increase in KYNA in ADX-rats following stress causes rats to generalize fear to otherwise innocuous stimuli. This has led us to propose that a stress-induced increase in KYNA may contribute to abnormal fear behavior in persons with a compromised stress response system, such as those with PTSD. If so, increased levels of KYNA may also impair fear extinction. Extinction is the basis for exposure-based therapy in humans and consists of repeated presentations of the fear-eliciting stimulus in the absence of aversive consequences, thereby

reducing the fear-provoking ability of the stimulus. Interestingly, extinction is often ineffective in persons with PTSD; in addition, a wealth of research in rodents and humans has revealed that the prefrontal cortex has a pivotal role in fear extinction. In the present study, rats underwent a standard fear conditioning procedure in Context A and received presentations of a tone followed by footshock. Extinction training began the next day and consisted of four daily sessions in which the tone was presented without shock in a new context (Context B). Two hours before each extinction session half of the rats were treated with 100mg/kg l-kynurenine (L-KYN; i.p.), the precursor of KYNA, which significantly elevates brain KYNA levels. The other half of the rats were treated with a vehicle solution. During the subsequent extinction test sessions, rats received presentations of the tone (no shock) in the original training context (A) and in the extinction context (B). No injections were made before the test sessions. Control rats exhibited low levels of fear to the tone during the Context B test session, while fear renewed (high levels) when the tone was presented in Context A, reflecting the normal context-dependency of fear extinction. However, rats that had been treated with L-KYN during extinction training exhibited high levels of fear to the tone in both contexts. A subsequent re-acquisition procedure showed that this effect was not simply due to an inability to discriminate between the two contexts. This suggests that increased levels of KYNA impair the extinction of conditioned fear, providing further support for the notion that increased levels of KYNA may contribute to impairments in fear learning and extinction associated with stress-related disorders.

Disclosures: C.H. Puskas: None. **M.C. Eddy:** None. **N.E. DeAngeli:** None. **K.B. Cochran:** None. **D.J. Bucci:** None.

Poster

415. Fear and Aversive Learning and Memory: Extinction

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 415.03/BBB8

Topic: G.01. Appetitive and Aversive Learning

Support: NSF Grant IOS1456706
NSF GRFP Grant DGE144083

Title: Diurnal examination of infralimbic prefrontal cortex neuronal activity: Encoding of distinct behaviors relevant to conditioned fear extinction learning

Authors: *M. J. HARTSOCK, J. R. RAVENEL, K. S. MCCONOMY, H. K. STRNAD, A. B. FAUSNAUGHT, M. P. SADDORIS, R. L. SPENCER
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Abstract: Stress-related mental disorders are associated with circadian disruptions and with impairments in fear extinction learning. In rats, projections from the infralimbic prefrontal cortex

(IL) to the basomedial amygdala are believed to mediate extinction. We have shown recently that rats exhibit a diurnal rhythm in fear extinction recall that is abolished by disruption of the IL circadian system, raising the possibility that circadian disruptions in this pathway—such as those produced by stress—may lead to learning impairments similar to those observed in mental disorders. To determine whether diurnal rhythms in extinction are driven by diurnal rhythms of neuronal activity in the IL, we recorded IL neuronal activity during fear extinction in the daytime (inactive phase) or nighttime (active phase). Adult male Sprague-Dawley rats on a 12h:12h light:dark cycle were administered bilateral microinjections of CaMKII-promoter-driven channelrhodopsin-2 (ChR2) in the IL to enable photo-tagging of IL pyramidal neurons. One week later, an 8-channel electrode array surrounding an optical fiber was implanted in the IL in each hemisphere. After recovering from surgery, rats were trained and tested in a delayed fear conditioning protocol consisting of tone-shock pairing on Day 1, fear extinction on Day 2, and fear extinction recall on Day 3. Neurophysiological recording of IL neurons was conducted on Days 2 and 3, and 470nm blue light was used on Day 3 to stimulate ChR2 during extinction recall. We have identified changes in phasic activity corresponding to two different behaviorally relevant events: 1) cue presentation after tone-shock pairing and 2) behavioral responding (i.e. freezing or unfreezing) to the paired cue. Of neurons associated with behavioral responding, one subpopulation increased its firing rate for freezing onset and decreased its firing rate for freezing offset, while another subpopulation exhibited an opposite activation pattern. Photo-tagging demonstrated that some recorded cells were positive for ChR2, establishing proof of principle that recorded cells can be distinguished by phenotype and subsequently manipulated. This study is the first to record diurnal firing within the IL and to differentiate neuronal subpopulations within the IL associated with freezing onset versus freezing offset.

Disclosures: M.J. Hartsock: None. **J.R. Ravenel:** None. **K.S. McConomy:** None. **H.K. Strnad:** None. **A.B. Fausnaught:** None. **M.P. Saddoris:** None. **R.L. Spencer:** None.

Poster

415. Fear and Aversive Learning and Memory: Extinction

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 415.04/BBB9

Topic: G.01. Appetitive and Aversive Learning

Support: CNPq Brazil - Project 207530/2014-9
Irish Research Council
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Title: The effects of pharmacological blockade of PPARs on formalin-evoked nociceptive behaviour, fear-conditioned analgesia and conditioned fear in the presence or absence of nociceptive tone

Authors: *J. C. GASPAR[1,2,3], B. OKINE[1,2,3], D. DINNEEN[1], A. LLORENTE-BERZAL[1,2,3], M. ROCHE[4,2,3], D. P. FINN[1,2,3]
[1]Natl. Univ. of Ireland Galway, Galway, Ireland; [2]Galway Neurosci. Ctr., Galway, Ireland; [3]Ctr. for Pain Res., Galway, Ireland; [4]Physiol., Natl. Univ. of Ireland, Galway, Ireland

Abstract: Introduction Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors[1]. There is evidence for their involvement in pain[2], cognition[3], and anxiety[4]. However, their role in pain-fear interactions is unknown. The amygdala plays a key role in pain, conditioned fear and fear-conditioned analgesia (FCA). **Aims** Investigate the effects of systemic and intra-basolateral amygdala (BLA) and central amygdala (CeA) administration of PPARα, PPARβ/δ and PPARγ antagonists on nociceptive behaviour, fear-conditioned analgesia (FCA), and conditioned fear in presence or absence of nociceptive tone in rats. **Methods** Male Sprague-Dawley (SD) rats received footshock (FC) or no footshock (NFC) in a conditioning arena. 23.5h later, rats received intra-plantar injection of formalin and intra-peritoneal vehicle, PPARα (GW6471), PPARβ/δ (GSK0660) or PPARγ (GW9662) antagonists, before re-exposure to the arena for 15 minutes, and behaviour recorded. In subsequent experiments, rats underwent a similar protocol except PPAR antagonists or vehicle were microinjected intra-BLA or intra-CeA 15 minutes prior to formalin or saline administration. Pain- and fear-related behaviours were assessed for 30 minutes and amygdalar neurotransmitters/endocannabinoids were measured post-mortem. **Results** Systemic administration of all antagonists potentiated context-induced freezing in the presence of formalin-evoked nociceptive tone, with no effect on nociceptive behaviour. Intra-BLA administration of PPARα or PPARγ antagonists potentiated freezing in the presence of nociceptive tone in FC rats. Blockade of all PPARs in the BLA increased freezing and BLA dopamine levels in NFC rats in the absence of nociceptive tone. Administration of PPARα, PPARβ/δ or PPARγ antagonists intra-CeA did not affect freezing duration in FC rats in the presence of nociceptive tone. Formalin-injected FC rats receiving PPARα, PPARβ/δ and PPARγ antagonists systemically had lower levels of CeA dopamine, while those receiving intra-BLA PPARα and PPARγ antagonists had higher levels of dopamine in the BLA, compared with vehicle-treated counterparts. **Conclusions** PPARs, particularly PPARα and PPARγ, in the BLA play a role in expression or extinction of conditioned fear in the presence or absence of nociceptive tone. **References** [1]Blanquart C. et al. (2003) J. Steroid Biochem. Mol. Biol. *85*: 267-73. [2]Okine et al. (2018) Br J Pharmacol. 2018 [3]Panlilio L. et al. (2013) Pharmacol Ther 84-102 [4]Domi E et al (2016) J. Neurosci. 4;36(50):12611-12623

Disclosures: J.C. Gaspar: None. **B. Okine:** None. **D. Dinneen:** None. **A. Llorente-Berzal:** None. **M. Roche:** 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.; Alkermes. **D.P. Finn:** 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.; Alkermes.

Poster

415. Fear and Aversive Learning and Memory: Extinction

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 415.05/BBB10

Topic: G.01. Appetitive and Aversive Learning

Support: NSF CAREER 1565410 (IAM)

Title: Selective manipulation of ventral hippocampus projections to the prelimbic cortex selectively facilitates fear extinction generalization

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Abstract: Extinction is the mechanism through which an organism decreases fear responses to cues that no longer predict danger. A major characteristic of extinction memory is that it is context-dependent, in that extinction memories formed in one context is not necessarily recalled in a different context, leading to fear renewal. The ability to extinguish fear memories and generalize these extinction memories to multiple contexts provides therapeutic potential, but little is known about the specific mechanisms that mediate fear renewal and extinction generalization. Several studies point at the ventral hippocampus (VH) as a key brain region mediating contextual gating of emotional behavior, yet the functional role of VH projections remains unknown. VH cells project to the prelimbic (PL) region of the medial prefrontal cortex, an area that is primarily involved in fear expression. In this study, we examined the effect of pathway-specific chemogenetic manipulations on VH cells projecting to the PL to determine their effect on fear extinction and generalization. First animals underwent cued fear conditioning and extinction. By employing the use of intersectional DREADDs, we observed that targeted activation of projections from the VH to the PL during extinction memory retrieval was sufficient to attenuate fear renewal after extinction and generalize extinction behaviors across contexts. Furthermore, chemogenetic manipulation of this pathway during cued fear retrieval had no effect on fear expression of subsequent extinction memory, suggesting that other pathways are involved in mediating fear expression prior to extinction. These findings suggest that VH projections to the PL cortex may be critical to increase extinction generalization across contexts. Understanding the functional role of VH projections may contribute to determine the etiology of anxiety disorders and the role that contextual information plays in emotional memories.

Disclosures: K. Leong: None. **J.H. Vasquez:** None. **I.A. Muzzio:** None.

Poster

415. Fear and Aversive Learning and Memory: Extinction

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 415.06/BBB11

Topic: G.01. Appetitive and Aversive Learning

Support: DARPA ElectRx N66001-15-2-4057

Title: Extinction-paired vagus nerve stimulation reduces avoidance of a conditioned odor

Authors: *C. R. OLEKSIAK, M. N. TABET, S. ARORA, M. P. SRIVASTAV, R. R. SOUZA, C. K. MCINTYRE
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Abstract: Vagus nerve stimulation (VNS) enhances extinction of auditory fear conditioning in a rat model of posttraumatic stress disorder (PTSD). Olfactory cues are described as powerful triggers of avoidance, panic attacks, and flashbacks in those with fear disorders. Here, we examined whether VNS paired with a conditioned olfactory cue could reduce avoidance and second order conditioning. To test this, adult male Sprague Dawley rats were implanted with a stimulation cuff around the vagus nerve. After recovery, the rats received two days of fear conditioning where the olfactory cue (CS; 100 µl of 10% amyl actetate) was paired with eight footshocks per day (US; 1-sec, 0.8 mA). Seventy-two hours later, the rats were given three days of sham (n=11) or VNS-paired extinction (5x 30-sec VNS at 0.4 mA/day; n=14). Finally, rats underwent an avoidance paradigm that consisted of habituation to a new context, an odor avoidance test, and a context avoidance test where approach, hiding time, and transits between the neutral chamber and the odor chamber were assessed. We found that sham- and VNS-treated rats did not differ in extinction acquisition or in the habituation to the avoidance chamber. However, VNS rats spent significantly more time approaching the odor source ($t=3.09$, $p=0.01$) and significantly less time hiding ($t=3.05$; $p=0.01$) than the sham-stimulated rats. These results suggest that VNS can be used to enhance extinction of conditioned fear to cues that span different sensory modalities. Neither group demonstrated second order conditioning. Further studies are needed to determine the optimal VNS parameters for enhancing extinction and possible VNS effects on extinction generalization across modalities.

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Poster

415. Fear and Aversive Learning and Memory: Extinction

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 415.07/BBB12

Topic: G.01. Appetitive and Aversive Learning

Support: Australian Government Research Training Program (AGRTP)
Career Development Fellowship from the National Health and Medical Research Council of Australia

Title: Impact of chronic early life adversity on fear extinction in juvenile and adolescent rats

Authors: *K. DRUMMOND[1,2], M. BLEWITT[3,2], G. FAULKNER[4,5], J. H. KIM[1,2]
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Abstract: Early life is viewed as a developmental period in which adversity could cause long-term impairments in fear inhibition. Exposure to adversity in both the neonatal period (Postnatal Day (P) 2-13) and adolescence period (P21-42) has previously been shown to cause robust changes to anxiety and fear-related behaviors. We hypothesized that chronic adversity early in life would be deleterious to fear inhibition throughout development in male and female Sprague-Dawley rats. In Experiment 1: We subjected male and female neonatal rats to a limited bedding stress environment from P2-13. Five days later, animals underwent fear conditioning, extinction and were tested to see whether their fear would renew. In Experiment 2: We reared rats in social isolation from P21-42, at which they were either behaviorally tested or resocialized and then tested in adulthood at P70. All animals underwent fear conditioning, extinction and were tested for their extinction retrieval ability. In Experiment 1, we found that juvenile male rats exposed to a limited bedding stress environment displayed a robust relapse of fear following extinction compared to controls. However, juvenile female rats behaved no differently to controls as both groups displayed a relapse of fear. In Experiment 2, relative to group-housed controls, both male adolescent and resocialized adult rats displayed higher freezing at test. In contrast, female adolescent and resocialized adult rats displayed enhanced extinction acquisition compared to controls but showed similar levels of freezing at test. Overall, these results demonstrate that exposure to adversity during early post-natal and adolescence development impacts the ability of rats to inhibit fear. Future work will determine whether exposure to adverse environments during development led to alterations in the activity of transposons (particularly long interspersed nuclear elements) in the rat genome.

Disclosures: K. Drummond: None. **M. Blewitt:** None. **G. Faulkner:** None. **J.H. Kim:** None.

Poster

415. Fear and Aversive Learning and Memory: Extinction

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 415.08/BBB13

Topic: G.01. Appetitive and Aversive Learning

Support: NIH R01 AT006344-01
NIH R01 AG048351

Title: Learning not to fear: Mindfulness meditation improves retention of fear extinction

Authors: *G. SEVINC[1], B. HOLZEL[2], T. GARD[3], J. GREENBERG[1], J. A. HASHMI[4], M. R. MILAD[5], S. W. LAZAR[1]
[1]Massachusetts Gen. Hosp., Charlestown, MA; [2]Tech. Univ. of Munich, Munich, Germany; [3]Univ. Zurich, Zurich, Switzerland; [4]Anesthesia, Pain Mangement and Perioperative Med., Dalhousie Univ., Halifax, NS, Canada; [5]Psychiatry, Univ. of Illinois at Chicago, Chicago, IL

Abstract: Mindfulness based stress reduction (MBSR) programs have been widely utilized to ameliorate stress-related symptoms, however the neural mechanisms that underlie the improvements are still largely unknown. Mindfulness meditation involves refraining from cognitive avoidance and thus provides a basis for internal exposure to aversive stimuli. We hypothesized that mindfulness-based interventions create a context akin to behavioral exposure and thereby alter neurobiological responses to aversive stimuli. We tested this hypothesis in a randomized longitudinal study design using a 2-day fear conditioning and extinction protocol with skin conductance as the index of extinction retention. Meditation-naïve participants completed either an 8-week MBSR program (n=42), or a stress management education program (n=25), as an active control. Alterations in brain structure and function from pre to post were assessed using fMRI. Both interventions decreased stress levels, while MBSR resulted in further improvements in anxiety, emotion regulation, rumination, and self-compassion. Both interventions improved memory for extinguished stimuli. Compared to the control, MBSR resulted in enhanced activity in right supramarginal gyrus during retrieval of extinction memory, and neural activity within this cluster correlated with total practice time. Changes in emotion regulation were associated with the differential functional coupling of right supramarginal gyrus to ventral posterior parietal cortex during early phases of recall. An investigation of hippocampal coupling patterns further revealed enhanced connectivity between hippocampus and postcentral gyrus following MBSR. Changes in hippocampal structure further predicted the degree of functional connectivity between the hippocampus and dorsolateral prefrontal and retrosplenial cortices. The results suggest that MBSR improves extinction predominantly though enhancing neural activity in regions associated with attentional input to memory during extinction recall. Structural and functional changes in the hippocampus suggest a role for enhanced contextual

retrieval in stress reduction following MBSR. Enhanced activity and connectivity with regions associated with dynamic control of attention suggest that MBSR may enhance emotion regulation skills through modulating attention and memory interactions. Considering that the ability to recall that a stimulus is no longer associated with threat is critical for healthy emotional functioning, the results suggest the improvement in this ability may be a key mechanism through which mindfulness meditation ameliorates stress-related symptoms.

Disclosures: B. Holzel: None. **T. Gard:** None. **J. Greenberg:** None. **J.A. Hashmi:** None. **M.R. Milad:** None. **S.W. Lazar:** None.

Poster

415. Fear and Aversive Learning and Memory: Extinction

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 415.09/BBB14

Topic: G.01. Appetitive and Aversive Learning

Support: GM111725-02

Title: Neural mechanisms underlying impaired extinction learning in a wild-type inbred mouse strain

Authors: *V. A. CAZARES[1], K. GORDON[3], G. G. MURPHY[2]
[1]Mol. and Behavioral Neurosci. Inst., [2]MBNI/Physiology, Univ. of Michigan, Ann Arbor, MI; [3]Biol., Kalamazoo Col., Kalamazoo, MI

Abstract: The ability to learn that neutral environmental stimuli can become predictive of an aversive outcome heightens an organism's chance for survival. Equally important for survival, is the ability to learn to diminish a fearful response (known as fear extinction) when stimuli no longer predict an aversive outcome. Deficits in extinction learning are often used to model maladaptive behavior in humans that are experiencing generalized anxiety or post-traumatic stress disorder. Previous work (Camp et al., 2009) has identified strong variations in the capability for fear extinction learning between *wild-type* inbred mouse strains. This work seeks to leverage deficits in the 129S1-inbred mouse strain, which is significantly impaired in extinction learning relative to the C57BL/6 mouse strain, to define neurophysiological substrates of fear suppression. Previous work has shown that distinct cell populations in the medial-prefrontal cortex (mPFC) respond to changes in the contingency between environmental stimuli and aversive outcomes. Our hypothesis is that instructive neural signals generated by mPFC to modulate downstream regions like the amygdala are degraded in 129S1 relative to C57BL/6. Impaired executive prefrontal neuromodulation in the 129S1 strain may account for deficits in fear extinction. To test this hypothesis, we first examined if neural activation in the mPFC between these mice strains differs depending on the amount of extinction training. For these

studies, we used the expression of *cfos,* an activity-dependent immediate-early gene as an indirect measure of neuronal activation. Consistent with prior results, we find that 129S1 mice are impaired in extinguishing freezing responses compared to the C57BL/6 strain. Moreover, using both immunolabeling and *cfos*-driven YFP expression our results show that for both strains of mice, reductions in fear responses are inversely correlated with the level of activation in the infralimbic subregion of the mPFC ($R = -0.5$, $p < 0.05$). Additionally, 129S1 mice also had reduced overall *cfos* expression in IL after three days of extinction training. Interestingly, we find that animals of the 129S strain while impaired in extinguishing fear responses can discriminate stimuli that was previously paired with an aversive outcome from neutral stimuli, suggesting that they possess a specific deficit in fear suppression. Based on this preliminary data, our current efforts deploy the use *in vivo* optical techniques to monitor neuronal activation in the mPFC during extinction learning to discern if changes in cell firing dynamics can account for the strain-specific behavioral phenotypes. <!--EndFragment-->

Disclosures: V.A. Cazares: None. **K. Gordon:** None. **G.G. Murphy:** None.

Poster

415. Fear and Aversive Learning and Memory: Extinction

Location: SDCC Halls B-H

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Program #/Poster #: 415.10/CCC1

Topic: G.01. Appetitive and Aversive Learning

Support: Max-Planck Society (MPG)
ANR / DFG French-German project SAFENET
European Union's Horizon 2020 research and innovation programme (ERC-2017-STG, grant agreement 502 n° 758448)

Title: Dissecting the role of interoceptive insular cortical circuits in emotionally enhanced learning

Authors: *A. S. KLEIN[1], N. DOLENSEK[1], D. A. GEHRLACH[1], C. HERRY[2], N. GOGOLLA[1]
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Abstract: Extinction therapy is the most commonly used clinical method to treat a variety of psychiatric conditions, such as addiction, trauma, and stress-related disorders. Importantly, fear extinction, the basis of exposure-based therapy, is a primary treatment strategy for anxiety disorders. However, extinction learning in clinical settings is conducted under exceptional internal states of the subjects, which, especially in patients suffering from psychiatric disorders, may hamper treatment success. Indeed, stress as well as internal feeling and emotional states have long been known to affect learning and memory performance. Since the posterior insular

cortex (pIC) is crucially involved in interoception and plays a prominent role in the detection and processing of feelings and emotions, we here addressed the role of the pIC in extinction learning. Using optogenetic activity manipulations we found an important role for the pIC in regulating extinction-learning abilities, which depended on the internal fear-state of the animal. Further, *in vivo* electrophysiological recordings revealed state-dependent processing of fear-related behaviors and cues in pIC neurons. These data suggest that the insula potently shapes fear extinction learning dependent on the internal state. To start addressing how internal state may affect the pIC's role in learning, ongoing research in the lab addresses the link between pIC-mediated interoception, vagal afferents carrying bodily signals to the brain, and fear extinction performance.

Disclosures: A.S. Klein: None. **N. Dolensek:** None. **D.A. Gehrlach:** None. **C. Herry:** None. **N. Gogolla:** None.

Poster

415. Fear and Aversive Learning and Memory: Extinction

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 415.11/CCC2

Topic: G.01. Appetitive and Aversive Learning

Support: DFG Grant SFB 1520 ZL
DFG Grant ZL 59/2-1
DFG Grant ZL 59/2-2

Title: Fear conditioning and exposure therapy benefit

Authors: *A. ZLOMUZICA, C. MERZ, D. ADOLPH, S. SCHNEIDER, J. MARGRAF, F. PREUSSER
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Abstract: Patients with anxiety disorders exhibit systematic changes in the acquisition and extinction of conditioned fear which might constitute a potential mechanism contributing to the development and maintenance of these debilitating disorders. Extinction learning has been increasingly acknowledged as a translational tool which can be used to refine current treatment options for anxiety disorders such as exposure-based therapies. Both, exposure-based therapy benefit and conditionability are individually variable. The present study sought to determine whether individual variability in fear acquisition and extinction predicts exposure therapy outcome in specific phobia. Participants with spider fear underwent a differential fear conditioning procedure before undergoing a standardized one session exposure therapy. In the differential fear conditioning task, skin conductance responses (SCR), subjective valence and contingency ratings of the CS were measured as dependent measures of conditionability. As a

measure of exposure therapy outcome, several variables were collected including reductions in self-reported fear, spider-phobic cognitions, as well as changes in behavioral avoidance (i.e. Behavioral Approach Test, BAT) from pre to post-treatment. Participants who successfully completed the exposure session showed more pronounced reductions in different self-reported fear measures relative to participants who did not master exposure. Most importantly, an association between the extinction learning rate and exposure success was found. Specifically, participants who benefited more from exposure showed superior extinction learning relative to participants who benefited less from exposure. Our findings indicate that individual differences in treatment benefit can be predicted on the basis of extinction learning performance.

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Poster

415. Fear and Aversive Learning and Memory: Extinction

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Program #/Poster #: 415.12/CCC3

Topic: G.01. Appetitive and Aversive Learning

Support: Medical Research Council Studentship
Engineering and Physical Sciences Research Council Studentship

Title: Neural and behavioural dynamics of pain learning

Authors: *O. ZIKA[1], B. CRITTENDEN[2], R. BOGACZ[2], K. WIECH[2]
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Abstract: Introduction Aversive experiences such as pain are known to lead to fast associative learning but can be highly resistant to extinction. This asymmetric learning is discussed as a key psychological mechanism involved in the maintenance of chronic pain. We used functional magnetic resonance imaging together with computational modelling to examine differences between acquisition and extinction of pain-related associations in a reversal learning paradigm.
Methods 28 healthy volunteers (14 male; mean age: 25.75) were tested in a 3T MR scanner. On each trial, participants were presented with one of three visual cues which were either followed by pain on 75% of trials, on 25% of trials or with probability switching between 75% and 25%. Switching cue probabilities changed unbeknownst to the participant every 28 to 32 trials (270 trials total). Upon cue presentation, participants had to indicate the subjective probability of pain (0%-100%) prior to delivery or omission of a noxious electrical stimulus.
Results Our results show that learning occurred faster when stimulus delivery became more likely (25% to 75%; acquisition) than when it became less likely (75% to 25%; extinction).

Probability ratings consistently exceeded the true probability during extinction. This over-prediction of pain during extinction increased over the course of the experiment and was best characterised by a computational model with dynamic learning rate. Critically, over-prediction was more pronounced in low-anxious individuals. The over prediction in low-trait anxious participants was due to increasing learning from shocks while learning from shock omissions remained constant. In contrast, high trait anxious individuals kept learning from both outcomes in a similar fashion.
Our fMRI results identified several regions that showed an increase in outcome-related activity in the second compared to the first half. The signal level in the posterior insula, precuneus and left dorsolateral prefrontal cortex increased during learning from shocks while thalamic activity decreased over time.
Conclusion Our data suggest a time-dependent mechanism involved in aversive learning. While learning during acquisition remains high, learning during extinction becomes increasingly slower. This decrease in extinction learning is modulated by trait anxiety with high trait anxious individuals being better at following the environmental statistics.

Disclosures: O. Zika: None. **B. Crittenden:** None. **R. Bogacz:** None. **K. Wiech:** None.

Poster

415. Fear and Aversive Learning and Memory: Extinction

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 415.13/CCC4

Topic: G.01. Appetitive and Aversive Learning

Support: CSUS RCA mini grant to S.C.F.

Title: A possible role for perirhinal cortex in fear extinction learning

Authors: *N. POTTER, C. A. CALUB, S. C. FURTAK
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Abstract: Several studies suggest that the perirhinal cortex (PER) may function to unitize stimulus components across time or modalities (Kent & Brown, 2012). For example, multiple findings have supported the involvement of the PER in stimulus unitization during fear conditioning to discontinuous auditory stimuli, such as discontinuous tones and pre-recorded rodent ultrasonic vocalizations. However, the role of the PER in processing such stimuli during other aspects of fear learning, including fear extinction, have not been fully evaluated. The current study assessed the involvement of the PER during a fear extinction paradigm using a discontinuous or a continuous conditioned stimulus (CS). Based on the stimulus unitization hypothesis, we predicted that the PER would be necessary to support fear extinction to the discontinuous CS but not to the continuous CS. Sprague-Dawley derived male rats were

randomly assigned to one of four groups based on two factors: CS type (a continuous light versus a discontinuous light) and PER manipulation (Inactivation group or Control group). All rats were bilaterally implanted with cannulae targeting the PER. Upon surgical recovery, subjects were evaluated on a three-day fear extinction paradigm conducted in the same context. On Day 1, Fear Acquisition, rats received 5 presentations of the CS that co-terminated with a foot shock unconditioned stimulus (US). On Day 2, Extinction Training, rats were bilaterally infused with muscimol, a GABA agonist (Inactivation group), or a saline vehicle (Control group). Approximately 40 mins following infusions, rats received 20 CS-alone presentations. On Day 3, Extinction Recall, rats were presented with an additional 15 CS-alone presentations. Behavioral sessions were recorded digitally for offline analysis using customized software to assess the level of freezing, defined as no movement for at least 1 sec. In a subset of rats, infusions of fluorescently-conjugated muscimol were analyzed to assess the spread of inactivations. Rats in the Inactivation group displayed significantly more freezing than the Control group during Extinction Recall regardless of whether the CS was a continuous light or discontinuous light. A subset of these results was previously presented (discontinuous CS only; Calub et al., 2015). Possible interpretations of the results are discussed within the framework of the stimulus unitization hypothesis.

Disclosures: N. Potter: None. **C.A. Calub:** None. **S.C. Furtak:** None.

Poster

415. Fear and Aversive Learning and Memory: Extinction

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 415.14/CCC5

Topic: G.01. Appetitive and Aversive Learning

Support: NARSAD Brain and Behavior Foundation Independent Investigator Grant

Title: Investigating the neural correlate of TMR-dependent fear extinction

Authors: *I. C. HUTCHISON[1], L. K. SHANAHAN[1], J. A. GOTTFRIED[1,2]
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Abstract: Sleep plays a crucial role in the consolidation and integration of memories, particularly those with emotional salience. Recent studies indicate that memories can be selectively targeted and reactivated in sleep via presentation of a sound or smell associated with the learning task in the prior wake state. Further, the reactivation of fear memories during sleep, in the absence of negative reinforcement, facilitates fear extinction (Hauner et al., 2013). In the current study we have used an olfactory contextual conditioning paradigm in which face images (conditioned stimuli; CS+) are paired with mild electric shocks (unconditioned stimuli; US) with

a 50% reinforcement rate in the presence of neutral background odors. During fMRI scanning, one of the odors (the target odor) was repeatedly delivered during non-REM sleep (measured using MR-compatible EEG recordings) in 16s intervals separated by 16s odor-free periods. This was followed by a second conditioning period with a reinforcement rate of 12.5%. Preliminary data suggest a selective reduction in response to target CS+ across sleep compared to the non-target CS+. In addition, analysis of functional MRI data in sleep indicates activity in limbic areas supportive of fear memory replay which appears to diminish with repeated cue exposures. We plan to use multivariate pattern analysis to further investigate how the representational content of odor-cued emotional memories develops as a function of contextual odor-cue delivery in sleep. In addition, we aim to assess to which degree this development relates to physiological extinction evidenced by skin conductance responses. We anticipate that our findings will yield important new insights into the plasticity of emotional memory storage that hold potential for future translational research in the area of psychiatric illness and pathological fear. Given the relative scarcity of effective therapeutic interventions, the use of smell cues in sleep represents a highly unique and innovative approach for tackling this problem.

Disclosures: I.C. Hutchison: None. **L.K. Shanahan:** None. **J.A. Gottfried:** None.

Poster

415. Fear and Aversive Learning and Memory: Extinction

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 415.15/CCC6

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant MH114026

Title: The role of mTOR signaling in enhanced fear extinction produced by acute, voluntary exercise

Authors: *N. A. MOYA[1], M. K. TANNER[2], J. JAIME[2], E. C. LOETZ[1], H. S. HAKE[2], B. N. GREENWOOD[1]
[1]Psychology, [2]Univ. of Colorado Denver, Denver, CO

Abstract: Exercise can benefit cognition, learning and memory, and mental health. In rats, beneficial effects of acute exercise include enhancing the extinction of traumatic memories, wherein a single bout of voluntary exercise after fear extinction training can enhance fear extinction memory and reduce relapse. Identifying mechanisms by which acute exercise augments fear extinction could reveal novel targets for the treatment of trauma-related disorders, such as Post-Traumatic-Stress Disorder (PTSD), the etiology of which depends on memories of traumatic events. The mechanisms by which acute exercise enhances fear extinction are unknown. One factor that could contribute to enhanced fear extinction memory following

exercise is the mammalian target of rapamycin (mTOR). mTOR is a translation regulator involved in synaptic plasticity, cell growth, and proliferation. mTOR signaling is sensitive to many exercise signals such as monoamines, growth factors, and metabolic signals, and is increased after chronic exercise in brain regions involved in learning and emotional behavior. mTOR is therefore a compelling potential facilitator of the memory-enhancing and overall beneficial effects of exercise on mental health. The goal of the current study was to test the hypothesis that mTOR signaling is critical for the enhancement of fear extinction memory produced by acute, voluntary exercise, in adult, male Long-Evans rats. We observed that, like chronic exercise, a single session of voluntary exercise increased mTOR signaling in extinction-related brain areas. Moreover, intracerebral-ventricular (ICV) administration of the mTOR inhibitor rapamycin reduced mTOR signaling and eliminated the enhancement of fear extinction memory produced by acute exercise, without reducing voluntary exercise behavior or altering fear extinction learning. These results suggest that mTOR signaling contributes to the memory-enhancing effects of acute exercise. Factors that increase mTOR signaling could be useful novel targets for the treatment of psychiatric disorders like PTSD.

Disclosures: N.A. Moya: None. **M.K. Tanner:** None. **J. Jaime:** None. **E.C. Loetz:** None. **H.S. Hake:** None. **B.N. Greenwood:** None.

Poster

415. Fear and Aversive Learning and Memory: Extinction

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 415.16/CCC7

Topic: G.01. Appetitive and Aversive Learning

Support: NIH MH114026

Title: Effects of optogenetic activation on nucleus accumbens projecting ventral tegmental neurons during fear extinction on fear extinction memory and relapse

Authors: *J. WISEMAN[1], E. C. LOETZ[2], E. B. OLESON[2], B. N. GREENWOOD[2]
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Abstract: Exposure therapy relies on the process of fear extinction, which is learning that a prior conditioned fear stimulus no longer predicts danger. One limitation of exposure therapy is that fear extinction memory is labile, and fear often returns even after successful extinction. Identification of novel strategies to prevent fear relapse after extinction is of utmost importance to mental health. Manipulations that enhance dopamine (DA) signaling can strengthen fear extinction, but the specific DA pathways involved, and whether fear extinction enhanced by DA is resistant to relapse, remain unclear. Midbrain DA neurons originating in the ventral tegmental

area (VTA) and projecting to the nucleus accumbens (NAc) encode prediction error. Activation of these neurons during extinction could, therefore, facilitate extinction by enhancing the learning that the conditioned stimulus no longer predicts an aversive event. The goal of the current study was to test the hypothesis that activation, during fear extinction, of midbrain DA neurons that project to the NAc, can enhance fear extinction and reduce relapse. Adult, male Long-Evans rats received bilateral intra-NAc microinjections of either AAV2retro-Cre-eGFP or CAV-Cre. These viruses travel retrograde to midbrain cell bodies of origin and express the enzyme Cre-recombinase. A second virus (AAV-EF1a-DIO-hChR2(H134R)-EYFP) that expresses a light-sensitive ion channel (ChR2) in a Cre-dependent manner was then injected into the VTA, allowing the expression of ChR2 in VTA neurons projecting to the NAc. VTA neurons projecting to the NAc were then optogenetically stimulated during auditory fear extinction. Fear extinction memory and relapse were subsequently assessed in the absence of stimulation. Preliminary results indicate that optogenetic stimulation of NAc-projecting VTA neurons during fear extinction reduces the renewal of fear in a novel context. These data suggest that novel therapeutic strategies aimed at the mesolimbic DA circuit could be effective adjuncts to exposure therapy.

Disclosures: J. Wiseman: None. **E.C. Loetz:** None. **E.B. Oleson:** None. **B.N. Greenwood:** None.

Poster

415. Fear and Aversive Learning and Memory: Extinction

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 415.17/CCC8

Topic: G.01. Appetitive and Aversive Learning

Support: NIH MH114026
Multidisciplinary Association for Psychedelic Studies

Title: 3,4-methylenedioxymethamphetamine (MDMA) impairs the extinction and reconsolidation of fear memory in rats

Authors: *R. R. WOOD[1], H. HAKE[1], A. SANCHEZ[1], E. C. LOETZ[1], M. OSTROVSKYY[1], E. B. OLESON[1], J. GRIGSBY[2], R. DOBLIN[3], B. N. GREENWOOD[1]
[1]Psychology, [2]Psychology and Med., Univ. of Colorado Denver, Denver, CO; [3]Multidisciplinary Assn. for Psychedelic Studies, Santa Barbara, CA

Abstract: 3,4-methylenedioxymethamphetamine (MDMA) paired with psychotherapy can reduce symptoms of post-traumatic stress disorder (PTSD) more effectively than psychotherapy or typical pharmacotherapy, either alone or in combination. However, the mechanisms by which MDMA might enhance psychotherapy remain unclear. Given that fear memories contribute to

PTSD symptomology, MDMA could augment psychotherapy by neurochemically targeting fear memories. We have investigated the effects of a single administration of MDMA on extinction and reconsolidation of fear memory in adult male Long-Evans rats. Our initial results indicate that low dose MDMA (1 or 2 mg/kg), administered 30 min before cued fear extinction, has no effect on fear extinction recall or fear renewal, whereas high dose MDMA (3 or 10 mg/kg) impairs cued fear extinction recall. In contrast, 5 mg/kg MDMA, but not 3 mg/kg MDMA, administered immediately after contextual fear memory reactivation, interferes with the reconsolidation of contextual fear memory. These data suggest that the therapeutic effects of MDMA could be mediated by a reconsolidation impairment, rather than an enhancement of fear extinction as previously suggested by studies in mice. However, it is unclear from these results whether the therapeutic effects of MDMA are specific to reconsolidation, or affect contextual fear memory in general. Our current studies are investigating the effects of 5 mg/kg MDMA (the dose previously observed to interfere with contextual fear memory reconsolidation) on contextual fear extinction and cued fear reconsolidation. Data collection is ongoing.

Disclosures: R.R. Wood: None. **H. Hake:** None. **A. Sanchez:** None. **E.C. Loetz:** None. **M. Ostrovskyy:** None. **E.B. Oleson:** None. **J. Grigsby:** None. **R. Doblin:** None. **B.N. Greenwood:** None.

Poster

415. Fear and Aversive Learning and Memory: Extinction

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 415.18/CCC9

Topic: G.01. Appetitive and Aversive Learning

Support: NIH MH114026

Title: Role of the dorsal striatum in fear extinction and relapse

Authors: *J. JAIME[1], M. K. TANNER[1], N. A. MOYA[1], J. DAVIS[1], E. C. LOETZ[2], B. N. GREENWOOD[2]
[2]Psychology, [1]Univ. of Colorado Denver, Denver, CO

Abstract: Fear extinction-based exposure therapy has poor long-term success for illnesses like PTSD. Previous work has shown that activation of substantia nigra dopamine (DA) neurons projecting to the dorsal striatum during cued fear extinction training enhances fear extinction recall and reduce relapse; however, the striatal circuits underlying enhanced extinction learning remain unknown. The striatum consists of two specific subregions; the dorsomedial striatum (DMS) and the dorsolateral striatum (DLS). The DMS supports “goal-directed” learning, while the DLS supports inflexible “habit learning”. The goal of this study is to investigate whether these dorsal striatum subregions differentially contribute to fear extinction learning and memory.

Following surgical implantation of bilateral cannulae into either the DMS or DLS, adult male Long-Evans rats were conditioned to fear a tone that terminated with a foot shock. After fear conditioning, rodents were injected with a $GABA_A/GABA_B$ agonist concoction (0.03 nmol Muscimol/0.3nmol Baclofen) to temporarily inactivate the DMS or DLS during fear extinction training. Preliminary data indicate that temporary inactivation of the DMS has no effect on extinction acquisition or recall, but reduces fear renewal in a novel context. In contrast, temporary inactivation of the DLS enhances fear extinction recall 24 h after extinction training, but has no effect on fear renewal. These results suggest that unique DA-striatal circuits could support different components of fear extinction. DLS inactivation could increase the DMS "goal-directed" contribution to extinction learning, resulting in an extinction memory strongly modulated by context. In contrast, DMS inactivation could increase the DLS "habit learning" contribution to extinction learning, rendering the extinction memory impervious to contextual modulation. These results have implications for novel strategies aimed at reducing the relapse of fear after extinction. <!--EndFragment-->

Disclosures: J. Jaime: None. **M.K. Tanner:** None. **N.A. Moya:** None. **J. Davis:** None. **E.C. Loetz:** None. **B.N. Greenwood:** None.

Poster

416. Reward Neurophysiology

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 416.01/CCC10

Topic: G.02. Motivation

Support: R01 MH108653-01
NARSAD Young Investigator Grant

Title: OFC glutamate activity and reward processing: Implications for schizophrenia

Authors: *S. BARNES[1], J. W. YOUNG[1], D. RAMANATHAN[1,3], L. FAGET[1], D. DILLON[4], T. S. HNASKO[2], M. A. GEYER[1]
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Abstract: Introduction: Reward deficits in schizophrenia are unresponsive to available medications and contribute significantly to poor functional outcome. A deeper understanding of the neural mechanisms underlying reward processing may identify novel therapeutic targets to improve reward function in schizophrenia. The ability to regularly update representations of reward value and use this information to guide decisions is necessary to maximize reward payoff. These processes can be quantified across species using the probabilistic reversal learning (PRL) task. The orbitofrontal cortex (OFC) is involved in detecting reward outcomes and is

disrupted in schizophrenia. Our aim was to determine how OFC glutamate neurons respond during PRL performance and identify how manipulation of OFC glutamate activity influences PRL performance.
Methods: Male Wistar rats were trained in the PRL task. In separate cohorts, AAV-CaMKIIa-GCaMP6f or AAV-CaMKIIa-ChR2-eYFP was injected into the medial OFC. Optic fibers were implanted dorsal to injection site to enable delivery of excitation light. Fiber photometry (FP) was used to record mOFC glutamate activity and optogenetics was used to activate mOFC glutamate neurons during PRL performance. PRL data was fitted to a Q-learning computational model according to $Qt+1 = Qt + \alpha \times PE$. α-Gain and α-Loss are learning parameters while β is a parameter in the softmax function that reflects choice behavior (exploratory or exploitative).
Results: FP revealed that mOFC glutamate neuron activity is suppressed when animals made a response that was rewarded. In contrast, no change in neural activity was observed when animals made a response that was punished. Optogenetic activation of mOFC glutamate neurons impaired PRL performance. When stimulation was delivered during accurate feedback presentation, animals completed fewer reversals, required more trials to complete the first reversal, and had a reduction in Win-Stay responding. Moreover, optogenetic stimulation elevated exploratory behavior (increased β) without affecting the ability to assign value to a stimulus (α-Gain or α-Loss).
Conclusions: These findings suggest that suppression of mOFC glutamate neuron activity is necessary for rewarding actions to be exploited. mOFC hyperactivity did not disrupt the ability to maintain accurate representations of value (indicated by normal learning parameters) but instead impaired the ability to appropriately use this information to guide choice behavior (reduced Win-Stay and increased β). These findings suggest that OFC hyperactivity in schizophrenia may contribute to impaired feedback-driven decision-making.

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Poster

416. Reward Neurophysiology

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 416.02/CCC11

Topic: G.02. Motivation

Support: CIHR FDN-147473

Title: Modulation of endocannabinoid-mediated plasticity within the orbitofrontal cortex by a palatable diet

Authors: *B. K. LAU, C. MURPHY-ROYAL, G. R. GORDON, J. S. BAINS, S. L. BORGLAND
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Abstract: The orbitofrontal cortex (OFC) plays a key role in the cognitive and emotional processing of decision-making. It is well established that the endogenous cannabinoid (endocannabinoid) system in the brain is important for appetite regulation. However, it is unknown if endocannabinoid signalling in the OFC is altered by an obesogenic diet. Using in-vitro patch clamp electrophysiology, we show that inhibitory GABAergic synaptic inputs onto pyramidal neurons within layer II/III of the OFC are sensitive to endocannabinoids. Specifically, they exhibit endocannabinoid-mediated short-term depression (depolarization-induced suppression of inhibition, DSI) and long-term depression (iLTD, via theta-burst stimulation). We then examined whether consumption of a palatable, energy-dense cafeteria diet altered endocannabinoid signalling within the OFC. We found there was a reduction in GABAergic synaptic transmission onto OFC pyramidal neurons following extended access (24 hr), but not restricted access (1 hr) to a cafeteria diet. This suppression of inhibition was partly reversed by the neutral CB1 receptor antagonist, NESS-0327, indicating the presence of tonic levels of endocannnabinoids. Associated with this endocannabinoid tone was an impairment of iLTD. We further showed that these obesity-induced synaptic alterations were mediated by upstream activation of Group 1 metabotropic glutamate receptors (mGluRs). Specifically, mGluR-iLTD induced by the Group 1 mGluR agonist, DHPG was impaired in obese animals, while endocannabinoid tone was blocked in the presence of the mGluR5 antagonist, MTEP. Interestingly, the impairments in iLTD were rescued in obese animals by restoring glutamate homeostasis, via the cystine/glutamate exchanger and glutamate transporter 1 (GLT-1) in astrocytes. Together, our findings suggest that long-term exposure to a palatable diet alters astrocyte modulation of glutamate homeostasis within the OFC, resulting in enhanced endocannabinoid signalling and tone via Group 1 mGluR activation, which then leads to decreased GABAergic synaptic transmission.

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Poster

416. Reward Neurophysiology

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Program #/Poster #: 416.03/CCC12

Topic: G.02. Motivation

Support: 1R01MH114882-01
2R01MH090264-06

P50 MH096890
P50 AT008661
F31 MH111108-01A1
T32 MH096678
T32 MH087004

Title: Orexin inputs to GABAergic lateral habenula neurons control aggression valence

Authors: *M. FLANIGAN[1], H. ALEYASIN[1], K. LECLAIR[1], E. K. LUCAS[3], B. A. MATIKAINEN-ANKNEY[4], A. TAKAHASHI[5], C. MENARD[1], S. BOUCHARD[1], M. L. PFAU[1], S. A. GOLDEN[6], E. S. CALIPARI[7], E. J. NESTLER[1], R. J. DILEONE[8], A. YAMANAKA[9], G. W. HUNTLEY[2], R. L. CLEM[1], S. J. RUSSO[1]
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Abstract: Heightened aggression is characteristic of multiple neuropsychiatric disorders and can have a wide variety of negative effects on patients, their families, and the public. Recent studies in humans and animals have implicated brain reward circuits in aggression and suggest that, in subsets of aggressive individuals, repeated domination of subordinate social targets is reinforcing. Here, we use a multidisciplinary approach to show that orexin neurons originating from the lateral hypothalamus activate a small population of GABAergic interneurons in the lateral habenula via orexin receptor 2 to promote aggression and conditioned preference for aggression-paired contexts. Our study suggests that the orexin system is a promising target for the development of novel therapies aimed at reducing aggressive behaviors and provides the first functional evidence of a local inhibitory circuit within the lateral habenula.

Disclosures: M. Flanigan: None. **H. Aleyasin:** None. **K. Leclair:** None. **E.K. Lucas:** None. **B.A. Matikainen-Ankney:** None. **A. Takahashi:** None. **C. Menard:** None. **S. Bouchard:** None. **M.L. Pfau:** None. **S.A. Golden:** None. **E.S. Calipari:** None. **E.J. Nestler:** None. **R.J. DiLeone:** None. **A. Yamanaka:** None. **G.W. Huntley:** None. **R.L. Clem:** None. **S.J. Russo:** None.

Poster

416. Reward Neurophysiology

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Program #/Poster #: 416.04/CCC13

Topic: G.02. Motivation

Support: PHS award R01-DA006214
NHMRC CJ Martin Award 1128089
NHMRC CJ Martin Award 1072706

Title: Chemogenetic activation of the PRAM pathway prevents light deprivation-induced augmentation of orexin/hypocretin expressing neurons and depressive-like behavior

Authors: *H. E. BOWREY[1], M. H. JAMES[2], G. S. ASTON-JONES[1]
[1]Brain Hlth. Inst., Rutgers Univ., Piscataway, NJ; [2]Brain Hlth. Inst., Piscataway, NJ

Abstract: ***Introduction:*** The 24 h light-dark (LD) cycle has a robust influence on behavior and mood. Deviations from this light cycle induce depression and depressive-like behavior, which can be ameliorated by artificially restoring normal light pattern information via phototherapy. We have recently demonstrated that the activation of a trisynaptic (retina->supra chiasmatic nucleus (SCN)->dorsomedial hypothalamus (DMH)->locus coeruleus (LC)) pathway, termed the PRAM (photic regulation of arousal and mood) pathway, prevents light deprivation-induced depression-like behavior. Previous research indicates that an orexin-dependent mechanism may underlie this depression-like behavior. As the DMH is a critical node in the PRAM pathway, we analyzed the expression of orexin in the DMH and the surrounding perifornical area (PF) and lateral hypothalamic (LH) orexin cell fields in animals that received PRAM stimulation during chronic light deprivation. ***Methods:*** Male Sprague Dawley rats received intraocular injections of an AAV encoding a Gq-linked designer receptor exclusively activated by designer drugs (DREADD: AAV2-hSyn-hM3D(Gq)-mCherry; *n*=12) or control virus (AAV2-hSyn-EGFP; *n*=10) . Rats were placed in continuous darkness for 8 wk, and those that received virus were concurrently subjected to daily i.p. injections of the DREADD agonist clozapine-*N*-oxide (CNO). A control group (*n*=10) received no virus and was maintained on a regular 12:12 light/dark cycle. Rats were then subjected to assays of mood (saccharin preference test, elevated plus maze and forced swim test) and vision (electroretinagram: ERG). Rats were perfused using 4% paraformaldehyde, and brains were cut into 40 µm thick cryosections. Hypothalamic tissue was immunohistochemically stained for orexin-A and c-Fos. ***Results:*** PRAM pathway stimulation enhanced retinal ERG signals and activated key PRAM pathway structures, including orexin neurons. Constant light deprivation induced a depression-like phenotype in control animals, which was augmented in DREADD animals given daily CNO. The abundance of orexin-A-immunoreactivity (IR) in DMH, LH and PF was affected by chronic light deprivation. Activation of the PRAM pathway prevented the light deprivation-induced reduction of orexin-A-IR in DMH, LH and PF. ***Conclusions:*** Dysfunctional orexinergic signaling may underlie light deprivation-induced depression-like behavior. PRAM pathway stimulation may prevent the augmentation of orexin-A-IR that occurs as a result of light deprivation. The PRAM pathway presents a novel circuit for the regulation of mood, and thus a possible new direction for the treatment of depression in humans.

Disclosures: M.H. James: None. **G.S. Aston-Jones:** None.

Poster

416. Reward Neurophysiology

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Topic: G.02. Motivation

Support: USDA-NIFA NJ06180
NHMRC CJ Martin Award 1072706
PHS award R01-DA006214
NHMRC CJ Martin Award 1128089

Title: Signaling at the orexin/hypocretin-1 receptor mediates food motivation in female rats after excessive weight gain and binge-like eating

Authors: *S. LIU[1], M. H. JAMES[1,3], S. WALSH[1], H. E. BOWREY[1,4], N. T. BELLO[2], G. ASTON-JONES[1]
[1]Brain Hlth. Inst., Rutgers, The State Univ. of New Jersey, Piscataway, NJ; [2]Dept. of Animal Sci., Rutgers, The State Univ. of New Jersey, New Brunswick, NJ; [3]Florey Inst. of Neurosci. and Mental Hlth., Parkville, Australia; [4]Save Sight Inst., Sydney, Australia

Abstract: ***Introduction:*** Binge eating disorder (BED) is characterized by a progressive escalation of intake of highly palatable food and increased responsivity to food-associated cues that induce craving and overeating. In this way, many of the clinical characteristics of BED closely resemble symptoms of drug abuse disorders, pointing to possible commonalities in the neural circuitry underlying these disorders. We and others have reported that the hypothalamic orexin (hypocretin) system is critically involved in the expression of highly-motivated drug seeking behavior, however its role in compulsive food seeking is not well understood. Here, we tested the hypothesis that orexin-1 receptor signaling mediates food motivation in female rats with a history of binge-like eating. In addition, we examined the role of obesity on our outcomes. ***Methods*****:** Using a within subjects design, female Long-Evans rats were assessed for baseline economic demand for sucrose using a novel behavioral economics paradigm. Binge-like eating was induced by exposing rats to sweetened fat (vegetable shortening/10% sucrose) for 30 min, twice/wk for 4wk, before being re-assessed for sucrose demand and following injections of the orexin-1 receptor antagonist SB-334867 (0,10,30mg/kg, ip). Rats were then exposed to a high fat diet (HFD; 45% fat) for 8wk, and the experiment was repeated. Sweetened fat binge intake was measured following SB dosing. ***Results****:* Binge eating increased sucrose demand only after HFD-exposure, which was dose-dependently reversed by SB. Binge intake was not altered by HFD exposure. SB also dose-dependently decreased sweetened fat binge intake after HFD exposure. ***Conclusions****:* Our findings indicate an interaction between binge-like eating and excessive weight gain with respect to motivation for food. This effect was blocked by an orexin-1

antagonist, highlighting the orexin system as a potential novel target for pharmacotherapies for controlling overeating episodes in individuals with obesity. Ongoing studies are investigating the effect of excessive weight gain and binge-like eating on other properties of the orexin system.

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Poster

416. Reward Neurophysiology

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Program #/Poster #: 416.06/DDD1

Topic: G.02. Motivation

Support: PHS award R01-DA006214
NHMRC CJ Martin Award 1072706
NHMRC CJ Martin Award 1128089

Title: Variable episodic self-administration enhances economic demand for cocaine and sensitivity to the orexin/hypocretin receptor antagonist SB-334867

Authors: *M. H. JAMES[1,2], H. E. BOWREY[1,3], J. E. FRAGALE[1], G. ASTON-JONES[1]
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Abstract: Introduction: Cocaine addicts rarely report using drug on a daily basis; in most cases, addicts use 3-4 times/week. This can be due to self-imposed abstinence, or related to issues that are difficult to predict, including drug supply, financial constraints or legal intervention. Despite this, models of cocaine self-administration typically involve giving animals access to drug on a highly predictable daily schedule 5-7d/week. Here, we tested whether introducing periods of abstinence of varying duration between self-administration sessions produces stronger addiction-like endophenotypes. We also tested the effect of the orexin-1 receptor antagonist SB334867 (SB) on the expression of these endophenotypes. **Methods:** Male Sprague-Dawley rats were assessed for 'baseline' economic demand for cocaine using our within-session threshold procedure (Bentzley et al., 2014). Rats were then given short (ShA; 1h) or intermittent (IntA; 5min access every 30min for 6h) access to cocaine on either a daily basis (14 sessions over 14 consecutive days) or a variable episodic basis (14 sessions over 28d, with 1-4d between sessions). Rats were then re-assessed for economic demand, as well as several other addiction endophenotypes. The effect of SB (0, 10, 30mg/kg) on demand and reinstatement behavior was assessed. **Results:** As per our recent studies (James et al., 2018), daily IntA was associated with greater escalation of intake, higher economic demand, higher compulsive responding and higher depression-like behavior compared to daily ShA. This IntA-induced multiphenotype was

exaggerated even further by variable episodic IntA. In contrast, variable episodic access did not affect the expression of addiction endophenotypes in ShA animals. SB was most effective at reducing demand and cued reinstatement behavior in variable episodic-IntA rats. **Conclusions:** These data indicate that IntA and variable episodic self-administration patterns interact to produce an exaggerated addiction-like phenotype, which is hyper-reliant on signaling at the orexin-1 receptor. Ongoing studies are investigating the effect of variable episodic self-administration patterns on orexin peptide expression in discrete subregions of the lateral hypothalamus, and orexinergic innervation of reward and stress centers.

Disclosures: M.H. James: None. **H.E. Bowrey:** None. **J.E. Fragale:** None. **G. Aston-Jones:** None.

Poster

416. Reward Neurophysiology

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Title: Intermittent access to fentanyl increases economic demand through an orexin/hypocretin mechanism

Authors: *J. E. FRAGALE[1], M. H. JAMES[1], V. BEHMAN[1], A. POLO[1], G. S. ASTON-JONES[2]
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Abstract: Our lab recently found that the intermittent access (IntA) self-administration model (Zimmer et al., 2012) produces a multifaceted addiction-like phenotype for cocaine that includes escalation of intake, increased motivation, greater cue-induced reinstatement, and a negative emotional state (James et al., 2018). While the IntA model has been successfully applied to the study of cocaine addiction, it has yet to be applied to opioids. Here, we extend the IntA model to the study of fentanyl self-administration. Rats were either given IntA (6 hr sessions with 5 min access to fentanyl every 30 mins) or short access (ShA; 1 hr) to the opioid fentanyl for 14 consecutive days. Following IntA or ShA, economic demand for fentanyl was assessed using a within-session behavioral economics procedure (Bentzley et al., 2012). IntA and ShA rats were also tested for a variety of other addiction-like behaviors. Like IntA to cocaine, we found that IntA to fentanyl produced a robust and persistent increase in motivation (decreased demand elasticity) without effecting hedonic set point (Q_o). IntA rats also showed increased cue-induced reinstatement of drug seeking and a depressive-like phenotype. We recently reported that IntA to

cocaine is associated with the persistent augmentation of lateral hypothalamic orexin/hypocretin function and that the addiction-like behaviors produced by IntA to cocaine are reversed by blocking orexin-1 receptor signaling (James et al., 2018). To determine the importance of the orexin system in the expression of addiction-like behaviors produced by IntA to fentanyl, IntA and ShA rats were treated with the orexin-1 receptor antagonist SB-334867(SB; 3, 10, and 30 mg/kg i.p.) 30 min prior to testing. We found that SB dose-dependently decreased motivation for fentanyl (increased demand elasticity) and blocked cue-induced reinstatement in IntA rats but not in ShA rats. Our results indicate that the IntA model can be extended to opioids and is a useful tool in the study of opioid addiction. Moreover, the data indicates that the orexin system is a potential target for the treatment of addiction across various drugs of abuse.
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Poster

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Topic: G.02. Motivation

Support: NHMRC CJ Martin Award 1128089
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PHS award R01-DA006214

Title: Cocaine self-administration disrupts the diurnal fluctuation in orexin (hypocretin) cell number and activity

Authors: *A. CHANG[1], M. H. JAMES[1,2], H. E. BOWREY[1,3], K. PENG[1], S. L. O'CONNOR[1], J. E. FRAGALE[1], G. ASTON-JONES[1]
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Abstract: ***Introduction:*** Addiction is associated with disruptions in sleep and circadian rhythmicity, and several addiction-related behaviors exhibit clear circadian regulation. The brain systems underlying these phenomena remain poorly understood. We and others have demonstrated that the hypothalamic orexin (hypocretin) system is a critical regulator of drug-seeking behavior. Orexin neurons exhibit significant diurnal fluctuations in cell number and activity; both measures are significantly higher during the active period relative to the inactive period. Here, we tested whether these regular fluctuations in orexin function are disrupted by cocaine self-administration. ***Methods***: Male Sprague Dawley rats were trained to self-administer

cocaine on a fixed-ratio 1 (FR1) schedule in 2h daily sessions with each session beginning precisely at ZT (Zeitgeber Time) 5. Following acquisition, rats progressed to 4h daily sessions (ZT5-9) that continued for 21d. A control group was disturbed at an equivalent time each day but was not given access to cocaine. Homecage cameras recorded activity in the 2h preceding the self-administration sessions (ZT3-5) in both cocaine and control animals. These videos were manually scored based on six different behaviors: grooming, ambulation, eating, drinking, rearing, and nesting, producing an aggregate activity score or an index of 'cocaine anticipatory activity' (CAA). The day after the final self-administration session, rats were perfused at 4h intervals and brains were processed for orexin and Fos immunoreactivity. ***Results:*** Cocaine intake escalated across the 21d self-administration period. This was associated with a concomitant increase in the expression of CAA. The magnitude of CAA was positively correlated with cocaine-seeking behavior. No change in homecage activity was observed in control rats across the same period. Diurnal fluctuations in orexin cell number and activity were disrupted in cocaine animals but were unaffected in controls. ***Conclusion:*** Rats exhibit an increase in general activity in period immediately preceding cocaine self-administration, indicating that cocaine may act as an entrainable cue. Cocaine self-administration disrupts regular diurnal fluctuations in orexin function, indicating that this system may contribute to circadian regulation of drug-seeking behavior.

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Poster

416. Reward Neurophysiology

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Program #/Poster #: 416.09/DDD4

Topic: G.02. Motivation

Title: Temporal modulation of TANs and PANs in monkey ventral striatum related to reward size and delay to obtain it

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Abstract: We recorded neuronal responses from 50 TANs (Tonically Active Neurons) and 101 PANs (Phasically Active Neurons) from ventro-medial part of the striatum of two monkeys while they performed a task in which 9 combinations of reward were offered by mixing 3 sizes (2, 4 or 6 drops of water) and 3 delays (1, 4 or 7s). A visual cue predicting the combination being offered was presented throughout the trial. The monkeys were required to respond in one of two periods represented by the appearance of a yellow or a purple dot. On the appearance of yellow

dot, the monkeys could refuse the offer by releasing a bar immediately or accept by releasing when a purple dot appeared. If a purple dot appeared, the monkeys could accept by releasing immediately or refuse by waiting for the yellow dot. To capture response feature that might represent the information, we used principal component (PC) analysis of the responses elicited by the visual cue. The principal components from all conditions regardless of cue were extracted and the scores of those were examined. We focused on the period immediately following the onset of the visual cue, but before any action were required. We found that for basically all of the TANs (44/50) and almost all of the PANs (72/101) the cue-elicited responses were related to the two factors, reward size and delay as seen through ANOVAs with reward size and delay as factors and spike count or coefficient for the first principal component (PC1) as dependent variable. The information about the offered reward captured by PC1 for both types of neurons expressed as percentage of variance explained by the two factors from the ANOVA, was similar between the two groups (N=116; mean ± SD: 17.9% ± 17.7 and 17.6% ± 11.8 for PANs and TANs respectively; two-sample t-test: 114 df, p=0.92). However, there was striking difference between TANs and PANs in how the neural activity was modulated according to the offered reward. For 24/44 TANs and for all 72 PANs the firing modulation was represented by counting the number of spikes. For the other 20 TANs the modulation was greatly underestimated using the spike count; it was, however, strongly represented as a temporal modulation of the response. That is, for the group having basically no correlation between PC1 and spike count, there is a temporally modulated code carrying information about reward size and delay to reward.

Disclosures: R. Falcone: None. **D. Weintraub:** None. **B. Richmond:** None.

Poster

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Topic: G.02. Motivation

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Title: Attentional allocation predicts reward anticipation during appetitive Pavlovian conditioning

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Abstract: Neural value representations underlie a wide range of behaviors, from simple consumatory actions to complex economic decisions. In a prior report, we identified frontal lobe value representations that were modulated by overt shifts of attention towards value-associated visual cues (i.e. by shifts of gaze during free viewing). Here, we investigate the behavioral sequelae of this attentional modulation. Specifically, we ask how overt shifts of attention influence a behavioral indicator of reward anticipation, and whether this influence is mediated by attention-driven modulation of the orbitofrontal cortex (OFC).
Two macaque monkeys performed an appetitive Pavlovian conditioning task in which simple color cues were presented on a display for 4 seconds, followed by a juice reward whose volume depended on the cue color. Fixation was not enforced during the 4-second cue presentation, and overt shifts of attention (gaze) towards or away from the cues were measured using an eye tracker. Also measured during cue presentation were reward anticipation, indicated by Pavlovian conditioned licking responses (CRs) before reward delivery; and single neuron activity in OFC, recorded using standard methods.
In general, attention predicted reward anticipation: the longer the monkeys spent looking at a cue at a given time point in a trial, the more likely they were to produce a CR at a later time point in that trial. To address neural mechanisms, mediation analysis measured the extent to which the attention-CR correlation could be statistically explained by the concurrently recorded firing of single OFC neurons. The resulting mediation effects, averaged across 105 OFC neurons, were indistinguishable from chance, meaning there was no evidence that OFC participated in the neural mechanism linking attention and reward anticipation. Finally, to determine whether OFC activity could predict reward anticipation independent of attention, we performed a partial correlation analysis in which linear effects of attention were removed. We found a weak, but significant correlation between OFC firing and CR probability; as with the attention-CR correlation, OFC activity at a given time point in a trial predicted CR probability at a later time point in that trial.
We conclude that overt attentional shifts and OFC activity both predict reward anticipation, and appear to do so independently from one another. However, it is still unclear what neural mechanism links attention and reward anticipation, and whether a similar mechanism may underlie the well-documented influence of attention on economic choice.

Disclosures: V.B. McGinty: None. **W.T. Newsome III:** None.

Poster

416. Reward Neurophysiology

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Program #/Poster #: 416.11/DDD6

Topic: G.02. Motivation

Title: Neural correlates of intrinsic motivation and performance during training

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Abstract: Intrinsic motivation can influence task performance and may be observable via electroencephalography (EEG) of brain activity. In our first study, task-based performance during both a feedback-based go/no-go training and subsequent transfer tasks was found to relate to components of intrinsic motivation. A second, follow-up study found a neural relationship with the same component of intrinsic motivation. Subjective data on intrinsic motivation were collected across 90 participants and correlated with performance metrics for both training and transfer tasks. EEG was used to identify the relationship between spectral power across electrodes and measures of intrinsic motivation across 32 participants.
The first study focused on the relationship of accuracy and various subscales of the Intrinsic Motivation Inventory (IMI) including enjoyment, perceived competence, and effort. This study identified a positive correlation between correct rejection and perceived competence in the training task ($r(88) = .316$, $p < .005$) and overall accuracy and both perceived competence ($r(88) = .414$, $p < .005$) and enjoyment ($r(88) = .400$, $p < .005$) in the subsequent target identification transfer task. The second study correlated the same subscales of IMI with a time-frequency decomposition of EEG data acquired using a similar training and transfer task as the previous experiment. Only the perceived competence subscale produced a significant relationship with time-locked spectral power of the last 100 trials of the task. A cluster-based non-parametric correction identified a single cluster ($p< 0.05$) of significant coefficients extending from -0.5 to 1.0s around the stimulus onset within the beta band. The focal points of this cluster resided in channels over the left anterior and posterior brain regions along with channels over the anterior midline. The topography of this cluster suggests a distributed network with beta-band activity that decreases in power as perceived competence increased across participants.
Previous studies have demonstrated that perceived competence after receiving feedback-based training relates to performance metrics on that task for specific situations. The results reported here from the first study corroborate those findings and EEG results from the second experiment extend those findings by identifying neural correlates. Taken together, findings from both studies suggest that neural patterns emerge as the task progresses that reflect both performance and perceived competence during this feedback based task. Future work can assess whether these brain regions can be monitored directly to assess effectiveness of feedback in real-time.

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Poster

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Topic: G.02. Motivation

Title: Modulation of motor resonance by the reward value associated with observed actions

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Abstract: The ability to interpret the goals and intentions of others' actions plays a significant role in our every-day interactions. An electrophysiological study by Caggiano et al., (2012) proved that mirror neurons, which discharge when a monkey executes a particular action and when it observes another individual perform the same or a similar action, are modulated by the value associated with the observed movement. In humans, action observation related motor activity, also refered to as 'motor resonance', is modulated by contextual information accompanying others' actions. Interestingly, we recently demonstrated that even in the absence of movement kinematics, reliable contextual information is enough to trigger significant muscle-specific motor resonance in primary motor cortex (M1) of observers. However, it is unknown whether motor resonance in human M1 is modulated by the value associated with an observed action.

For this purpose, we employed single-pulse transcranial magnetic stimulation (TMS) and measured changes in motor-evoked potential amplitude (MEP) in the index (FDI) and little finger (ADM) muscles while participants observed either full view or partly occluded grasping videos that were associated with either high or low reward. The reward was contigent on whether the upcoming grasping action would involve a whole-hand (WHG) or precision-grip (PG), respectively. A number written on the cues indicated whether the current trial was associated with a high or low reward (e.g.,'100'= participant could receive 100 points; '1'= participant could receive 1 point), while the colors of the cues indicated which grip type would lead to winning the reward.

Our prelimimary statistical results show that the reward value influences muscle-specific motor resonance in M1 (reward x muscle specificity $p = .048$). Post hoc analyses revealed a significantly higher motor resonance during the observation of high- versus low-reward trials in the ADM muscle ($p=.003$) but not in the FDI muscle ($p=.68$). These findings support the vital role of the action observation system to the understanding of other's actions.

Disclosures: N. Wenderoth: None. **A. Cretu:** None.

Poster

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Topic: G.02. Motivation

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Title: Dopamine regulation of A-type potassium channels underlies individual differences in motivation for reward

Authors: *B. O'DONOVAN[1], A. ADELUYI[3], S. A. SAMARANAYAKE[2], A. GALLOWAY[1], P. HASHEMI[2], J. R. TURNER[3], P. I. ORTINSKI[1]
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Abstract: Motivational drive for reward is an important component of appetitive behavior that varies significantly between individuals. While it is known that dopamine signaling and medium spiny neuron (MSN) firing patterns in the nucleus accumbens (NAc) shell play a prominent role in response to reward; the relationship between endogenous dopamine levels, neuronal output and individual differences in motivation is unclear. In this study we find that, among naïve Sprague-Dawley rats, individual performance on a sucrose-reinforced progressive ratio task is linked to pronounced differences in the NAc dopamine levels in vivo. Rats motivated to complete higher ratios of active lever responding, have increased phasic NAc shell dopamine levels, but similar rates of dopamine clearance, relative to rats completing low ratios of responding. Whole-genome RNA sequencing highlights substantially divergent transcriptome profile among rats that differ in motivational response to sucrose, with notable enrichment at genes related to dopamine signaling and at voltage-gated, including A-type, potassium channel genes. Furthermore, NAc shell MSNs of highly motivated rats have decreased action potential output and increased action potential afterhyperpolarization, which is mediated by A-type potassium current activity. Animals with low motivation for sucrose show increased sensitivity to cocaine effects on behavioral, dopamine signaling, and neuronal excitability measures, whereas cocaine effects are blunted in animals with high motivation for sucrose. This work identifies molecular and functional adaptations that reflect individual motivation for reward in an effort-based task. We show that motivational response to reward is determined by an interaction between dopamine signaling and A-type potassium channels in the NAc.

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Poster

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Title: Nucleus accumbens shell single-unit and local field potential activity encoding of outcome probability estimation

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Abstract: The estimation of reward probability allows an organism to make predictions, adjust its action selection and maximize the cost-benefit of the available response options. This flexible behavior is essential during foraging where, by estimating the probability that a food source is replenished or depleted, the animal needs to minimize energy expenditure and maximize the energy obtained. The capacity to assess reward probability relies on a complex network that includes the Nucleus Accumbens (NAc). Indeed, dopamine levels in the NAc Core are sensitive to reward rate. However, it remains unexplored the role of the NAc Shell in the estimation of reward probability at the single neuronal activity level. To address this issue, we recorded single units and local field potentials (LFP) of the NAcSh, while rats performed an adaptive decision-making task. During a trial, the subject had to approach and place its nose into a central port for 0.7s; subsequently, an auditory stimulus was delivered (Go cue), indicating the subject to press the left or the right lever, each one was associated with an independent reward probability: 10-90, 10-50, 50-90, 50-50%, and counterbalanced. Then the rat had to move to the opposite panel to emit two dry licks in the sipper, followed by three drops of sucrose (one per lick) if rewarded, or dry licks, if unrewarded. The probabilities assigned to each lever were changed periodically every 50 trials without any explicit signal. Thus, animals must continuously estimate reward probability across the session. We found neurons that were selectively modulated by the Go cue, the lever press, or the consummatory behavior; while other neurons encoded more than one feature of the task, displaying a more sensoriomotor integrative profile. Interestingly, some units closely covaried with reward probability. Indeed, a principal component analysis (PCA) confirmed that most of the NAcSh neurons were sensitive to dynamic changes in reward probability across the session, suggesting an abstract representation of reward expectancy. Finally, the LFPs displayed an increase in power at delta, theta, beta, and gamma oscillations in

rewarded trials; furthermore, there were a ramping increase in LFP power as the subject approached to the impending reward. Nevertheless, no modulation in LFP oscillations were observed when reward was omitted neither to the movement direction. We concluded that single unit responses in the NAcSh estimated, trial by trial, reward probability, as well as other variables which could help the subject to increase the chance to obtain a reward; while LFP oscillations were selectively tracking reward expectancy.

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Poster

416. Reward Neurophysiology

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Topic: G.02. Motivation

Support: Wellcome Trust

Title: Effort disutility measured in behaviour and dopamine neuron activity of non-human primates

Authors: *M. H. BURRELL, A. PASTOR-BERNIER, W. SCHULTZ
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Abstract: Introduction: Reward follows work, but with finite energy, all life must decide what reward is worth the work. Economic theory provides a framework of how rational decision makers optimise this choice: agents should maximise the possible utility (subjective benefit) discounted by the disutility (subjective cost) of the required effort. The dopamine reward prediction error signal is a potential neuronal substrate of this utility calculation as it is known to capture subjective value of rewards, risk and time. Other work has demonstrated pathologies of midbrain dopamine neurons and drugs that target dopaminergic signalling affect the willingness to expend effort. However, it is not clear how effort disutility is represented in the reward prediction error signal. Here, we provide experimental evidence of the fundamental assumption of a subtractive cost of effort and use this to investigate a biological basis of this valuation process in midbrain dopamine neurons. Methods: Two rhesus macaques (*Macaca mulatta*) made repeated binary choices using a custom-built joystick, in which the reward and effort to obtain the reward were varied. From these choices, we independently estimated the utility of juice reward and disutility of effort using random utility models, validating these models with out-of-sample tests. The activity of dopamine neurons in the Substantia Nigra and Ventral Tegmental Area in one animal was measured using single-cell extracellular recordings during choice. Dopamine neuron activity was compared to the subjective values of reward and effort within a temporal-difference learning model. Results: Empirical choice behaviour was closely matched to

that predicted by the estimated net utility (reward utility minus effort disutility) of choices providing evidence for the subtractive economic model of effort disutility. Dopamine neuron activity captured the utility of reward, as previously demonstrated, but critically also correlated with the estimated disutility of effort at the time of the conditioned stimulus. Moreover, this signal followed subtle shifts in effort disutility over time as predicted by behavioural data. These data suggest dopamine neurons capture the cost of effort by encoding net utility and hence convey value signals that are appropriate for decision-making involving effort.

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Poster

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Support: Wellcome Trust

Title: Satiety changes neuronal correlates of revealed preference in monkeys

Authors: *A. PASTOR-BERNIER[1], A. STASIAK[2], W. SCHULTZ[3]
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Abstract: Introduction Rewards or goods contain typically at least two attributes or components, such as taste and calories. We often trade-in some amount of one component to gain one unit of the other component. Experimentally, these components can be modeled as distinct rewards in bundles of two goods. By trying to obtain the most preferable combination of two goods, we are expressing our preferences (revealed preference) and are aiming to maximize their utility. Our previous study demonstrated that monkeys' choices conform with revealed preference theory. Revealed preference was assessed in choices between a reference bundle and a variable bundle. In the variable bundle, a quantity of one juice is given up in order to gain one unit of the other juice without losing overall value. This trade-off is fair when each bundle is chosen by the animal with equal frequency, as assessed by psychophysics (indifference point). Repeated tests with systematic trade-offs resulted in a series of indifference points that conformed to an indifference curve. In the current study, we used revealed preference tests to investigate behavioral and neuronal changes in relation to the satiety of one of the bundle rewards (sensory-specific satiety). Methods The animals were presented with two bundles, each containing two juices on which they became sated to different degree. We assessed sensory-specific satiety as change in preference between the two juice rewards, which was manifested as difference in the slope of the indifference curve. We studied single neuron activity in orbitofrontal cortex (OFC)

during choices among two bundles across unsated and sated states. A support vector machine classifier (SVM) was trained on neuronal responses during unsated and sated states. Results Indifference curves showed slope changes for the juice reward on which the animal was more sated, reflecting a relatively lower preference for that juice. OFC responses to the bundles showed correspondingly stronger changes to the juice on which the animal was more sated, resulting in similar slope changes of neuronal indifference curves. SVM analysis showed that neuronal responses predicted well the satiety-induced, changed behavioral preferences. These results demonstrate that revealed preference theory provides valuable tools to demonstrate neuronal correlates of sensory specific satiety.

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Poster

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Title: Cell type-specific and non-specific mechanisms of cholinergic interneuron-mediated plasticity onto MSNs

Authors: *W. T. FLEMING, J. LEE, I. B. WITTEN
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Abstract: Cholinergic (ChAT) interneurons in the nucleus accumbens (NAc) bidirectionally regulate the extinction of cocaine associations, likely through their modulation of plasticity on medium spiny neurons (MSNs). In particular, cocaine extinction is associated with a gradual reduction of glutamatergic presynaptic plasticity onto MSNs, and the activation of ChAT cells has been shown to hasten this plasticity (Lee et al., Neuron 2016). However, whether ChAT cells differentially control plasticity onto the two major subtypes of MSNs--those that express D1 receptors (D1Rs) versus D2 receptors (D2Rs)--has not been examined. This is an important question because D1R and D2R MSNs are thought to undergo different forms of dopamine-dependent plasticity, and contribute differentially to reward-related behaviors. Therefore, to compare ChAT-mediated plasticity in D1R and D2R MSNs, we used double transgenic mice (either ChAT::Cre/Drd1a::tdTomato or ChAT::Cre/Drd2::EGFP) to allow for modulation of ChAT cells during behavior as well as identification of subtype-specific MSNs during

electrophysiological recordings. We bilaterally injected an AAV expressing Cre-dependent ChR2-eYFP into the NAc and optogenetically activated ChAT cells during extinction of a cocaine conditioned place preference (CPP). Immediately after, we performed ex vivo whole-cell recordings of D1R or D2R MSNs to measure changes in plasticity mediated by ChAT cells. Consistent with previous findings, ChAT activation during extinction testing significantly reduced cocaine CPP (mean reduction 115.6 ± 27.8 secs in 15 min test; p=0.014 for group, two-way ANOVA; n=18 stim, n=28 control). In addition, ChAT activation during extinction testing reduced the frequency of mini excitatory post-synaptic currents (mEPSCs) at both D1R MSNs (p=0.002, linear, mixed-effects regression (LME); n=8 stim; n=10 control) and D2R MSNs (p=0.001,LME; n=12 stim, n=12 control), suggesting that ChAT activation has a presynaptic effect on glutamatergic transmission that is not specific to MSN subtype. We also observed that ChAT activation reduced mEPSC amplitudes at D2R MSNs (p=0.008, LME; n=12 stim; n=12 control), but not D1R MSNs (p=0.893, LME; stim n=8; control n=10), suggesting that ChAT activation may have cell type-specific postsynaptic effects. Together, these findings begin to clarify both cell type-specific and non-specific mechanisms by which ChAT interneurons regulate synaptic plasticity and behavior.

Disclosures: W.T. Fleming: None. **J. Lee:** None. **I.B. Witten:** None.

Poster

416. Reward Neurophysiology

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

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Topic: G.02. Motivation

Support: NYSCF-Robertson Neuroscience Investigator Award
NIH DP2 DA035149-01
NIH 5R01MH106689-02

Title: Spatial segregation of movement and reward encoding across the basal forebrain cholinergic system

Authors: *J. CHOI[1,2], J. AU[3], H. JANG[1], E. A. ENGEL[1], I. B. WITTEN[1,2]
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Abstract: Ascending cholinergic transmission from the basal forebrain (BF) has been implicated in numerous aspects of behavior and cognition, including learning, memory, attention and reward. However, due to the sparse and distributed nature of these cholinergic neurons, it remains unclear if across different BF subregions these neurons respond in a homogeneous or heterogeneous manner. To address this question, we performed fiber photometry to record from

cholinergic neurons across two BF subregions (medial septum, MS, and nucleus basalis, NB) in ChAT::Cre rats that were trained to perform a delayed non-match to position task in operant chambers (MS: n = 8 recordings, NB: n = 17 recordings, 16 ChAT::Cre males and 1 female, ~3-8 months of age).
In the MS, a substantial fraction of the variability in cholinergic activity was explained by the animal's locomotor behavior, while task events provided little additional explanation of neural activity (average variance explained by speed: R2 = 0.32 + 0.02; explained by task events and speed: R2 = 0.40 + 0.024; mean+se). In contrast, in the NB, cholinergic activity was poorly explained by locomotion, while task events accounted for a substantial fraction of response variability (average variance explained by speed: R2 = 0.087 + 0.03; explained by task events; R2 = 0.33 + 0.028; mean+ se). Further, within the NB, responses were spatially organized, as medial recording sites (within the internal capsule) displayed transiently elevated activity during the reward-seeking action and reward onset, whereas lateral recording sites (within the posterior globus pallidus) exhibited elevated activity throughout reward consumption. These findings provide some of the first evidence in support of spatial organization of heterogeneous functional properties within the BF cholinergic system.

Disclosures: J. Choi: None. **J. Au:** None. **H. Jang:** None. **E.A. Engel:** None. **I.B. Witten:** None.

Poster

416. Reward Neurophysiology

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Program #/Poster #: 416.19/DDD14

Topic: G.02. Motivation

Support: NIMH Grant RO1MH110594
NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation, the Edward Mallinckrodt, Jr. Foundation, and the Department of Neuroscience, Washington University School of Medicine.

Title: A neural pathway for information seeking: A cingulate-striatum-pallidum network predicts gaze shifts to objects associated with uncertain rewards

Authors: *E. S. BROMBERG-MARTIN[1], J. K. WHITE[1], K. ZHANG[2], J. PAI[3], I. E. MONOSOV[1]
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Abstract: When faced with delayed and uncertain rewards, both humans and animals search for information from their environment to learn what the future holds. However, the neural systems

that motivate information seeking have been primarily investigated in economic choice tasks. Less is known about the most fundamental form of natural information seeking behavior: shifting the eyes to inspect the source of uncertainty. We hypothesized that information seeking is motivated by neurons that we recently discovered in anatomically connected regions of the anterior cingulate cortex (ACC), dorsal striatum (DS), and ventral pallidum (VP). These neurons are selectively activated by visual objects associated with uncertain future rewards, with sustained ramping activity anticipating the moment the uncertainty can be resolved by getting information about the future outcome. This activity would be ideal to motivate gaze shifts to the source of uncertainty to gain information about future outcomes. To test this, we examined neuronal activity and eye movements while monkeys freely gazed at visual stimuli associated with different levels of reward uncertainty and different times of receiving information to resolve the uncertainty. Animals learned that visual conditioned stimuli (CSs) predicted juice reward with different probabilities and amounts, including both certain and uncertain CSs (e.g. 100% vs. 50% chances of reward). In an "information anticipation" version of the conditioning procedure, one set of *informative CSs* were followed by an informative cue that appeared 1s after CS onset and indicated the upcoming reward size in advance of its delivery (thus resolving any uncertainty). A second set of *non-informative CSs* were followed by non-informative cues that did not indicate the upcoming reward size (and hence did not resolve uncertainty). We found that the expectation of gaining information had strong, correlated effects on neurons and eye movements. (1) Many neurons in all three brain areas had activity ramping to the expected time of receiving information. (2) Eye movements to gaze at the CS had a similar information-anticipatory time course. (3) Neural information signals had strong moment-to-moment correlations with gaze (stronger signals during gaze at uncertain CSs). (4) This enhancement of neural information signals started hundreds of milliseconds *before* animals shifted their gaze onto the CS. Thus, the ACC-DS-VP network is ideally positioned to guide information seeking: it anticipates information about future rewards and activates before gaze shifts toward the source of uncertainty.

Disclosures: J.K. White: None. **K. Zhang:** None. **J. Pai:** None. **I.E. Monosov:** None.

Poster

416. Reward Neurophysiology

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Program #/Poster #: 416.20/DDD15

Topic: G.02. Motivation

Support: NIMH RO1MH110594
NARSAD Young Investigator Grant

Title: A neural pathway for information seeking: Causal manipulations of regions in the cingulo-striatum-pallidum network and their effects on the motivation to resolve uncertainty

Authors: *I. E. MONOSOV[1], J. K. WHITE[2], E. S. BROMBERG-MARTIN[1], K. ZHANG[3], J. PAI[4]
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Abstract: What brain systems motivate us to resolve our uncertainty and gain information about the future? While little is known about the underlying neural pathways, we recently discovered a promising candidate. A subpopulation of neurons in an anatomically connected network comprising of the anterior cingulate cortex (ACC), dorsal striatum (DS), and ventral pallidum (VP) are selectively activated by visual objects associated with uncertain rewards, and have sustained ramping activity that anticipates the moment of gaining information about the future outcome (i.e. resolving the uncertainty). This raises a crucial question: is their activity *passively* anticipating the gain of information, or *actively* motivating animals to seek information and resolve their uncertainty? Our linked poster by Bromberg-Martin et al. is consistent with an active role, showing that fluctuations in the activity of these neurons predict the motivation to shift gaze to inspect uncertainty-related objects. Here we put this hypothesis to a direct test by pharmacologically inactivating the basal ganglia regions of the uncertainty-related network. These inactivation experiments were done while monkeys performed a saccadic reaction time task to measure their motivation to obtain information about future rewards. On each trial, a visual conditioned stimulus (CS) was presented peripherally and predicted an uncertain, 50% chance for juice reward to be delivered 3 sec after CS onset. There were two types of CSs. CS1 was *informative*: when the animal shifted gaze onto the CS, the CS was replaced with an informative cue that indicated whether the trial was going to be rewarded (and hence immediately resolved the uncertainty). CS2 was *non-informative*: when the animal gazed at the CS, it was replaced with a non-informative cue that did not predict the upcoming outcome (and hence caused uncertainty to remain until the end of the trial when the outcome was delivered). Importantly, the animal's reaction time to gaze at the CS had no influence on the timing or amount of juice reward; it only influenced their speed of gaining access to the informative or non-informative cues. We found that monkeys displayed a strong motivational bias related to gaining information about future rewards: they fixated the informative CS much earlier than the non-informative CS, and therefore obtained uncertainty-resolving information earlier. Pharmacological manipulation of the targeted striatal and pallidal subregions diminished this reaction time bias. Therefore, basal ganglia play a causal role in animals' motivation to resolve their reward uncertainty and gain information about the future.

Disclosures: J.K. White: None. **E.S. Bromberg-Martin:** None. **K. Zhang:** None. **J. Pai:** None.

Poster

416. Reward Neurophysiology

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Topic: G.02. Motivation

Support: supported by the intramural research program of the National Institute on Drug Abuse

Title: Corticotropin releasing factor (CRF) increases excitability of Dorsal Raphe glutamatergic neurons

Authors: *J. A. MIRANDA-BARRIENTOS, B. LIU, M. F. MORALES
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Abstract: The dorsal raphe (DR) contains serotonin, GABA and glutamate neurons that express the vesicular glutamate transporter 3 (VGluT3) (Gras et al., 2002; Herzog et al., 2004; Jackson et al., 2009; Qi et al., 2014). We had recently demonstrated that axons from DR VGluT3 neurons establish excitatory synapses on ventral tegmental area (VTA) dopamine neurons that innervate the nucleus accumbens, and that VTA activation of inputs from DR VGluT3 neurons is rewarding (Qi et al., 2014). To have a better understanding of cellular properties of DR VGluT3 neurons that may play a role in their regulation, we began analyzing the electrophysiological and pharmacological properties of these neurons. By electrophysiological analysis of the membrane intrinsic properties of DR VGluT3 neurons, we did not find electrophysiological properties that distinguish VGluT3 neurons from neighboring serotonin neurons. Given that the DR has high levels of expression of corticotropin releasing factor (CRF) receptor 2 (CRF-R2), by in situ hybridization we looked for expression of CRF-R2 within DR VGluT3 neurons. We detected expression of CRF-R2 mRNA in some DR neurons expressing VGluT3 alone or in combination with Triptophan Hydroxylase (enzyme for the production of serotonin). Consistent with the presence of CRF-R2 mRNA in some VGluT3 neurons, by patch clamp recordings on genetically identified DR VGluT3 neurons, we found that CRF increased the excitability of a subpopulation of DR VGluT3 neurons. The effect of CRF on DR VGluT3 neurons excitability was blocked by a CRF-R2 antagonist. These findings indicate that DR glutamatergic neurons are modulated by CRF, suggesting a role of stress on controlling the neuronal activity of DR glutamatergic neurons.

Disclosures: J.A. Miranda-Barrientos: None. **B. Liu:** None. **M.F. Morales:** None.

Poster

416. Reward Neurophysiology

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Program #/Poster #: 416.22/DDD17

Topic: G.02. Motivation

Title: Selective activation of GABAergic inputs from the pedunculopontine tegmental nucleus to the ventral tegmental area drives reward

Authors: *H.-L. WANG, Q. SHEN, Y. ZHANG, M. F. MORALES
IRP/NIDA/NIH, Baltimore, MD

Abstract: The pedunculopontine tegmental nucleus (PPTg) is composed of cholinergic, GABAergic, and glutamatergic neurons (Wang & Morales, 2009), which provide inputs to ventral tegmental area (VTA, Wang et al., 2010 SfN 366.4). Recent optogenetic studies have shown that VTA optical activation of PPTg glutamatergic or cholinergic axons produces a reinforcing effect or place preference (Wang et al., 2015 SfN; Yoo et al., 2017; Xiao et al., 2016). However, the functional role of PPTg GABA inputs to VTA is unclear. To determine the role of PPTg GABA inputs to the VTA, PPTg GABA neurons were selectively tagged with either eYFP (VGAT-eYFP) or ChR2 tethered to eYFP (VGAT-ChR2-eYFP) in VGAT::Cre mice. Mice were implanted with optic probes aimed at the VTA. To determine if VTA activation of PPTg GABA inputs is positively reinforcing, mice were trained to earn VTA optical stimulation by rotating one of two response wheels. VGAT-ChR2-eYFP mice, but not VGAT-eYFP mice, rotated the reinforced wheel significantly more than the non-reinforced wheel. To confirm that wheel-turning was goal-directed, we switched the contingencies between the wheels for 5 days after the initial 8 days of training. VGAT-ChR2-eYFP mice quickly increased their responding on the new reinforced wheel, completely reversing their operant performance by the third reversal session. Mice continued to respond on the new reinforced wheel for two subsequent reversal testing sessions. Next, to further characterize the rewarding properties of PPTg GABA inputs to VTA, we conducted real-time place preference studies. VGAT-ChR2-eYFP and VGAT-eYFP mice were tested in a three chambers apparatus and were given continuous trains of optical stimulation when they entered the laser-paired chamber. VGAT-ChR2-eYFP mice spent significantly more time in the laser paired chamber in training and test days. Thus, VGAT-ChR2-eYFP mice not only earned the stimulation by entering the laser-paired chamber when stimulation was available (training day); they also acquired a conditioned place preference for the stimulation-associated chamber when the stimulation was no longer available (test day). Then, we examined c-Fos expression in VTA neurons induced by local activation of PPTg GABA fibers and found that this activation induced c-Fos expression in dopamine and non-dopamine neurons. However, only a small fraction (10%) of the c-Fos-positive neurons expressed tyrosine hydroxylase (a dopaminergic marker). We conclude that VTA selective

excitation of PPTg GABA inputs elicits reward by a mechanism that involves the excitation of mostly non-dopamine neurons.

Disclosures: H. Wang: None. **Q. Shen:** None. **Y. Zhang:** None. **M.F. Morales:** None.

Poster

416. Reward Neurophysiology

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Topic: G.02. Motivation

Support: NIDA-IRP

Title: Monosynaptic inputs to rat GABA neurons of the ventral tegmental area or the rostromedial tegmental nucleus

Authors: *C. MEJIAS-APONTE[1], A. KUPPA[2], Z. H. FUSFELD[2], M. F. MORALES[3]
[1]Neuronal Networks Section, INRB, Natl. Inst. On Drug Abuse, Baltimore, MD; [3]Integrative Neurosci. Res. Br., [2]Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: It is well established that the neuronal activity of dopamine neurons in the ventral tegment area (VTA) is inhibited in part by inputs from local GABA neurons or from the neighboring rostromedial tegmental nucleus (RMTg). Findings from a recent rat study using chemical retrograde track tracers (fluorogold and cholera toxin subunit b) showed differences between the brain structures innervating the VTA and those innervating the RMTg (Yetnikoff et al., 2015). This study showed a stronger innervation from the nucleus accumbens to the VTA than to RMTg, and a stronger innervation from the superior colliculus to the RMTg than to the VTA. While these findings provide crucial circuitry information, it remains to be determined the extent to which neurons from the identified brain structures (innervating the VTA or RMTG) synapse on GABA neurons. To selectively map the rat synaptic inputs to GABA neurons within the VTA or RMTg, we used a monosynaptic viral-vector rabies based tract-tracing and the recently developed GAD1::Cre transgenic rats (provided by NIDA-IRP). Given that the VTA and RMTg are adjacent structures, we used transgenic rats (instead of mice) to maximize the selective viral vector targeting to GABA neurons within the VTA or RMTg. We found that neurons distributed in the basal forebrain, hypothalamus, midbrain and brainstem establish monosynaptic synapses on GABA neurons of the VTA or RMTg. We are currently quantifying the total number of neurons synapsing on GABA neurons of the VTA or RMTg, and determining the phenotypes of these projection neurons.

Disclosures: C. Mejias-Aponte: None. **A. Kuppa:** None. **Z.H. Fusfeld:** None. **M.F. Morales:** None.

Poster

416. Reward Neurophysiology

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Program #/Poster #: 416.24/DDD19

Topic: G.02. Motivation

Support: R01DA036612

Title: Ventral tegmental area glutamate neurons drive reinforcement independent of dopamine

Authors: *V. ZELL[1], N. G. HOLLON[2], T. STEINKELLNER[1], E. SOUTER[1], L. FAGET[1], X. JIN[2], T. S. HNASKO[1]
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Abstract: The ventral tegmental area (VTA) is a midbrain dopamine center implicated in the control of motivated behaviors. The VTA is a highly heterogeneous structure that also contains GABA and glutamate neurons, subsets of which can co-release more than one of these small molecule transmitters. In a previous study we reported that optogenetic stimulation of VTA VGLUT2 (glutamate) cell bodies or terminals can drive behavioral reinforcement. We further showed that mice displayed marked preference for brief over sustained stimulation, resulting in behavioral responses that were notably distinct when compared to dopamine neuron stimulation. Still, a subset of VTA glutamate neurons co-release dopamine and may directly recruit dopamine neuron activation through intra-VTA connectivity. Thus, a fundamental question remains: is behavioral reinforcement driven by VTA glutamate neuron stimulation dependent on increased dopamine release? To address this we conditionally disrupted the gene encoding tyrosine hydroxylase (*Th*) in VGLUT2-expressing neurons to selectively block dopamine synthesis in and release from VTA glutamate neurons. Co-expression of DA and glutamate markers is abolished in these Th cKO mice. ChR2-mediated stimulation of VTA glutamate neurons leads to robust evoked DA release in the nucleus accumbens (NAc) shell of control mice, and this too is abolished in the cKO. Nevertheless, both groups showed identical patterns of self-stimulation for optogenetic stimulation of VTA glutamate cell bodies or their terminals in the NAc. These data support the hypothesis that activation of VTA glutamate neurons can drive positive reinforcement through a mechanism parallel to mesolimbic dopamine projections and independent of their ability to increase DA signaling in the medial NAc shell.

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Poster

416. Reward Neurophysiology

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Topic: G.02. Motivation

Support: Intramural research program at NIDA/NIH

Title: Distinct expression of calcium binding proteins in glutamatergic neurons in the ventral tegmental area

Authors: *S. MONGIA[1], T. YAMAGUCHI[2], H. WANG[1], J. MIRANDA-BARRIENTOS[1], B. LIU[1], M. MORALES[1]
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Abstract: The ventral tegmental area (VTA) has three major subclasses of neurons: dopaminergic, GABAergic and glutamatergic (expressing the vesicular glutamate transporter 2; VGluT2). The VTA dopaminergic and GABAergic neurons have been further characterized based on the expression of calcium-binding proteins (CaBPs). Here we determined whether the VTA VGluT2 neurons express any of the known CaBPs (calbindin, CB; calretinin, CR or parvalbumin, PV). By using a combination of *in situ* hybridization (for the detection of VGluT2 mRNA) and immunohistochemistry (for detection of CB, CR or PV), we found that among the total population of VTA VGluT2 neurons, 30% co-expressed CB, almost 3% co-expressed PV and less than 1% co-expressed CR and were concentrated in the medial aspects of the VTA. We found that within the total population of CaBPs, almost one-third of CB neurons co-expressed VGluT2 mRNA, CR neurons rarely co-expressed VGluT2 mRNA, and over half of the PV neurons co-expressed VGluT2 mRNA. We concluded that one-third of the population of VTA VGluT2 neurons co-express a CaBP, and that about one third of the VTA CB neurons express VGluT2 and approximately half of the population of VTA PV neurons express VGluT2. These findings further provide evidence for heterogeneity among VTA VGluT2 neurons.

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Poster

416. Reward Neurophysiology

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Topic: G.02. Motivation

Support: This work was supported by the Intramural Research Program (IRP) of the National Institute on Drug Abuse

Title: Ventral tegmental area glutamate neurons are functionally diverse

Authors: *D. J. ESTRIN[1], D. H. ROOT[1,2], M. MORALES[1]
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Abstract: The Ventral Tegmental Area (VTA) comprises different neuronal populations; dopaminergic, GABAergic and glutamatergic. The VTA glutamatergic neurons express the Vesicular Glutamate Transporter 2 (VGluT2) for the vesicular accumulation of glutamate. Optogenetic studies have provided evidence for the involvement of VTA-VGluT2 neurons in both reward and aversion learning. Findings from these optogenetic studies have shown that optical stimulation of VTA-VGluT2 cell bodies is rewarding, whereas optical stimulation of VTA-VGluT2 terminals in the Nucleus Accumbens or Lateral Habenula is aversive. To determine the natural electrophysiological role that VTA-VGluT2 neurons play in motivation, we recorded from channelrhodopsin 2 phototagged VTA-VGluT2 neurons (n=27) in response to rewarding or aversive stimuli. We identified two major functionally distinct subpopulations of VTA-VGluT2 neurons: (1) VTA-VGluT2 neurons that increased their firing during an aversive air puff but decreased their firing during sucrose consumption, and (2) VTA-VGluT2 neurons that increased their firing during both sucrose consumption and air puff presentation. Additionally, we found minor subpopulations of VTA-VGluT2 neurons that increased their firing rates in response to learned cues and reward-approach behavior. We identified separate VTA VGluT2 subpopulations which differ in firing by signaling either outcome evaluation or reward expectation. From these findings, we deduce that there is functional diversity among the VTA-VGluT2 neurons. This functional diversity may reflect the molecular heterogeneity within the VTA-VGluT2 population.

Disclosures: D.J. Estrin: None. **D.H. Root:** None. **M. Morales:** None.

Poster

416. Reward Neurophysiology

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Program #/Poster #: 416.27/DDD22

Topic: G.02. Motivation

Support: NIDA Intramural Program

Title: Opponent signals from the lateral preoptic utilize the heterogeneity of the ventral tegmental area to drive motivated behavior

Authors: *D. J. BARKER, R. JUZA, S. ZHANG, J. MIRANDA-BARRIENTOS, B. LIU, C. MEJIAS-APONTE, S. MONGIA, M. MORALES
Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: The lateral preoptic area (LPO) is comprised of a mixture of glutamatergic and GABAergic neurons and participates reward, aversion, stress, and drug addiction. It has long been established that the LPO provides substantial input to the ventral tegmental area (VTA), however the exact nature of this connection has remained elusive. Here we show that LPO inputs to the VTA establish a complex functional network with the VTA that is necessary for generating simple, but functionally opposing behavioral responses. By the combination of retrograde tracing and *in situ* hybridization, we determined that 65.6 ± 3.9% of lateral preoptic area inputs to the VTA neurons expressed the vesicular GABA transporter mRNA, while 42.4 ± 2.4% expressed the vesicular glutamate transporter mRNA. Further, by psuedorabies monosynaptic tracing, we determined that LPO neurons synapse on each major cell type in the ventral tegmental area: glutamate, GABA, and dopamine. To determine the exact nature of these connections, we then used triple immuno-electron microscopy to label both pre- and post-synaptic cell types simultaneously, revealing that the lateral preoptic area glutamate and GABA neurons each target dopamine, glutamate, and GABA neurons in the ventral tegmental area, but with relatively different proportions. In spite of this synaptic complexity, activation of LPO glutamatergic or GABAergic inputs to the VTA drives simple, yet opposing behaviors: activation of LPO glutamatergic inputs to the VTA is aversive while activation of GABAergic inputs is rewarding. Thus, our ongoing work is focused on the types of information being encoded by LPO glutamatergic and GABAergic inputs to the VTA and to determine how their integration by VTA neurons might yield opposing behavioral states.
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Poster

416. Reward Neurophysiology

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Topic: G.02. Motivation

Support: Supported by the Intramural Research Program of the National Institute on Drug Abuse

Title: VTA glutamatergic neurons play a role in defensive behaviors via glutamatergic inputs from the lateral hypothalamus

Authors: *M. F. BARBANO, H.-L. WANG, S. ZHANG, J. A. MIRANDA-BARRIENTOS, M. F. MORALES
Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: The selection of the adequate strategy to cope with a life-threatening situation is vital for the survival of both the individual and the species. Defensive behavior is characterized by different responses, such as freezing, fighting or escape. The brain circuits underlying freezing and fighting behaviors have been extensively studied but those underlying escape are not well understood. Findings from electrical stimulation and lesion studies performed over 30 years ago suggest that interactions between the lateral hypothalamus (LH) and the ventral tegmental area (VTA) play a role in escape responses. Since then, optogenetic studies have implied that glutamatergic LH inputs activate VTA GABA neurons to induce escape responses (Nieh et al. 2016). However, it is unclear if other types of VTA neurons (dopaminergic or glutamatergic) play a role in escape responses. To investigate the role of LH glutamate neurotransmission to VTA, we injected Cre-dependent viral vectors encoding ChR2 tethered to mCherry in the LH of VGluT2::Cre mice to tag LH axons expressing the vesicular glutamate transporter 2 (VGluT2). By immunoelectron microscopy, we found that VGluT2-axons from LH neurons more frequently established synapses on VTA glutamatergic than dopaminergic neurons (Zhang et al., SfN 2018). By electrophysiological recordings, we found that VTA photoactivation of VGluT2 terminals from LH neurons induced excitatory postsynaptic currents and evoked action potential firing on VTA VGluT2 neurons. These findings indicate that LH glutamatergic neurons provide a glutamatergic input to VTA glutamatergic neurons. Next, to evaluate the behavioral consequences of VTA optical stimulation of VGluT2 LH inputs, we conducted optogenetic studies while mice were trained in real time place aversion (RTPA) or open field (OF) tests. We found that VTA photoactivation of VGluT2 LH terminals elicited active avoidance and long-lasting aversion in the RTPA test, as well as escape responses in the OF test. To determine the participation of VGluT2 LH inputs to VTA in innate escape responses, we inhibited glutamate release from LH onto VTA neurons, and found that VTA photoinhibition of VGluT2 terminals

derived from LH decreased escape responses in mice confronted to predator odor or forced swimming. From these behavioral findings, together with the observation that LH VGluT2 neurons preferentially synapse on VTA glutamatergic neurons, we conclude that glutamatergic neurotransmission from LH to VTA glutamatergic neurons plays a role in defensive behaviors.

Disclosures: M.F. Barbano: None. **H. Wang:** None. **S. Zhang:** None. **J.A. Miranda-Barrientos:** None. **M.F. Morales:** None.

Poster

416. Reward Neurophysiology

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 416.29/DDD24

Topic: G.02. Motivation

Support: NIDA-IRP

Title: VTA glutamatergic neurons receive major glutamatergic inputs from the lateral hypothalamus

Authors: *S. ZHANG[1], M. F. BARBANO[2], H.-L. WANG[3], J. A. MIRANDA-BARRIENTOS[4], M. F. MORALES[5]
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Abstract: Emerging evidence indicates that different neurons of the ventral tegmental area (VTA) participate in specific aspects of behavior. The VTA has three major classes of neurons: dopaminergic, GABAegic and glutamatergic. The VTA glutamatergic neurons express vesicular glutamate transporter 2 (VGluT2) for accumulation of glutamate into synaptic vesicles. We recently found that glutamate released in VTA from lateral hypothalamic (LH) glutamatergic neurons (expressing VGluT2) plays a role in defensive behaviors (Barbano et al., 2018 SfN). To determine the synaptic connectivity established by LH VGluT2 neurons in VTA, we used a combination of cell specific viral vector tracing and immunoelectron microscopy. To tag LH VGluT2 neurons and their axons, we injected into the LH of VGluT2::Cre mice a Cre-inducible adeno-associated virus (AAV) with a double-floxed inverted open reading frame (DIO) expressing ChR2 (AAV-DIO-ChR2) tethered to mCherry. In the same mice, we tagged VTA VGluT2 neurons by local injection of AAV-DIO tethered to eYFP. By immunolabeling and electron microscopy, we detected in the VTA many fibers and axon terminals containing mCherry, all mCherry axon terminals expressed VGluT2, indicating that mCherry terminals were originated from LH VGluT2 neurons. Through the analysis of VTA sections labeled for the detection of mCherry (VGluT2 LH inputs) and eYFP (VTA VGluT2 neurons), we detected

mCherry in both axons and axon terminals, and eYFP in both cell bodies and dendrites. We found that 43.98 ± 4.27% of the LH mCherry terminals made asymmetric (excitatory type) synapses on both eYFP-dendrites and eYFP-cell bodies. Further, we observed that multiple mCherry-terminals synapsed on a single eYFP-dendrite. These findings indicate that VTA glutamatergic neurons are highly innervated by LH glutamatergic neurons. Through the analysis of VTA sections labeled for the detection of mCherry (VGluT2 LH inputs) and tyrosine hydroxylase (TH; a marker of dopaminergic neurons), we found that 34.72 ± 0.79% of the LH mCherry terminals made asymmetric synapses on TH dendrites. We conclude that within the VTA, LH glutamatergic terminals establish synapses on VTA glutamatergic cell bodies and multiple synapses on single glutamatergic dendrites. In contrast, LH glutamatergic terminals establish fewer synapses on single dopaminergic dendrites, and do not establish synapses on dopaminergic cell bodies. From these ultrastructural findings, we suggest that release of glutamate in the VTA evoked by photostimulation of LH glutamatergic inputs is likely to have a stronger excitatory influence on VTA glutamatergic neurons than on dopaminergic neurons.

Disclosures: S. Zhang: None. **M.F. Barbano:** None. **H. Wang:** None. **J.A. Miranda-Barrientos:** None. **M.F. Morales:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.01/EEE1

Topic: G.08. Drugs of Abuse and Addiction

Support: KAKENHI KIBAN C 16K10197

Title: Fingolimod (FTY720) suppresses cocaine-induced hyper locomotion, which attenuate striatal D_1-type medium spiny neurons of PKA/DARPP-32 signaling

Authors: *K. UEMATSU[1], T. SHUTO[2], Y. SYOJI[1], N. UCHIMURA[1], A. NISHI[2]
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Abstract: Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid metabolite that regulates critical cellular processes such as proliferation, survival and migration as well as immune responses. S1P exerts its function by activating S1P receptors (S1PRs). There are five S1PR subtypes: $S1P_1R$, $S1P_2R$, $S1P_3R$, $S1P_4R$ and $S1P_5R$. It has been reported that S1PRs are expressed in many cell types including neurons in the brain. Fingolimod (FTY720) is an agonist of S1PRs and a new oral drug for multiple sclerosis. Fingolimod binds to all S1PR subtypes except $S1P_2R$. There are few reports about a possible role of S1P and fingolimod in neurons. In this study, we investigated the effect of S1P and fingolimod on cAMP/PKA signaling in mouse

striatal slices by monitoring the phosphorylation states of an intracellular phosphoprotein, dopamine- and cAMP-regulated phosphoprotein of M_r 32 kDa (DARPP-32) at Thr34 (PKA-site). In striatal slices prepared from D_1-DARPP-32-Flag/D_2-DARPP-32-Myc transgenic mice, fingolimod (100 nM) increased DARPP-32 Thr34 phosphorylation in D_2-type/striatopallidal neurons at 30 sec by 2-fold, but decreased P-Thr34 DARPP-32 in D_1-type/striatonigral neurons at 30 min to 60% of control. The increase in DARPP-32 Thr34 phosphorylation in striatopallidal neurons was mimicked by treatment with S1P (10 μM) (3-fold increase at 1 min of incubation) and with a selective $S1P_1R$ agonist SEW2871 (10 μM) (2.2-fold increase at 1min of incubation). The cocaine (100 μM)- and a dopamine D_1 agonist (±)-SKF-81297 (1.0 μM)-induced increases in P-Thr34 DARPP-32 were abolished after 30 min of pre-treatment with fingolimod (100 nM). In behavioral studies, pretreatment with fingolimod (1.0 mg/kg, i.p.) attenuated cocaine (20 mg/kg, i.p.)- and R(+)-SKF-81297 (0.7 mg/kg, i.p.)-induced locomoter activities. AAV injection to striatal result to over-expression of $S1P_1R$ on D_1-cre mice, is further decrease cocaine-induced locomotion by pretreatment with fingolimod (1.0 mg/kg, i.p.) than wild-type. Thus, fingolimod inhibits cAMP/PKA signaling in striatonigral neurons, resulting in the suppression of psychostimulant-induced activation of dopamine signaling and behavior.

Disclosures: K. Uematsu: None. **T. Shuto:** None. **Y. Syoji:** None. **N. Uchimura:** None. **A. Nishi:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.02/EEE2

Topic: G.08. Drugs of Abuse and Addiction

Title: Impaired social interactions in the offspring of cocaine-exposed fathers

Authors: *A. YAW[1], J. D. GLASS[2,1], H. K. CALDWELL[2,1]
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Abstract: It is well established that the damaging effects of drugs of abuse, such as cocaine, can extend beyond the user to their offspring. While most preclinical models of the generational effects of cocaine abuse have focused on maternal effects, we, and others, see distinct effects on offspring sired by fathers that have used cocaine. However, little is known about the effects of paternal cocaine use on first generation (F1) offspring social behaviors. Thus, we examined anxiety-like behaviors (open field and elevated plus) in both sexes, as well as aggressive behavior (resident-intruder task) and social recognition memory (social habituation/dishabituation task) in male F1 offspring. We found that there were sire-dependent effects on anxiety-like behaviors and social memory. Specifically, cocaine-sired male offspring had reduced anxiety, as measured by increased time spent in the open arms of an elevated plus

maze (%), while there were no differences in female offspring. Further, in the social recognition test, while F1 males were able to recognize a novel mouse, they did show marked increases in sniffing times during later trials − which could be indicative of altered social motivation or cognitive deficits. Taken together, these data suggest that both anxiety-like and social behaviors in F1 males are altered by paternal cocaine use.

Disclosures: A. Yaw: None. **J.D. Glass:** None. **H.K. Caldwell:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.03/EEE3

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA IRP
NIDA DA025636
VA BLR&D 1IO1BX000782

Title: Linking cocaine-induced structural brain changes to altered cognition in rhesus monkeys

Authors: *H. P. JEDEMA[1], X. SONG[1], H. J. AIZENSTEIN[2], Y. YANG[1], C. W. BRADBERRY[1]
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Abstract: Altered cognitive performance and structural changes in brains of patients suffering from cocaine dependence have been reported in multiple clinical studies. Due to the cross-sectional nature of these studies, it remains unclear whether these differences are caused by chronic stimulant use or whether they preceded use and may instead reflect a predisposition to development of drug dependence. Longitudinal preclinical studies can provide insight into the nature of these changes. In this study, we obtained structural MRI scans from 14 adult male rhesus macaques and established baseline performance on a visual working memory task (delayed match to sample; DMS) and a stimulus reversal learning task (RevL). Subsequently, 8 subjects self-administered cocaine intravenously, 4 days a week (up to 4.8mg/kg/day) while control subjects performed similar tasks for water reward. Impairment of cognitive performance on drug free days for these subjects was published previously. After 12 months of cocaine self-administration a second set of MRI scans were obtained. We used voxel-based morphometry (VBM) to examine the treatment group by time structural interaction on gray matter density (GMD). We observed clusters of decreased GMD in the medial and lateral temporal lobe (parahippocampal fusiform gyri and along the superior temporal sulcus) in the cocaine group. In addition, clusters of decreased GMD were observed in the thalamus, superior parietal cortex,

insula, and orbitofrontal and superior frontal cortex. Clusters of increased GMD in the cocaine group were observed in cerebellum, bilateral temporal poles and ventral frontal cortex, superior parietal, precentral and postcentral cortex, and rostral caudate nucleus. The impaired performance on the DMS task showed a significant correlation with the reduced GMD in the temporal lobe and superior frontal cortex in the cocaine group. Impairments in performance on the RevL task correlated significantly with the reduced GMD in orbitofrontal and superior frontal cortex, and the superior temporal and parietal cortex. These findings are consistent with altered metabolic activity in non-human primates after chronic cocaine. Furthermore, most of the observed GMD interactions are consistent with the clinical gray matter alterations in stimulant-dependent patients, suggesting these alterations are the result of prolonged stimulant exposure rather than a pre-existing condition.

Disclosures: H.P. Jedema: None. **X. Song:** None. **H.J. Aizenstein:** None. **Y. Yang:** None. **C.W. Bradberry:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.04/EEE4

Topic: G.08. Drugs of Abuse and Addiction

Support: MH079201
MH093092

Title: The effects of genetic brain serotonin deficiency on responses to repeated cocaine

Authors: *B. D. SACHS[1], M. M. KARTH[2], M. D. KARTH[2], D. VANDYK[2], G. PEREZ[2], M. G. CARON[3]
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Abstract: Substance abuse disorders are poorly understood conditions that exert a devastating toll on society. While the exact causes of these disorders are not known, the fact that substance abuse is highly heritable highlights the important role of genetics in the development of these conditions. Although many factors are likely involved in the progression from drug use to addiction, long-term drug-induced changes in gene expression have been hypothesized to play a major role. Thus, it is possible that genetic factors contribute substance abuse risk at least in part by influencing transcriptional responses to repeated drug use. The current study sought to determine the effects of genetically induced brain serotonin (5-HT) deficiency on a subset of behavioral and transcriptional responses to repeated cocaine. Cocaine is known to significantly impact multiple neurotransmitter systems, including the dopamine, 5-HT, and norepinephrine

systems, but whether genetic reductions in brain 5-HT synthesis lead to significant alterations in cocaine responses has not been established. To model 5-HT deficiency, this study took advantage of a genetically modified mouse line that harbors a partial loss of function mutation in the brain 5-HT synthesis enzyme, tryptophan hydroxylase 2 (Tph2). Tph2(R439H) knock-in (Tph2KI) mice have been shown to exhibit 60-80% reductions in the levels of brain 5-HT compared to their wild-type littermates. Prior work has shown that elevation of 5-HT levels via the administration of 5-hydroxytryptophan, the precursor to 5-HT, reduces locomotor responses to acute cocaine, whereas pharmacological reductions in 5-HT levels potentiate the locomotor-stimulating effects of acute cocaine. Thus, we had hypothesized that brain 5-HT deficiency would lead to exaggerated locomotor responses to cocaine. In contrast to our initial hypothesis, our results revealed no significant genotype differences in locomotor activity following acute or repeated cocaine administration in male mice. However, brain 5-HT significantly impacted the effects of cocaine on the striatal expression of several genes that have been previously implicated in cocaine responses, including BDNF, the D2 dopamine receptor, FosB, and CREB. Although our data reveal that genetically impaired brain 5-HT synthesis is not sufficient to alter cocaine-induced locomotor sensitization, future research will be required to determine whether other behavioral responses to cocaine are impacted by brain 5-HT deficiency.

Disclosures: B.D. Sachs: None. **M.M. Karth:** None. **M.D. Karth:** None. **D. vanDyk:** None. **G. Perez:** None. **M.G. Caron:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.05/EEE5

Topic: G.08. Drugs of Abuse and Addiction

Support: German DFG Grant AM 488/1-1

Title: Role of D1 and D2 medium spiny neurons responses to psychostimulants and antipsychotics, understading drug addiction vulnerability in schizophrenia

Authors: *D. AMATO[1,2], J. A. HEINSBROEK[3], P. W. KALIVAS[4]
[1]Neurosci., Med. Univ. of South Carolina., Charleston, SC; [2]Dept. of Psychiatry and Psychotherapy, Friedrich-Alexander Univ. Erlangen-Nürnberg, Germany, Erlangen, Germany; [3]Med. Univ. of South Carolina, Charleston, SC; [4]Neurosci. Res., Med. Univ. S Carolina, Charleston, SC

Abstract: Nearly half of patients with schizophrenia abuses addictive substances such as cocaine. The neurobiology underlying this high prevalence is unknown. A possible interpretation for this comorbidity is linked with cocaine efficacy to enhance rewarding responses in

schizophrenia, which are impaired by chronic antipsychotics. D1 and D2 medium spiny neurons (MSNs) in the nucleus NAc (NAc) differently mediate the rewarding properties of cocaine. Using in vivo Ca2+ miniature microscopic imaging, we measured NAc Ca2+ transients using head mounted miniature microscope to monitor D1 and D2 MSNs responses in mono- and a cross- sensitization protocols. To target specific cell-type responses, we injected Cre dependent Ca2+indicator (gCaMP6f) in D1- and D2-Cre mice. Cocaine sensitization was induced by 2 injections of cocaine separated by 7 days-interval, performed after an independent session with saline treatment to set out baseline MSNs responses. The cross-sensitization between haloperidol (HAL) and cocaine was induced by a single cocaine injection given after chronic pretreatment with clinical-like doses of HAL. These measurements were performed after recording locomotion and MSNs responses to an acute injection of HAL. Cocaine sensitization: Two cocaine injections induced locomotor sensitization and differently modulated D1 and D2 MSNs. Sub-populations of D1-MSNs responses decreased after acute cocaine. This suppression reversed during locomotor sensitization. D2-MSNs responses were also suppressed after acute cocaine, but this inhibition persisted during locomotor sensitization. At baseline, D2-MSNs were largely active while the majority of D1-MSNs activity was depressed. Acute HAL effects: A single HAL injection stimulated most of D1-MSNs responses, but depressed locomotion and D2-MSNs responses. Cross-sensitization: the pretreatment with HAL evoked a locomotor response to cocaine higher than the response observed in the mono-sensitization protocol. D1- and D2-MSNs responses were now equalized by HAL pretreatment, in that a similar percentage of both cell-types were now activated. This outcome is opposite to that one observed in the mono-sensitization protocol in which D2-MSNs responses were abolished. Locomotor sensitization is accompanied by increased D1-MSNs and decreased D2-MSNs activity in the mono-sensitization protocol. Instead, both the D1- and D2-MSNs are activated during cross-sensitization. These results reveal important new insights into the role of NAc MSNs as putative moderators of drug addiction in schizophrenia.

Disclosures: D. Amato: None. **J.A. Heinsbroek:** None. **P.W. Kalivas:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.06/EEE6

Topic: G.08. Drugs of Abuse and Addiction

Title: Deletion of the trpc4 gene reduces cocaine induced impulsivity

Authors: M. GEREAU, D. SHIVAPUJA, B. GOULD, *W. D. KLIPEC
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Abstract: The canonical transient receptor potential (TRPC) family of calcium permeable, non-selective cation channels is abundantly expressed throughout the mammalian brain, playing a pivotal role in modulating cellular excitability. We have developed a *trpc4* knockout rat that shows a brain-wide elimination of TRPC4 channels, including within a subpopulation of TRPC4 bearing dopamine neurons in the ventral tegmental area (VTA). Our previous research has shown that knockout rats (*trpc4* KO), compared to normal (WT) rats, exhibit reduced cocaine self-administration and reduced dopamine cell firing rates in the VTA, with no differences in simple or complex learning using food or water rewards. Here, we measured the effects of acute cocaine administration (15, 10, 5 and 0 mg/kg i.p.) on early response errors (impulsivity) made by *trpc4* KO and WT rats during a differential reinforcement of low rate (DRL) reinforcement schedule. The DRL schedule required rats to withhold a response for 14-sec to obtain water reinforcement while responses before 14 second reset the clock without providing water reinforcement. Cocaine administration produced a significant dose dependent increase in early responding (impulsiveness) in WT compared to *trpc4* KO rats. These data, in conjunction with our previous research demonstrating that the deletion of the *trpc4* gene does not impair learning involving natural rewards but does affect cocaine self-administration, further demonstrate a novel role for the TRPC4 channel in cocaine reinforcement as well as other dopamine disorders. The findings further suggest that functional TRPC4 expression may be an important model for investigating cocaine addiction, and also suggest that novel drug therapies targeting the subpopulation of TRPC4 VTA DA neurons may be useful in treating cocaine addiction, without side effects on learning and performance of behaviors controlled by natural rewards.

Disclosures: M. Gereau: None. **D. Shivapuja:** None. **B. Gould:** None. **W.D. Klipec:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.07/EEE7

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA037744

Title: A persistence of habitual responding for cocaine underlies punishment resistance

Authors: H. F. SPENCER[1], T. H. KIM[2], *R. J. SMITH[1]
[1]Dept Psychological and Brain Sci., Texas A&M Univ., College Station, TX; [2]Dept Psychological & Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Addiction is characterized by compulsive drug use that persists even when faced with negative consequences. In other words, addiction is defined by drug-seeking behavior that is resistant to punishment. This type of punishment resistance is also observed in animal models of

addiction, with a subset of rats continuing to self-administer cocaine despite receiving footshock. We sought to test the hypothesis that punishment resistance arises from failure to exert goal-directed control over habitual drug seeking. Male and female Sprague Dawley rats were trained on a seeking-taking chained schedule of cocaine self-administration (2 h/day), in which presses on a seeking lever gave access to a separate taking lever reinforced with intravenous cocaine (0.5 mg/kg/infusion). The seeking link of the chain required completion of a random ratio (RR20) or random interval (RI60) schedule, and the taking link was reinforced under a fixed ratio (FR1) schedule. Following at least 12 days on the final seeking-taking reinforcement schedule, animals were exposed to 4 days of punishment testing, in which footshock (0.4 mA, 0.3 s) was delivered randomly on one-third of trials, immediately following completion of seeking and prior to extension of the taking lever. Both before and after punishment testing (4 days prior to punishment, and then 4 days after rats had resumed self-administration), instrumental response strategy was assessed using an outcome devaluation procedure we developed for intravenous cocaine. Sensitivity to outcome devaluation indicated goal-directed responding, while insensitivity to outcome devaluation indicated habitual responding. We observed punishment resistance in a subset of animals under both reinforcement schedules (RR20 or RI60). Further, we found that punishment resistance was associated with a persistence of habitual responding, whereas punishment sensitivity was associated with a switch to goal-directed responding after punishment (even if animals showed habitual responding prior to punishment). These findings indicate that punishment resistance is related to a dominant habit system that persists under conditions that should encourage a transition to goal-directed behavior.

Disclosures: H.F. Spencer: None. **T.H. Kim:** None. **R.J. Smith:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.08/EEE8

Topic: G.08. Drugs of Abuse and Addiction

Support: DA031695-06

Title: Modulatory effects of cocaine intake and escalation on anterior cingulate encoding during reward-guided decision-making

Authors: *D. VAZQUEZ[1], A. C. BURTON[1], H. J. PRIBUT[2], S. S. TENNYSON[1], M. R. ROESCH[3]
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Abstract: Maladaptive decision-making, impulsivity, and impaired cognitive flexibility are defining features of cocaine addiction. The anterior cingulate cortex (ACC) has generally been implicated in functional decision-making and attention, and plays a role in encoding reward prediction errors. Previously, we have shown that ACC correlates are critical for online learning, signaling the need for attention to cues after error commission arising from reward expectancy violations. We have also found an exaggerated behavioral response bias in cocaine rats towards high-value rewards, demonstrating their enhanced sensitivity to reward value manipulations. As a result of these findings, we decided to examine the impact of cocaine self-administration on ACC error encoding. We hypothesized that chronic cocaine self-administration alters flexible reward-guided decision-making through disruption of attention for learning correlates in the ACC. To address this issue, we recorded ACC single-unit activity during rat performance of a reward-guided decision-making odor task involving reversal learning. In the functional ACC, outcome expectancy violations result in increased attention to predictive stimuli, subsequently facilitating online adaptations in behavior. In our task, cocaine rats displayed reversal learning deficits, a bias towards short delays to reward, and faster reaction times across all trial blocks; these behavioral effects were moderated by cocaine intake. Additionally, we found dosage-dependent modulation of ACC attention for learning correlates following unsigned prediction errors. There was an inverse correlation between cocaine intake and ACC firing rate to up-shifts in reward value, which also translated into an inverse correlation between escalations in cocaine intake and average ACC firing rates. These dosage-dependent effects on response bias and neural responsiveness to cues suggests that cocaine intake induces graded modulatory effects in the ACC, which translates into disrupted cognitive flexibility.
Keywords: anterior cingulate cortex, cocaine, prediction error, monitoring, attention, single unit

Disclosures: D. Vazquez: None. **A.C. Burton:** None. **H.J. Pribut:** None. **S.S. Tennyson:** None. **M.R. Roesch:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.09/EEE9

Topic: G.08. Drugs of Abuse and Addiction

Support: Doctoral scholarship of Collectivité Territorial de la Martinique

Title: Effect of N-acetylcysteine on the expression of cocaine-induced locomotor sensitization and cocaine-enhanced brain stimulation rewards in rats

Authors: *R. HODEBOURG[1], P.-P. ROMPRÉ[2]
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Abstract: Repeated exposure to cocaine decreases basal extracellular levels of glutamate in the nucleus accumbens, a brain region that plays a key role in reward and motivation. This reduction in glutamate, which is due to downregulation of the cysteine/glutamate antiporter located on glial cells, is correlated with a vulnerability to cocaine relapse in rats. It has been shown that N-acetylcysteine (NAC), a cysteine prodrug, restores the function of the cysteine/glutamate exchanger and reduces the vulnerability to relapse. Furthermore, our previous studies have shown that acute NAC treatment reduces the motivation to self-administer cocaine in rats. Since the motivation to take cocaine and sensitization to the psychomotor effect of cocaine share common neural substrates, we carried out a first study to determine whether acute NAC administration decreases the expression of cocaine-induced sensitization. Adult male rats received daily cocaine (20 mg/kg) or vehicle injections for 5 consecutive days. After 7 days of withdrawal, rats received a NAC (60 mg/kg) or vehicle injection 3 h before a single cocaine challenge (15 mg/kg). Locomotor activity was recorded for 1 h immediately after each cocaine injection. Acute NAC administration decreased the expression of cocaine sensitization, but not acute locomotor stimulant effect of cocaine. A second study evaluated the effect of an acute NAC administration on the enhancement effect of cocaine on brain stimulation reward. A new cohort of rats was trained to self-administer an electrical stimulation in the lateral hypothalamus. Reward thresholds were measured before and after systemic injection of NAC (60 mg/kg) of its vehicle with or without cocaine (0, 4 mg/kg). NAC had no effect on reward and on the enhancement effect of cocaine on reward. These findings contribute to a growing literature suggesting that NAC deserves clinical attention as a treatment for cocaine addiction.

Disclosures: R. Hodebourg: None. **P. Rompré:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.10/EEE10

Topic: G.08. Drugs of Abuse and Addiction

Support: BP-Endure Grant 5R25NS080687

Title: Environmental modulation on oxytocin's anxiolytic effectiveness in cocaine treated animals

Authors: *D. M. OJEDA, S. FONSECA, G. MOLINA, E. TORRES, C. MALDONADO-VLAAR
Col. of Natural Sci., Univ. de Puerto Rico, Rio Piedras Campus, San Juan, Puerto Rico

Abstract: Drug addiction is a common and debilitating condition that effects an individual’s biological constitution and social stability. This condition has a high prevalence worldwide and

its coupled with anxiety-like symptoms. The neuropeptide oxytocin has been considered to be a potential therapy for this condition due to its use as an anxiolytic in cocaine treated subjects. However, the role of the environment on the anxiolytic effectiveness of oxytocin has yet to be further evaluated. The present set of experiments are aimed to address this question. The protocol consisted of 6-day systemic cocaine injections treatment schedule. Sprague-Dawley male rats were assigned to two separate groups: one exposed to "noise- environment" and the other to "no noise-environment" conditions. The "noise-environment" consisted of exposing animals, post-cocaine injection, to a three-minute period of 86 +/- dB white noise sound stressor within an operant chamber. The "no-noise-environment" consisted of cocaine pre-treated animals exposed to the operant chamber without any sound. On the 6th testing day, cocaine treatment was withheld and subjects were treated with either intranasal oxytocin or vehicle and divided in four groups: "oxytocin-noise", "vehicle-noise", "oxytocin-no noise" and "vehicle-no noise" and subsequently tested in an elevated plus maze (EPM) apparatus. On Day 6, we measured locomotion during the time in the operant chamber and EPM behavioral parameters. Results showed that both groups of the "no noise-environment" had higher levels of locomotion when compared to the "noise-environment" groups. In addition, we found that oxytocin decreased locomotion in the "no noise-environment" exposed animals but had no effect in the "noise-environment" animals when compared to subjects exposed to vehicle injections. Animals that received intranasal oxytocin treatment spent a significant more time in the open arms when compared to their respective controls. However, the "oxytocin-no noise" group spent significantly more time in the open arms than the "oxytocin-noise" group. These preliminary findings reveal differences in oxytocin's behavioral effects between subjects exposed to the two different environments. These results indicate a potential modulatory role of the stress environment on the anxiolytic potential of oxytocin in cocaine treated animals.

Disclosures: D.M. Ojeda: None. **S. Fonseca:** None. **G. Molina:** None. **E. Torres:** None. **C. Maldonado-Vlaar:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.11/EEE11

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA033358

Title: Effects of adenosine A_{2A} receptor antagonists on cocaine-induced behaviors

Authors: *N. HAYNES, R. BACHTELL
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Abstract: Cocaine addiction is a chronic and relapsing disorder with no current pharmacologic intervention. Repeated cocaine use affects the nucleus accumbens (NAc) by altering various neurotransmitter systems. Adenosine A_{2A} receptors ($A_{2A}R$) in the NAc modulate both dopamine and glutamate neurotransmission depending on their synaptic localization and may ultimately influence cocaine-induced behavior. The aim of these studies was to examine the role of $A_{2A}R$ subpopulations on cocaine-induced locomotor activity by using pharmacological antagonists targeting pre- and postsynaptic $A_{2A}R$. Pharmacologic inhibition of postsynaptically-expressed $A_{2A}R$ with KW 6002 (1.0 mg/kg, ip) increased locomotor activity and enhanced locomotion induced by an acute injection of cocaine. Interestingly, the effects of the presynaptic $A_{2A}R$ antagonist, SCH 442416 (1.0 mg/kg, ip), were distinct from KW 6002. In particular, SCH 442416 had no effect on acute cocaine-induced locomotion but blunted the expression of cocaine-sensitizated locomotor activity. Alternatively, KW 6002 had no effect on the expression of cocaine-sensitized locomotion. Together, these results suggest a differential role of $A_{2A}R$ in the NAc depending on synaptic localization. These studies ultimately suggest that blockade of presynaptic $A_{2A}R$ attenuates cocaine-induced behavior following a sensitizing cocaine regimen and may serve as targets for future pharmacologic therapy to treat cocaine addiction.

Disclosures: N. Haynes: None. **R. Bachtell:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.12/EEE12

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH/NIDA R15DA040809 (LIP).

Title: Estradiol's potentiation of the acquisition of cocaine place preference may involve mTOR signaling

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Abstract: Women show an increased vulnerability to drug abuse. They experience more intense subjective effects of psychostimulant drugs and stronger cravings during abstinence, consequently leading to higher rates of relapse. There is an abundance of empirical data supporting the idea that the ovarian hormone estradiol is responsible for mediating and potentiating these sex-specific responses to psychostimulants. Interestingly, previous data from our lab demonstrated that acutely elevating estradiol (EB) levels in cocaine-conditioned, ovariectomized (OVX) female rats prior to a test for conditioned place preference (cocaine-CPP)

increased the magnitude of the dose required for the expression of cocaine-CPP. However, the direct effects of acute elevations of estradiol at different stages of cocaine-CPP and their molecular underpinnings remain virtually unknown. Recently, mTOR signaling has been shown to be a key molecular mechanism by which psychostimulant drugs of abuse, including cocaine, exhibit their effects within the mesolimbic reward circuit of male rodents. Moreover, inhibition of mTOR has been demonstrated to attenuate cocaine-CPP. Thus, the goal of the present study was - a) to determine if estradiol potentiates cocaine-CPP via enhancing the drug-cue associations and b) to measure alterations in the expression of mTOR in mesolimbic reward circuit. We subjected adult OVX female rats to a cocaine-CPP paradigm over five days; we used a 3/3 (AM/PM) conditioning procedure with intraperitoneal injections of 10mg/kg of cocaine hydrochloride. To systematically compare the effects of EB on drug-cue associations that develop during the conditioning stage of the CPP paradigm (i.e. CPP acquisition), rats were treated with either EB (5 µg; s.c.) or peanut oil (PO; equal volume; s.c.) 30 minutes prior to the start of each daily conditioning session. Expression of cocaine-CPP was assessed under a drug- and hormone-free state 24h following the last conditioning session. Immediately after the CPP test, animals were euthanized and brain tissue comprising VTA, NAc and dorsal striatum was isolated. Results demonstrated that EB-treatment during the conditioning phase of CPP augmented the preference for the drug-paired compartment. Moreover, EB-treatment increased levels of mTOR protein expression in VTA, NAc, and dorsal striatum after cocaine-CPP. In conclusion, our results demonstrated for the first time that drug-cue associations are potentiated by estradiol and may be mediated by increased expression of mTOR.

Disclosures: S.S. Kokane: None. **L.I. Perrotti:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.13/EEE13

Topic: G.08. Drugs of Abuse and Addiction

Support: T32DA041349

Title: Combined effects of phenmetrazine and nor-binaltorphimine on dopamine function and motivation to self-adminster cocaine

Authors: *P. ESTAVE, S. R. JONES
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Abstract: Despite numerous clinical trials, the FDA has not approved any medication assisted treatment for cocaine addiction. Our laboratory has shown that chronic cocaine self-administration reduced overall dopamine (DA) neurotransmission, resulting in a

hypodopaminergic state. Other studies have shown that repeated, non-contingent cocaine exposure resulted in a hyper-functioning kappa opioid receptor (KOR) system. Thus, this study aims to examine the effectiveness of a combination therapy, targeting the dopamine transporter and the KOR, in reducing cocaine-seeking behaviors in rats chronically exposed to cocaine.
In order to understand the impacts of cocaine self-administration on the DA and KOR systems, we used *ex vivo* fast scan cyclic voltammetry in the nucleus accumbens core to examine DA dynamics after chronic cocaine exposure (40 infusions of cocaine for 5 consecutive days). We found that cocaine exposure reduced stimulated DA release and attenuated cocaine potency at the dopamine transporter. Additionally, we examined KOR function through activation of KORs using the agonist U50,488, which inhibited DA release. We observed that responses to U50,488 were augmented post-cocaine exposure.
Male Sprague Dawley rats were used to determine the individual and combined behavioral effects of the DA releaser phenmetrazine and the KOR antagonist nor-binaltorphimine (nor-BNI). After rats self-administered forty infusions of cocaine (1.5 mg/kg/infusion) on a fixed-ratio 1 schedule for five consecutive days, a progressive ratio schedule was used to determine cocaine breakpoints (0.1875 mg/kg/infusion) at baseline, and following phenmetrazine (25 mg/kg/day; osmotic mini-pump), nor-BNI (10 mg/kg; i.p.), or a combination of the two drugs.
Administration of phenmetrazine alone significantly reduced breakpoints (60% of baseline), while nor-BNI had no effect as a monotherapy. Interestingly, cocaine breakpoints were further attenuated by the combination (20% of baseline), indicating augmented effects of targeting both the DA and KOR systems.
In this study, voltammetry results showed that chronic cocaine exposure reduced DA transmission and augmented KOR function. Dual targeting of the DA and KOR systems through a combination therapy approach revealed a greater decrease in motivation to take cocaine compared to either monotherapy. Taken together, these data support the potential beneficial effects of the dopamine transporter and KOR as dual-cellular targets to treat multiple aspects of the withdrawal syndrome, ultimately decreasing the risk of relapse in individuals with cocaine dependence.

Disclosures: P. Estave: None. **S.R. Jones:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.14/EEE14

Topic: G.08. Drugs of Abuse and Addiction

Title: The ketogenic diet decreases behavioral responses to cocaine in male and female rats

Authors: *L. A. MARTINEZ[1], M. E. LEES[1], D. N. RUSKIN[2], S. A. MASINO[2]
[1]Neurosci., [2]Neuroscience/Psychology, Trinity Col., Hartford, CT

Abstract: The ketogenic diet (KD) is a high fat, low carbohydrate and adequate protein formulation that has traditionally been used as a treatment for epilepsy; however, there is growing evidence that this diet has broader therapeutic potential due to its diverse, positive effects on nervous system function. Recent drug addiction studies suggest that activation of the brain adenosine system decreases behavioral responses to many drugs of abuse, including cocaine. Given that one consequence of the KD is an increase in brain adenosine, we sought to address whether the KD has potential as a novel therapy for drug addiction. In this study, male and female Sprague-Dawley rats were placed on a strict 6.6:1 (fat:[carbohydrates+protein], by weight) KD or control diet at 5 weeks of age and then maintained on those diets for 3 weeks prior to behavioral testing. During testing, rats received daily i.p. injections of cocaine (15 mg/kg/ml) or saline vehicle for one week, were abstinent for a subsequent week, and then all animals received a final challenge injection of 15 mg/kg/ml cocaine. We found that both males and females on the KD exhibited decreased cocaine-induced stereotyped responses, during the week of repeated cocaine injections as well as on the final challenge day. These results suggest that the KD may indeed hold potential as a therapy for drug addiction. Future studies will focus on exploring the neural mechanisms underlying the behavioral effects of this diet.

Disclosures: L.A. Martinez: None. **M.E. Lees:** None. **D.N. Ruskin:** None. **S.A. Masino:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.15/EEE15

Topic: G.08. Drugs of Abuse and Addiction

Title: The effects of environmental enrichment or impoverishment on the development and extinction of cocaine and amphetamine conditioned place preference and nucleus accumbens c-Fos expression

Authors: C. IRVING[1], A. SAPERSTEIN[1], C. CHADWICK[1], A. F. SCHROEDER[2], E. ANDERSON[1], I. LAMPTEY[1], V. DUSZAK[1], A. SHAIKH[1], *J. A. SCHROEDER[1]
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Abstract: Environmental factors can increase or decrease a person's susceptibility to addiction to drugs of abuse. Childhood trauma and community poverty may increase the likelihood of drug abuse, and environmental enrichment can reduce drug-seeking behaviors. The same environmental factors may influence cravings and relapse potential during drug abstinence. In this study, conditioned place preference (CPP) was used to measure cocaine and amphetamine

reward behavior. Animals were exposed to environmental enrichment (group housing, novel objects, food treats) or impoverishment (single housing, food, bedding and water only) for 3 weeks prior to CPP. Environmental impoverishment prior to conditioning enhanced cocaine, but not amphetamine CPP. In a follow-up study, animals were exposed to enrichment or impoverishment following CPP and were re-assessed for their preference one week later to measure the extinction of CPP. Animals exposed to one week of enrichment following cocaine or amphetamine CPP displayed a weaker preference for the drug-paired environment after extinction. Immunohistochemical evaluation of the nucleus accumbens revealed significant differences in c-Fos expression in enriched versus impoverished animals. These results add to the body of evidence suggesting that environmental enrichment or impoverishment can have a significant impact on conditioning to psychostimulants and the activation of brain reward centers.

Disclosures: C. Irving: None. **A. Saperstein:** None. **C. Chadwick:** None. **A.F. Schroeder:** None. **E. Anderson:** None. **I. Lamptey:** None. **V. Duszak:** None. **A. Shaikh:** None. **J.A. Schroeder:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.16/EEE16

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA T32

Title: Psychomotor sensitization following an intermittent access schedule of cocaine self-administration

Authors: *C. CARR, J. XIA, J. HILDENBRAND, C. FERRARIO, T. ROBINSON
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Abstract: The ability of self-administration procedures to produce symptoms of addiction is critical to understanding the neural and behavioral plasticity associated with the transition from controlled to compulsive intake. Traditionally, the amount of drug consumed was considered critical to the development of addiction-like behavior. More recently, the temporal pattern of use has been shown to be an important factor. The Intermittent Access (IntA) model of cocaine self-administration produces a pharmacokinetic profile characterized by spikes in blood cocaine levels by alternating brief periods of drug availability with longer periods of unavailability. Similar to the oft-used Long Access (LgA) procedure, IntA is capable of producing an escalation of intake, a progressive increase in cocaine demand, robust drug- and cue-induced reinstatement of drug-seeking, and other hallmarks of addiction, despite far less total cocaine consumption. A

recent report has shown tolerance to the psychomotor activating effects of cocaine when tested soon after discontinuation of LgA, the opposite of which is hypothesized for IntA. Here we asked whether IntA to cocaine produces drug-induced psychomotor sensitization. Research has shown that repeated treatment with psychostimulant drugs produces sensitization to both their psychomotor activating and incentive motivational effects. This behavioral sensitization has been associated with neuroadapations in brain regions involved in reward processing and decision making, which are especially important for the transition to addiction. We report, relative to rats given LgA to cocaine (6 hours/day), rats given IntA were hypersensitive (sensitized) to the psychomotor activating effects of cocaine following one day of withdrawal, evidenced by increased locomotor activity. These findings add to the growing literature that the IntA procedure is capable of producing more robust addiction-like behavior and is especially valuable for studying associated neuroadaptations.

Disclosures: C. Carr: None. **J. Xia:** None. **J. Hildenbrand:** None. **C. Ferrario:** None. **T. Robinson:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

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Topic: G.08. Drugs of Abuse and Addiction

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Research and Education Initiative Fund, a component of the Advancing a Healthier Wisconsin Endowment at the Medical College of Wisconsin
Neuroscience Research Center, Medical College of Wisconsin

Title: The impact of addiction-associated risk factors on neuronal ensemble activation

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Abstract: Addiction is a chronic relapsing disorder, with many known risk factors such as novelty seeking and stress. High novelty seeking is a trait associated with increased vulnerability for addiction, while stress is a major cause of craving and relapse. Previous studies have shown that novelty, drugs of abuse, and stress engage mesocorticolimbic circuitry, however, it is unknown whether the same *cells* mediate both risk factors and addiction. Using TetTag H2B-EGFP mice, our lab has previously identified a drug-seeking neuronal ensemble within the prelimbic prefrontal cortex (PrL PFC) that could be reactivated by a subsequent drug-seeking session. The proportion of the ensemble that was reactivated was correlated to the persistence of

the drug-seeking behavior. However, it is still unclear how novelty or stress engages this ensemble. In the first experiment, mice were trained to self-administer cocaine and the ensemble was tagged during a drug seeking session. Mice then underwent an additional 2 wks of abstinence, during which time they were exposed to episodic social defeat stress. Mice underwent an additional drug-seeking test and tissue was analyzed for ensemble reactivation using c-Fos immunohistochemistry. In the second experiment, we examined the ensembles associated with novelty and cocaine in the nucleus accumbens (NAc) and PFC. A novel experience or continued home cage exposure was used to induce tagging to identify neurons involved with a novel experience. Three days later, animals were administered a single cocaine or saline injection in a habituated chamber, and ensemble reactivation was assessed. While we found significantly more c-Fos positive neurons in the PrL PFC, NAc core, and NAc shell in cocaine treated animals, it was only in the NAc shell that the novelty-tagged ensemble showed greater reactivation. This suggests that different types of rewards may engage a common ensemble in the NAc shell, while ensembles in the other areas may be more specific to reward type. Ongoing experiments are assessing whether this trend is observed following other classes of psychoactive drugs. Together, these studies provide further insight into how drug experiences and risk factors for addiction are processed in the brain.

Disclosures: N.N. Nawarawong: None. **M. Slaker:** None. **C.M. Olsen:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

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Program #/Poster #: 417.18/EEE18

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R21-DA021793
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UW ADAI

Title: Phasic dopamine release within the nucleus accumbens core differentially mediates drug-taking and drug-seeking

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Abstract: Altered dopamine (DA) transmission is implicated in most contemporary theories of drug abuse. However, the direction, timing, and context in which alterations of DA transmission occur remain a matter of debate. Both drug themselves, and the cues that are repeatedly paired with drugs are capable of driving DA release in the nucleus accumbens core (NAcc). Recent work from our lab identifies opposite trajectories of cue-evoked DA release in the NAcc over chronic drug use, depending on whether the cue is a result of an action by the subject to obtain the drug, or whether the cue was delivered by the investigator. Attenuation of phasic DA transmission to response-contingent drug cues was observed in animals that escalated their daily drug intake. Systemic administration of L-DOPA restored the neurochemical change and returned drug consumption to pre-escalated levels. Conversely, phasic DA transmission increased within the NAcc in response to non-contingent drug-cues, which coincided with increases of drug-seeking behaviors. Therefore, we postulated that phasic DA transmission in the NAcc mediates increased drug-taking and drug-seeking in a divergent manner. To investigate this hypothesis, we evoke phasic DA release with optogenetics during drug-taking and drug-seeking contexts. We injected a viral vector that expresses channelrhodopsin2 under the control of the CaMKIIδ promoter (AAV1-CaMKIIδ-ChR2-mCherry) bilaterally into the ventral tegmental area, and implanted optic fibers in the NAcc of male Wistar rats. Photostimulation (6 pulses, 30 Hz) paired with response-contingent drug cues decreased drug consumption in animals that escalate daily drug intake ($p<0.01$), but had no effect in their non-escalating counterparts ($p>0.05$), indicating that escalation of consumption is driven by low phasic DA. However, photostimulation paired with cues presented in a non-contingent manner prevented habituation of their ability to drive drug seeking ($p<0.05$), indicating that increased drug seeking is driven by high phasic DA. Therefore, these direct, temporally resolved manipulations indicate that diametric changes in NAcc phasic DA transmission mediate increased drug-taking and drug-seeking behaviors following chronic use.

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Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

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Program #/Poster #: 417.19/EEE19

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant DA011064

Title: The $5HT_{1B}$receptor agonist, CP 94,253, attenuates the reinforcing effects of cocaine self-administration during the estrus phase post-abstinence

Authors: *S. N. SCOTT[1], T. T. NGUYEN[2], S. M. DOYLE[3], J. L. NIESEWANDER[3]
[2]Sch. of Biol. Hlth. Systems Engin., [3]Sch. of Life Sci., [1]Arizona State Univ., Tempe, AZ

Abstract: We have found that serotonin 5-HT_{1B} receptors ($5HT_{1B}$Rs) modulate cocaine self-administration in opposite directions depending on the phase of the addiction cycle in male rats. Specifically, administration of a $5HT_{1B}$R agonist, CP 94,253 (CP), facilitates cocaine intake during the active drug-taking phase, while attenuating cocaine intake and drug seeking behavior following 21 days of protracted abstinence. We recently found that CP facilitates cocaine intake in female rats tested during the active drug-taking phase, similar to effects observed in male rats. In this study, we examined if female rats show the same abstinence-dependent change in CP effects as observed in males. Clinical and preclinical research suggests that females are more susceptible to cocaine craving and relapse, and this phenomenon is influenced by gonadal hormones. For instance, heightened sensitivity to the effects of cocaine is observed during the estrus phase compared to other phases of the estrous cycle, suggesting that estrogen enhances the reinforcing properties of cocaine. For this reason, the present study tested female rats during the estrus phase. Female Sprague-Dawley rats were trained to self-administer 0.75 mg/kg, IV cocaine on a fixed ratio (FR) 5 schedule of reinforcement for 2 hours/day. Once reinforcement rates stabilized, rats were given at least 21 days of abstinence during which daily vaginal smears were taken starting on abstinence day 14. Once rats were predicted to be in the estrus phase, they underwent pretreatment with CP (5.6 mg/kg, SC) or vehicle. Testing commenced 15 min later with access to 0.75 mg/kg, IV, cocaine on an FR5 schedule for one hour and then with access to 0.075 mg/kg for the second hour of testing. This study is ongoing and thus far, preliminary results indicate that CP decreases cocaine intake and response rates similar to the effects observed in male rats. This suggests that CP attenuates the reinforcing properties of cocaine. These findings have important implications for developing treatments for cocaine dependence in women.
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Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

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Program #/Poster #: 417.20/EEE20

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH/NIDA R15DA040809 (LIP)
University of Texas at Arlington Honors College Undergraduate Research Fellowship (JHA)

Title: Sex differences in nucleus accumbens CREB activity after cocaine-induced conditioned place preference

Authors: *B. D. BUTLER, J. H. ANTONIO, A. C. HOCH, J. C. HOLT, S. S. KOKANE, L. I. PERROTTI
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Abstract: Sex-differences in behaviors related to drug-craving and relapse have been observed to be more intense in addicted women than in addicted men. Cue-reactivity has been proposed to be an underlying cause of craving and relapse in addicted individuals. Preclinical evidence indicates involvement of the CREB signaling pathway in the nucleus accumbens (NAc) as a molecular basis of drug-cue associations. The main aim of this study was to determine dose-dependent sex differences in cocaine place preference and also to observe sex differences in the CREB activity across different cocaine conditioning doses and sex. Both male and female Long-Evans rats underwent a cocaine-conditioned place preference (cocaine-CPP) paradigm over eight days. Briefly, the first day consisted of a pre-test in which rats were allowed to roam freely in a CPP apparatus for 15 minutes. Twenty-four hours following the pre-test, rats were conditioned with one of three doses of cocaine (0, 5, 10, and 15 mg/kg) or 0.9% saline on alternate days and in alternate compartments of the CPP apparatus. Twenty-four hours after the last conditioning session animals were tested for cocaine-CPP by allowing them to freely roam throughout the CPP apparatus and the time spent in each compartment was measured. Thereafter, rats were euthanized, their brains extracted, and processed for immunohistochemistry (IHC) for phosphorylated CREB (pCREB). Overall, females exhibited significant cocaine-CPP across all three conditioning doses of cocaine. Interestingly, males exhibited a cocaine-CPP for only the 10 and 15 mg/kg doses. Respectively, our IHC results show increased pCREB expression in the NAc of both males and females when they exhibited significant cocaine-CPP (Females 5, 10, 15 mg/kg; Males 10, 15mg/kg). Interestingly, our data indicate a trend for higher NAc pCREB levels in cocaine-preferring females than in cocaine-preferring males (10, 15mg/kg). Males conditioned with 5 mg/kg of cocaine did not develop CPP and had comparatively lower levels of pCREB expression. In conclusion, our study shows that females develop CPP for cocaine at lower conditioning doses than males and that altered CREB activity in the NAc may be an underlying mechanism mediating this effect.

Disclosures: B.D. Butler: None. **J.H. Antonio:** None. **A.C. Hoch:** None. **J.C. Holt:** None. **S.S. Kokane:** None. **L.I. Perrotti:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.21/EEE21

Topic: G.08. Drugs of Abuse and Addiction

Title: Environmental enrichment facilitates cocaine abstinence in an animal conflict model

Authors: *S. EWING, R. RANALDI
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Abstract: An ongoing challenge of using animal subjects to explore novel interventions for drug addiction is the construct validity of animal addiction models. In this study, we tested the utility of a newer "conflict" model of abstinence using environmental enrichment (EE), a well-researched experimental intervention for reducing addiction-related behaviors. Long-Evans rats (n=16) were trained in 3-h daily sessions to self-administer a cocaine/saline solution (1 mg/kg/infusion) until each demonstrated a stable pattern of drug-seeking. Afterward, half were placed in EE cages equipped with toys, obstacles, and a running wheel, while the other half were given clean, standard laboratory housing (non-EE). All rats then completed daily 30-min sessions during which the 2/3 of flooring closest to the self-administration levers was electrified, causing discomfort should they approach the levers; current strength (mA) was increased after every day of drug seeking until the rat ceased activity on the active lever for 3 consecutive sessions. Rats housed in EE reached this "abstinence" criterion after fewer days and at lower current strengths than non-EE rats. These results suggest that EE administered after the development of a cocaine-taking habit may be an effective strategy to facilitate abstinence.

Disclosures: S. Ewing: None. **R. Ranaldi:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

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Topic: G.08. Drugs of Abuse and Addiction

Support: NARSAD Young Investigator Award
NIH Grant K08DA036657

Title: Investigating addiction and reward prediction in mice using a modular open-source operant behavior system

Authors: D. CASSATARO, A. CUMPELIK, *L. L. SJULSON
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Abstract: Due to the popularity of genetic tools in neuroscience, investigators increasingly are using mice in complex behavioral tasks that were previously used only with rats or primates. Here we describe the development of a modular open source operant behavior system for mice

and outline its application to drug self-administration and reward-guided behavior. This Arduino-based system is built entirely from low-cost or 3D-printed parts, enabling rapid reconfiguration, scalability, and straightforward integration with electrophysiological recording, fiber photometry, and closed loop optogenetic manipulation.

Disclosures: D. Cassataro: None. **A. Cumpelik:** None. **L.L. Sjulson:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

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Program #/Poster #: 417.23/EEE23

Topic: G.08. Drugs of Abuse and Addiction

Title: In-vivo visualization of Zn dynamics using PET and fiber photometry

Authors: *J. BONAVENTURA, S. LAM, J. GOMEZ, M. MICHAELIDES
NIDA/NIH, Baltimore, MD

Abstract: Zinc (Zn^{2+}) is a trace element involved in multiple biological functions. Besides numerous proteins (i.e. enzymes and transcription factors) containing Zn2+ binding sites where Zn^{2+} is required for either structural or catalytic purposes, there is also unbound Zinc putatively able to act as a signaling ion. Zinc transport is highly regulated by several transporters from the ZIP (Zrt- and Irt-like protein) family which control influx of Zn^{2+} into the cytoplasm, and the ZnT (or SLC30) family which mediate Zn^{2+} efflux from the cytoplasm. In particular, the ZnT-3 transporter is expressed in glutamatergic neurons in the prefrontal cortex, amygdala and hippocampus and the mediates the accumulation of Zn^{2+} in synaptic vesicles, where the ion is co-packaged (and co-released) with Glutamate and is able to modulate synaptic transmission. However, little is known about the dynamics of 'zincergic' transmission so we developed tools that allowed us to explore this topic. In this study we developed the use of a long-lived and positron-emitting isotope of Zinc (^{65}Zn) to non-invasively visualize Zinc dynamics in longitudinal studies via positron-emission tomography (PET) imaging. In parallel, we used a virally transduced FRET based biosensor to visualize acute free Zn^{2+} fluctuations upon neuronal activation through fiber photometry. These new approaches, combined with the use of ZnT-3 knock-out mice, allowed us to elucidate the role of synaptic Zinc release in the neurocircuitry involved in the rewarding and addictive properties of cocaine and other psychostimulants.

Disclosures: J. Bonaventura: None. **S. Lam:** None. **J. Gomez:** None. **M. Michaelides:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.24/EEE24

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH/NIDA

Title: Longitudinal changes in brain activity during acquisition of cocaine locomotor sensitization and its modulation by synaptic zinc

Authors: *J. L. GOMEZ, J. BONAVENTURA, M. MICHAELIDES
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Abstract: Zinc (Zn^{2+}) is an essential element and its 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. Chronic cocaine exposure leads to a phenomenon called behavioral sensitization where repeated injections of the same cocaine dose lead to elevation of locomotor activity with repeated exposure. This change in locomotor activity is believed to reflect cocaine-induced changes in brain plasticity. We investigated the role of synaptic Zn^{2+} in modulating cocaine locomotor sensitization via use of a transgenic mouse line lacking the gene (Slc30a3) encoding the neuronal zinc transporter (ZnT3). The ZnT3 packages Zn^{2+}into glutamatergic vesicles for synaptic co-release and is enriched in brain regions implicated in drug-induced sensitization. In parallel, we used PET and the [^{18}F]FDG radiotracer to identify changes in regional brain activity at different time points of the sensitization paradigm. Mice were scanned prior to any experimental manipulation (scan-1); after first exposure to cocaine (scan-2); after fifth exposure to cocaine (scan-3); and after one-week withdrawal plus re-exposure to cocaine (scan-4). *In vitro* assays were also undertaken in different cohorts to examine synaptic plasticity markers in brain areas indicated by PET experiments. These experiments aim to highlight the mechanistic involvement of neuronal Zn^{2+} in behavioral sensitization to cocaine.

Disclosures: J.L. Gomez: None. **J. Bonaventura:** None. **M. Michaelides:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.25/EEE25

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA R00 DA035322
NARSAD Young Investigator Award to MPS

Title: Stressor controllability alters the severity of cocaine-induced encoding deficits in accumbal neurons for reward-predictive cues

Authors: *K. A. SILETTI, K. S. MCCONOMY, A. K. MONTGOMERY, M. P. SADDORIS
Psychology & Neurosci., Univ. of Colorado Boulder, Boulder, CO

Abstract: Repeated experience with drugs of abuse can induce lasting changes in neural function. Even after enforced periods of drug abstinence, animals show heightened responsivity to cocaine-associated cues and impaired discrimination of natural reward-associated cues. Work from our lab and others has demonstrated dysregulation of mesolimbic circuitry as an underlying mechanism for deficits in reward-associated learning in cocaine-experienced rats. For example, electrophysiological activity in the nucleus accumbens (NAc) core and shell both exhibit abnormal and often attenuated encoding during Pavlovian conditioning for non-drug rewards weeks after the cessation of cocaine self-administration. Furthermore, these cocaine-induced cognitive deficits appear to significantly worsen after periods of drug abstinence, such that changes during this period induce robustly greater drug seeking and relapse. One potential mechanism by which this may occur is due to the chronic stress of withdrawal during abstinence. If so, providing animals with an active coping mechanism during this period could endow animals with resilience to prevent worsening cognitive deficits. To test this, rats with a history of cocaine self-administration (1 mg/kg iv; 2hr access, 14d) were given a single session of stressor controllability, wherein unsignaled tailshocks could be terminated with a wheel turn. Behavioral control learned in this situation is known to trans-situationally promote resiliency and was thus hypothesized to behaviorally immunize these subjects against other stressors (such as withdrawal or drug craving). These rats were compared to animals that received similar tailshocks but had no control over their termination, where the lack of control precipitates a more pathological stress phenotype. Here we demonstrate that neural encoding in the NAc in subsequent behavioral tasks is comparable to drug-naïve controls in rats with behavioral control, while the inescapable shock subjects show significantly worsened encoding. These findings suggest that stress exposure during drug abstinence significantly affects subsequent learning and, in particular, that controllability may impart resilience against drug-related cognitive deficits.

Disclosures: K.A. Siletti: None. **K.S. McConomy:** None. **A.K. Montgomery:** None. **M.P. Saddoris:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.26/EEE26

Topic: G.08. Drugs of Abuse and Addiction

Support: University of Colorado Startup Funds

Title: Differential encoding in prefrontal and accumbal neurons during a continuously updating risk-based decision making task

Authors: *M. SADDORIS, K. S. MCCONOMY
Psychology and Neurosci., Univ. of Colorado Boulder, Boulder, CO

Abstract: Value-based decisions involve assessing how potential gains are offset by a variety of costs. For example, animals may desire a valuable food, but the risks of obtaining that item (e.g., potential injury, predation, energy expended) may be sufficiently great to bias animals towards less desired (but less risky) options. Many studies have now shown an important role for limbic system structures in risk-based decision making, including contributions from prefrontal cortex, nucleus accumbens and mesolimbic dopamine afferents. However, in nearly all of these tasks, rats learn a stable dichotomy of options where one response is associated with a "safe" option, while a different response with a "risky" option. These binary choice models provide information about which option is preferred, but such all-or-none choices cannot determine how much an option is preferred or the extent to which risk tolerance is stable across trials or sessions. To overcome this limitation, we adapted a task used in human clinical settings for rodent use known as the Balloon Analogue Risk Task (BART). Here, rats can press a lever and each press "inflates" the size of the potential reward, and rats can "cash out" at the foodcup to obtain accumulated food rewards. However, there is a probability of the trial "busting" if the rat presses too much (entailing lost rewards and a timeout period), so rats must continuously update their risk tolerance in the press sequence to maximize rewards while avoiding busts. In this task we recorded from neurons in both the prelimbic cortex (PL) and nucleus accumbens core (NAc) of the same animals during the BART task. In general, both PL and NAc are highly engaged in the BART task, with most cells displaying phasic responses to at least one of the task events (e.g., press, cashout delay, reward/bust). Preliminary data suggests that PL neurons play a prioritized role in encoding activity related to the cashout decision, and thus may be important for disengaging in reward seeking behavior. In contrast, NAc showed more activity in the reward/bust period, suggesting a role in assessment of task outcome for behavioral updating.

These findings suggest that NAc and PL may coordinate activity and differentially contribute to discrete aspects of risk tolerance.

Disclosures: M. Saddoris: None. **K.S. McConomy:** None.

Poster

417. Drugs of Abuse and Addiction: Cocaine: Other Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 417.27/FFF1

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R00 DA035322

Title: Optical stimulation midbrain dopamine neurons drives self-administration in cocaine-experienced rats, but fails to elicit phasic neural encoding in nucleus accumbens shell

Authors: *K. MCCONOMY[1], M. SHAH[1], M. P. SADDORIS[2]
[1]Psychology and Neurosci., Univ. of Colorado Boulder, Boulder, CO; [2]Psychology and Neurosci., Univ. of Colorado, Boulder, Boulder, CO

Abstract: Repeated exposure to cocaine results in abnormal encoding for motivationally-salient stimuli in the nucleus accumbens (NAc). For example, we and others have shown that phasic responses of NAc neurons as well as phasic dopamine release within the NAc are strikingly altered in drug-abstinent rats with a history of cocaine self-administration. Furthermore, this effect is most pronounced in the NAc shell, a region important for mediating the reinforcing properties of cocaine, and is also thought to be important for supporting motivationally-related behavior. To date, investigations have largely focused on how prior experience with cocaine can produce altered encoding and behavior in subsequent Pavlovian or operant settings, but less is known about whether the function of of the dopamine system itself if appropriately engaged in motivated goal-directed action. To test this, TH::cre rats or littermate controls were first allowed to self-administer either cocaine iv or water to a port (2hr/d, 14d), followed by a period of abstinence. Later, all rats receive infusions of channelrhodopsin (AAV-DIO-ChR2-mCherry) into the ventral tegmental area (VTA), along with chronically implanted optical fibers into the VTA and electrophysiological arrays into the NAc shell. Rats were allowed to press a lever to receive optical stimulation of the VTA (5sec, 20Hz, 4ms pulsewidth, 20mW) and neural activity was recorded in the NAc shell during the task. TH::cre rats showed high levels of self-administration regardless of drug background compared to non-transgenic controls, though cocaine TH::cre rats showed excessive levels of pressing during the stimulation itself (lockout period). However, while drug-naïve TH::cre rats showed robust activity related to the press, this phasic activity was largely absent in cocaine TH::cre rats. Surprisingly, both cocaine and drug-naïve TH::cre groups showed rapid extinction of pressing when the laser was off, though only

the cocaine group also displayed rapid reinstatement post-extinction. Thus, stimulation of dopamine is sufficient to support self-administration after cocaine, but does not rescue phasic responses in mesolimbic target regions important for motivation.

Disclosures: K. McConomy: None. **M. Shah:** None. **M.P. Saddoris:** None.

Poster

418. Neural Mechanisms of Nicotine Addiction I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 418.01/FFF2

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH/NIDA grant R25DA035161 (TI, KSR)
NIH/NCI grant U54CA132378/ U54CA137788 (KSR)
NIH/RCMI grant RR03060 (KSR)
NIH/NINDS grant R25 NS076414-03 (KSR)

Title: Sex differences in expression of deltafosb in taurine and cocaine treated rat brains

Authors: *T. D. IRVING[1], A. COLE[1], C. VASQUEZ[1], T. ADEBOWALE[1], U. AKPARA[1], K. Y. SALAS-RAMIREZ[2]
[2]Physiology, Pharmacol. and Neurosci., [1]CUNY Sch. of Med., New York, NY

Abstract: Cocaine use is responsible for 15,000 yearly deaths in the United States, yet there are limited effective interventions for cocaine addiction. Chronic cocaine use stimulates the production of transcription factor deltaFosB in regions of the brain that mediate reward, particularly the nucleus accumbens (NAC). Accumulation of deltaFosB represents a molecular marker for addiction that increases sensitivity to compulsive drug-seeking behaviors. Taurine, a dietary and endogenous amino acid produced by the liver and glial cells, has neuroprotective capabilities. Studies have shown that taurine can effectively inhibit cocaine reward in both male and female rodents; therefore, this study aims to determine whether taurine has the capacity to downregulate deltaFosB expression in the NAC of cocaine treated rats and if these effects are sex-specific. Forty adult male and female rats were divided into treatment groups that were either exposed to a saline or taurine pretreatment for two weeks. Afterwards, they were divided into five groups: (1) pre-sal/saline, (2) pre-sal/cocaine (3) pre-tau/taurine, (4) pre-tau/cocaine and (5) pre-tau /taurine + cocaine while being tested for the psychomotor effects of cocaine using a behavioral sensitization protocol. Immediately after the behavioral assessments, animals were perfused and tissue was stored in cryoprotectant. We examined the expression of deltaFosB using immunocytochemical techniques, followed by microscopic analysis with Neurolucida software to trace regions of the NAC and determine the mean quantity of deltaFosB stained cells per area. As determined by a one-way ANOVA, cocaine significantly increased expression of

deltaFosB in the NAC core of female rats compared to control (p=.0339), and both pretreatment and co-administration with taurine reduced deltaFosB levels. Preliminary analysis of male rats similarly showed DeltaFosB attenuation in taurine pre-treatment and co-administration in the NAC core (p=.0212), however, these effects are seen in the NAC shell as well (p=.0403). There were no significant differences in deltaFosB in the NAC of animals treated with taurine independent of cocaine treatment. These findings support previous studies where cocaine increases deltaFosB expression in the transition of an non-addicted to an addicted brain; however, taurine effectively reduces the expression of deltaFosB in the NAC in both male and female rats with sex-specific effects on the core and shell deltaFosB expression. Further studies are needed to determine the sex specific efficacy of taurine as a treatment for cocaine addiction and relapse.

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Poster

418. Neural Mechanisms of Nicotine Addiction I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 418.02/FFF3

Topic: G.08. Drugs of Abuse and Addiction

Support: DoD W81XWH-11-2-0145
DoD W81XWH-12-2-0048

Title: The effects of the COMT inhibitor tolcapone and sex on alcohol consumption in individuals with alcohol use disorder (AUD)

Authors: *J. MITCHELL, A. R. COKER, D. WEINSTEIN, T. A. VEGA, C. S. MILLER, A. S. KAYSER
UCSF: Dept. of Neurol., San Francisco, CA

Abstract: Previous data suggest that hypo-dopaminergic tone in frontal brain regions contributes to impulsivity (1) and by extension to problems with alcohol and drug abuse (2). Consistent with these findings, variations in the dopamine-metabolizing enzyme catechol-O-methyltransferase (COMT) have been linked to behavioral differences in drug and alcohol consumption and decision making (3). The catechol-O-methyltransferase (COMT) inhibitor tolcapone, which increases cortical dopamine tone in frontal brain regions (4), could therefore prove useful in assessing the effects of frontal dopamine levels on behavior. Aims: We administered tolcapone to individuals meeting DSM-V criteria for alcohol use disorder (AUD) to assess whether tolcapone administration attenuates alcohol consumption and subjective high. Methods: Here we assessed the effects of a randomized double-blind administration of the catechol-O-

methyltransferase (COMT) inhibitor tolcapone (100mg TID for 5 days) on alcohol consumption, impulsive choice, and laboratory tasks assessing impulsivity and subjective high in 55 non-treatment seeking male and female AUD subjects. Results: We found that 5 days of 100mg TID tolcapone significantly attenuated self-reported alcohol consumption ($n = 55$, $F = 4.36$, $p = .041$), and this effect was driven by female subjects (drug x gender, $n = 55$, $F = 3.33$, $p = .07$). In addition, while tolcapone significantly attenuated subjective high across all subjects ($n = 51$, $F = 10.2392$, $p = .002$), it decreased intoxication (BAC) only in females ($n = 22$, $T = 2.045$, $p = .053$). The tolcapone effect on alcohol consumption was significantly correlated to different sub-types of impulsivity in males and females. Females with lower attentional impulsivity were more likely to decrease their consumption on tolcapone ($n = 23$, $F = 6.99$, $p = .015$), while males with greater motor impulsivity were more likely to decrease their consumption on tolcapone ($n = 32$, $F = 7.17$, $p = .012$). This suggests that males and females may be using different factors to impact their alcohol related decision making. Conclusions: These data provide preliminary evidence to suggest that tolcapone may be an advantageous therapeutic for females with AUD.

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Poster

418. Neural Mechanisms of Nicotine Addiction I

Location: SDCC Halls B-H

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Program #/Poster #: 418.03/FFF4

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant Z1A000069
NIDA IRP

Title: A closer look at transcranial magnetic stimulation through *in vivo* neuroimaging

Authors: *M. A. BOEHM[1,2], J. GOMEZ[1], J. BONAVENTURA[1], Z. JUSTINOVA[1], H. JEDEMA[1], E. STEIN[1], C. BRADBERRY[1], M. MICHAELIDES[1]
[1]Neuroimaging Res. Br., Natl. Inst. on Drug Abuse, Baltimore, MD; [2]Neurosci., Brown Univ., Providence, RI

Abstract: Transcranial magnetic stimulation (TMS) is a noninvasive technique used to induce electric fields in the brain. Over the past 30 years, TMS has emerged as a potential therapeutic tool for several disorders including depression, addiction, and Parkinson's disease. Despite increasing use in research and medicine, basic questions remain about the underlying mechanisms of TMS and how to optimize stimulation protocols for treating different disorders. Animal TMS studies have proven useful in revealing some mechanisms, but the wide range of parameters (e.g. intensity and frequency) and limited focality of rodent TMS make it difficult to

compare and translate findings. TMS in nonhuman primates (NHPs) allows for more focal stimulation and improved translatability to human applications of TMS. Previous TMS studies using NHPs have shown to be useful in investigating the effects of TMS parameters, but most studies have been limited to applications in the motor cortex. The objective of this pilot TMS study is to utilize [^{18}F]-fluorodeoxyglucose positron emission tomography (FDG-PET) to systematically assess the impact of varying intensity and frequency on motor and prefrontal TMS-induced brain activity in two *Saimiri sciureus* monkeys. Two male *Saimiri sciureus* monkeys will undergo acute (600 pulses) and short-term (1800 pulses) stimulation protocols targeting either motor or prefrontal cortex. FDG-PET will be used to compare TMS-induced brain activity at different intensities (25-150% resting motor threshold) and frequencies (1-20Hz, iTBS, cTBS). Brain activity will be normalized to within-subject sham control experiments. Acute experiments will assess brain activity during a 10 minute stimulation procedure, while short-term experiments will assess brain activity immediately following a 30 minute stimulation procedure. Overall, this project aims to improve our understanding of the role of intensity and frequency in rTMS targeting the motor and prefrontal cortex. This systematic characterization of TMS parameters could inform therapeutic applications for addiction and other disorders.

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Poster

418. Neural Mechanisms of Nicotine Addiction I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 418.04/FFF5

Topic: G.08. Drugs of Abuse and Addiction

Title: 5-methoxy-N,N-dimethytryptamine's influence on the right angular gyrus: Evidence for altered default mode functioning

Authors: *M. VILLANUEVA, P. GAST
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Abstract: Psychedelic tryptamines, pervasive in both plant and animal life, are widely used in non-US clinics for the treatment of opioid addiction as well as spiritual retreats. We conducted an EEG study on pre- and post-inhalation of 5-Methoxy-N,N-dimethyltryptamine (Bufo Alvarius toad) administered by Shamans in Mexico and Australia to reveal its influence on resting-state EEG. We performed independent component (IC) analysis (ICA) on individual data and used Measure Projection Toolbox, a group-level analysis scheme for EEGLAB, to cluster the results based on the estimated source locations weighted by power spectrum density. The results revealed that alpha and gamma band power levels within the right angular gyrus showed increased alpha and decreased gamma frequency power after administration. This pattern was

observed only in the eyes closed condition. Because the angular gyrus is one of nodes of the default mode network (DMN), the increased alpha band power during eyes closed indicates decreased activation of DMN. The result may be related to the decrease in, often negative, self-referential mentations and may explain the usefulness of DMT for treatment of psychiatric disorders, especially addictions.

Disclosures: P. Gast: None.

Poster

418. Neural Mechanisms of Nicotine Addiction I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 418.05/FFF6

Topic: G.08. Drugs of Abuse and Addiction

Support: FRM
Fondation Avenir
ANR

Title: High frequency but not low frequency deep brain stimulation of the subthalamic nucleus reduces motivation for cocaine, while increasing that for apple sauce in the monkey

Authors: *S. RAVEL[1], J. NACEF[2], J.-L. ANTON[1], I. BALANSARD[3], P.-Y. BORIUS[4], R. DESBRIÈRE[5], A. EUSEBIO[1,6], B. NAZARIAN[1], L. RENAUD[3], J.-M. RÉGIS[7], C. W. BRADBERRY[8], C. BAUNEZ[1]
[1]Inst. de Neurosciences de la Timone, CNRS & AMU UMR 7289, Marseille, France; [2]Inst. of Neurosci., Newcastle Univ., Newcastle upon Tyne, United Kingdom; [3]CE2F-PRIM, CNRS & AMU UMS 3537, Marseille, France; [4]AP-HP, Hop. de la Pitié-Salpétrière, Paris, France; [5]Hop. Saint-Joseph, Marseille, France; [7]Gamma Unit, Hop. de la Timone, AP-HM, [6]Hop. de la Timone, AP-HM, Marseille, France; [8]NIDA Intramural Res. Program, NIH, Baltimore, MD

Abstract: There is currently no pharmacological treatment for cocaine addiction, therefore it is important to look for alternative treatment strategies. One possibility could be a surgical approach. Indeed, it has been shown in the rat that the inactivation of the subthalamic nucleus (STN), by either lesions or high frequency Deep Brain Stimulation (DBS), reduces motivation for cocaine while increasing motivation for food. It has thus been suggested that STN high frequency DBS could be a good strategy to treat cocaine addiction. Before testing in human addicts, the aim of the present study was to validate this hypothesis in non-human primates. We have trained two monkeys to work under various schedules of reinforcement (Fixed Ratio 15 (FR15) and Progressive Ratio (PR)) for either apple sauce or cocaine (intraveinous 0.1 mg/kg/injection). After stabilisation of performances, electrodes have been implanted bilaterally in the STN, and high or low frequency chronic stimulation has been applied (HF : 130 Hz, 2V or

LF : 50 Hz, 3V respectively). All conditions (apple sauce-HF/LF stimulation ON, apple sauce-stimulation OFF, cocaine-HF/LF stimulation ON, cocaine-stimulation OFF) have been tested in alternance. Results have first shown that, after STN HF DBS, the motivation for apple sauce was significantly increased, while that for cocaine was significantly decreased. Conversely, STN LF DBS failed to show any modulation. These results confirm the opposite effect of STN HF DBS on motivation previously observed in rats, and further allowed us to demonstrate the selectivity of the HF over LF to elicit this effect. Since decreasing the motivation for the drug, without diminishing other forms of motivation is the goal for a possible treatment of cocaine addiction, STN HF DBS may thus be the appropriate strategy.

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Poster

418. Neural Mechanisms of Nicotine Addiction I

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 418.06/FFF7

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH intramural funding ZIA-AA000218
Brain and Behavior Research Foundation (BBRF) grant #17325

Title: A deeper insight into how GABA-B receptor agonism via baclofen may affect alcohol seeking and consummatory behaviors: Lessons learned from a human laboratory investigation

Authors: *M. FAROKHNIA[1], S. DESCHAINE[1], A. SADIGHI[2], F. AKHLAGHI[2], L. LEGGIO[1]
[1]NIH, Bethesda, MD; [2]Univ. of Rhode Island, Kingston, RI

Abstract: The GABA-B receptor agonist baclofen has been sugested as a potential pharmacotherapy for alcohol use disorder. Despite promising data from preclinical experiments, human studies with baclofen have shown mixed results. Additional research is needed to understand how this medication works from a biobehavioral standpoint; well-controlled human laboratory studies provide an informative platform to address this question. Here, we analyzed data from a laboratory experiment before which alcohol-dependent individuals (N = 34) received baclofen (30 mg/day) or placebo for a week. The experimental session included three consecutive phases: alcohol cue-reactivity (water trial followed by two alcohol trials), fixed-dose alcohol priming (40 minutes, target blood alcohol concentration: 0.03 g/dL), and alcohol self-administration (2 hours, up to 8 mini-drinks). Alcohol craving and subjective responses were also assessed throughout the session, and blood samples were collected for pharmacokinetic

measurements. Baclofen, compared to placebo, did not significantly attenuate cue-elicited craving or the amount of alcohol self-administration. However, baclofen moderated the relationship between alcohol priming and self-administration as shown by significant interaction effects between drug condition (baclofen vs. placebo) and priming variables (alcohol craving: $F_{3,9} = 6.03$, $p = 0.01$; alcohol sedation: $F_{3,6} = 7.16$, $p = 0.01$; breath alcohol concentration: $F_{1,25} = 5.22$, $p = 0.03$) on the total amount of alcohol self-administered. Baclofen pharmacokinetic parameters were calculated as follows (mean ± standard error): maximum plasma concentration (C_{max}) = 84.90 (13.58) ng/mL; time to C_{max} (t_{max}) = 2.47 (0.21) h; apparent plasma clearance at steady state (CL_{ss}/F) = 60866.52 (12641.72) mL/h; half-life ($t_{1/2}$) = 4.42 (0.29) h; area under plasma concentration - time curve (AUC) = 1033.49 (97.77) h*ng/mL. Bivariate correlation analyses between pharmacokinetic parameters and behavioral outcomes showed that baclofen C_{max} was negatively correlated with alcohol craving during cue-reactivity ($r = -0.57$, $p = 0.03$) and ratings of 'like more' during priming phase ($r = -0.59$, $p = 0.02$). These data suggest that baclofen may act through dissociating the link between an initial drink (priming) and subsequent alcohol consumption (self-administration). This study also sheds light on how baclofen pharmacokinetic parameters are correlated with behavioral outcomes assessed in a laboratory setting.

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Poster

418. Neural Mechanisms of Nicotine Addiction I

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 418.07/FFF8

Topic: G.08. Drugs of Abuse and Addiction

Support: R01 MH099085

Title: Sex differences in ketamine addiction-like behavior and nucleus accumbens spine morphology in chronic mild-stressed rats pre-treated with ketamine

Authors: K. N. WRIGHT, *D. P. HAGARTY, C. STRONG, K. J. SCHOEPFER, M. KABBAJ
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Abstract: Sub-anesthetic doses of ketamine provide rapid alleviation of depressive symptoms in individuals with Major Depressive Disorder (MDD). Important caveats that may hinder its widespread use are MDD is highly comorbid with drug addiction, and ketamine at higher doses is well-known for its abuse liability. Furthermore, women have a twofold higher rate of MDD and display a more rapid progression towards addiction than men. While ketamine at low doses has a clear therapeutic benefit, it is important to investigate the abuse potential along with the

therapeutic effects of ketamine in preclinical studies of both sexes displaying phenotypic MDD. Our lab was the first to show that female rats are more sensitive than males to ketamine's antidepressant-like and addictive-like effects, and at lower doses. Also, after intermittent intravenous ketamine self-administration (SA), both sexes exhibit ketamine-seeking behavior during extinction-reinstatement testing. These studies used stress-naïve rats, so it is uncertain if rats displaying the depressed-like phenotype will have a greater propensity to the SA of ketamine. To address these questions, male and female rats underwent chronic mild stress (CMS) and were treated with four intermittent, iv infusions of sub-anesthetic ketamine. Ketamine infusions ameliorated some of the depression-like traits as measured by the novelty-suppressed feeding test and sucrose preference test. Then, rats underwent SA of 0.5 mg/kg/inf ketamine under fixed-ratio 1 and progressive ratio schedules of reinforcement, and incubation of ketamine craving at multiple time points after the last exposure. Finally, alterations in dendritic spine morphology were measured in the nucleus accumbens, an area affected by both depression and addiction, using 3-dimensional reconstruction. CMS females displayed greater ketamine addiction-like behaviors than non-stressed females or males of either condition. Interestingly, CMS females pre-treated with ketamine display augmented addiction-like behavior compared to CMS females without ketamine pre-treatment, an effect not observed in male counterparts. The integration of these two models helps elucidate the complex relationship between ketamine's therapeutic and abusive properties in susceptible populations.

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Poster

418. Neural Mechanisms of Nicotine Addiction I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 418.08/FFF9

Topic: G.08. Drugs of Abuse and Addiction

Support: UTHSCSA President's Translational and Entrepreneurial Research Fund
Pilot award from CBN/CTSA

Title: Evaluation of a combination opioid/antipsychotic drug to reduce the abuse liability of opioid analgesics: A preclinical and clinical feasibility study

Authors: *A. M. BOLEY, M. E. CURTIS[1], G. T. COLLINS[2,5], M. S. ECKMANN[3], A. S. NAGPAL[3], J. S. POTTER[4], A. FRAZER[2], D. J. LODGE[2]
[1]Psychology, Univ. of Texas at San Antonio, San Antonio, TX; [2]Pharmacol., [3]Anesthesiol., [4]Psychiatry, UT Hlth. San Antonio, San Antonio, TX; [5]South Texas Veterans Hlth. Care Syst., San Antonio, TX

Abstract: Opioid abuse is a public health epidemic, with more than 115 overdose deaths each day in the United States. Opioid analgesics are routinely prescribed for pain, which is a debilitating condition that must be adequately treated to improve the quality of life of patients; however, this treatment puts the patient at risk by exposure to opioid medications that can lead to misuse. Thus, better treatment options are needed to help these patients without the risk of addiction and abuse. Given that the reinforcing properties of opioids are mediated, at least in part, by dopamine transmission, we posit that blocking dopaminergic D2 receptors will reduce the abuse liability of opioids without altering analgesic efficacy. Here we present initial feasibility data examining fixed dose combinations of oxycodone (OXY) with an atypical antipsychotic drug. Our initial preclinical studies suggest that the analgesic efficacy of OXY (2mg/kg i.p.), determined by the tail flick latency task, is not altered by its co-administration with an atypical antipsychotic drug. Similarly, respiratory depression, the primary reason for opioid-induced death, was not exacerbated by this combination. Interestingly, the antipsychotic drug reduced opioid self-administration, the gold standard for examining abuse liability in preclinical models. Taken together, these initial preclinical studies suggest that this drug combination may reduce the abuse liability of prescription opioids without altering analgesic efficacy or respiratory depression. To further confirm safety and demonstrate feasibility, we have now carried out initial clinical experiments. Specifically, healthy human subjects were enrolled in a double-blind crossover study involving three visits to the UT Health Pain Clinic. On visit 1, subjects became familiar with the experimental procedures and baseline pain thresholds were established using experimenter-administered pain. On visits 2 and 3, patients were given a single capsule containing either OXY plus placebo or OXY plus one of two atypical antipsychotics [risperidone (2mg) or ziprasidone (80mg)], with the order of the drug combinations randomized. Three different doses of OXY were examined in separate groups of subjects (5mg, 10mg, or 15mg). Preliminary data from these studies suggest that the drug combination did not alter the analgesic response of OXY or increase the incidence of adverse effects when compared to OXY alone. Future studies are underway to examine the effect of the combination on abuse liability in recreational drug users. If effective, this approach could be beneficial in the management of opioid treatments for disorders such as chronic pain.

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Poster

418. Neural Mechanisms of Nicotine Addiction I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 418.09/FFF10

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH grant DA035958
NIH grant AA02919

Title: Peripheral mechanoreceptor activation modulates mesolimibic gaba and dopamine neurons and dopamine release in the nucleus accumbens via delta opioid receptors

Authors: *K. BILLS, T. CLARKE, M. NELSON, Z. NEY, S. STEFFENSEN
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Abstract: Acupuncture has been shown to reduce drug-seeking behavior; however, the neurobiological mechanisms underlying its mechanism remain elusive. We have previously demonstrated that activation of the ulnar nerve at the Shenmen acupoint HT7 reduces cocaine, alcohol and methamphetamine self-administration and modulates GABA neurons in the midbrain ventral tegmental area (VTA) via an opioid receptor (OR)-dependent mechanism. A component of the neurological pathway mediating HT7's effects begin with activation of peripheral mechanoreceptors in the ulnar nerve. These receptors activate the dorsal column medial lemniscal (DCML) pathway and progressively synapse in the nucleus cuneatus, thalamus and lateral habenula before influencing the VTA. There, activation of opioid receptors produces a transient decrease in GABA neuron firing. The specific receptors mediating these effects, the optimal frequency of activation, regional specificity of activation and ultimate impact on dopamine (DA) release in the nucleus accumbens (NAc) have not been described. Here, we test a novel mechanism of activating the DCML via subcutaneous vibratory implants at the C7-T1 vertebral levels. We show that activation of peripheral mechanoreceptors at vibrational frequency of < 100 Hz, independent of the HT7 acupoint, is sufficient to depress VTA GABA neuron firing rate, increase DA neuron firing rate and produce a subsequent increase in DA release in the NAc. The effects are blocked with general and local administration of naltrindole, a selective delta opioid receptor antagonist. This suggests that summation of sensory input rather than acupoint based stimuli is responsible for the observed modulation of the mesolimbic system.

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Poster

418. Neural Mechanisms of Nicotine Addiction I

Location: SDCC Halls B-H

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Program #/Poster #: 418.10/FFF11

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA 1R21DA037556
VA 589-KG-0012

Title: Craving, cardiovascular, and cognitive effects of cocaine and lorcaserin, a serotonin 2C agonist with anti-addictive properties

Authors: *K. W. GRASING, M. D. HICKMAN, V. C. BOINPELLY, K. SURINENI, H. THAKUR, J. PIRTLE
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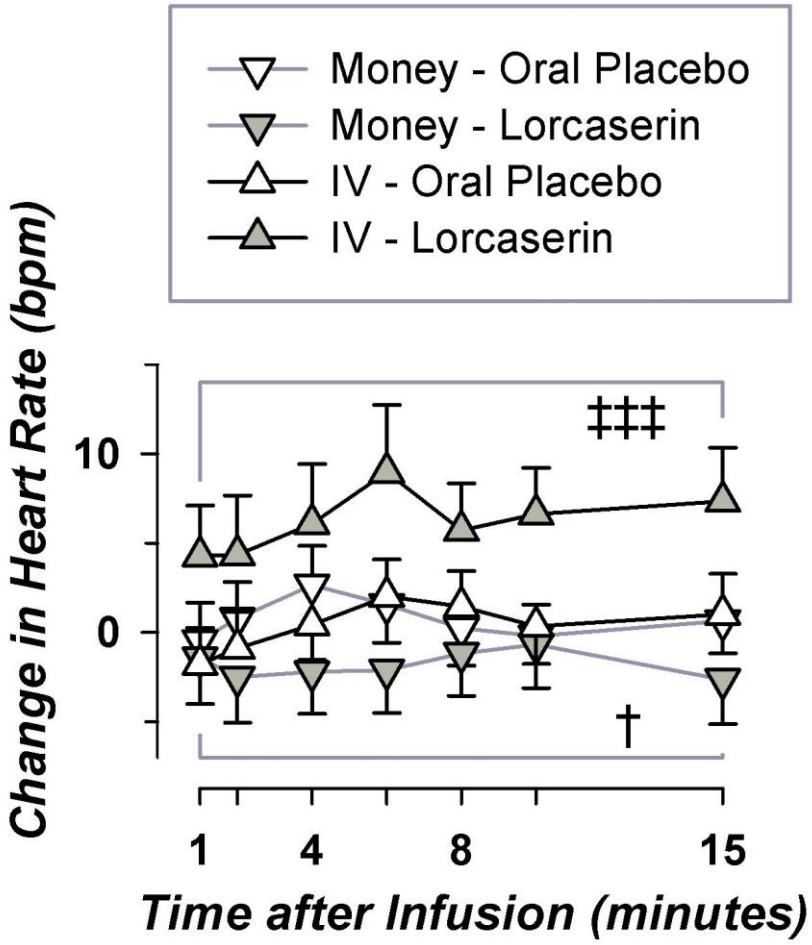
Abstract: AIM: Lorcaserin is a selective agonist for 2C serotonin receptors that can attenuate cocaine-reinforced behavior in rats and rhesus monkeys. Its interaction with cocaine-induced cardiovascular effects has not been described. The goal of this study was to determine whether lorcaserin modified craving, cognitive function, or cardiovascular effects of cocaine.
METHODS: Cocaine experienced users received either oral placebo (12 participants) or lorcaserin (n=9), followed by low- or high- doses of intravenous cocaine (0.23 or 0.46 mg/kg-injection). They were then allowed to self-administer the lower dose of cocaine, and afterwards reported script-induced craving and completed the Repeatable Battery for the Assessment of Neuropsychological Status.
RESULTS: As previously reported, oral lorcaserin did not modify cocaine self-administration. When collapsed across intravenous dose, lorcaserin treatment increased both systolic blood pressure and heart rate following noncontingent injections. During cocaine self-administration, lorcaserin decreased heart rate after a choice of monetary reinforcement, but increased heart rate following an intravenous choice. The availability of active cocaine increased systolic blood pressure, after a choice of either monetary or intravenous reinforcement, but was uneffected by lorcaserin. Lorcaserin did not significantly modify peak heart rate or blood pressure values, and no participant met stopping criteria for dosing because of elevated heart rate or blood pressure. No arrhythmias or electrocardiogram changes were identified, and lorcaserin did not modify script-induced craving or cognitive function.
CONCLUSION: Combined treatment with cocaine and lorcaserin had complex effects on cardiovascular measures. During cocaine self-administration, lorcaserin increased heart rate after an intravenous choice, but had an opposite effect after monetary reinforcement. Cardiovascular effects did not appear to be clinically significant. Lorcaserin treatment did not alter either craving or cognitive function.



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Poster

418. Neural Mechanisms of Nicotine Addiction I

Location: SDCC Halls B-H

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Program #/Poster #: 418.11/FFF12

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA13588
Harrington Scholar-Innovator Grant

Title: Novel small molecule inhibitors of protein kinase C epsilon for reducing consumptions of alcohol and nicotine

Authors: *J. WANG[1], A. BLASIO[2], M. B. POMRENZE[3], H.-Y. WANG[5], S. MCHARDY[5], R. O. MESSING[4]
[1]The Univ. of Texas At Austin, Austin, TX; [2]Univ. of Texas At Austin Col. of Pharm., Austin, TX; [3]Neurosci., [4]Neurology/Neuroscience, Univ. of Texas at Austin, Austin, TX; [5]Ctr. for Innovative Drug Discovery, Dept. of Chem., Univ. of Texas at San Antonio, San Antonio, TX

Abstract: Alcohol use disorder and smoking are highly comorbid and are among the top five preventative causes of death. Despite the large social and economic burden that they cause, current treatments for alcohol and nicotine addiction have limited efficacy. Based on prior biochemical and animal studies, we have proposed that protein kinase C epsilon is a drug target for reducing alcohol consumption. Thus, we derived several potent and novel small molecule inhibitors of protein kinase C epsilon (PKC epsilon) from the Rho-associated protein kinase (ROCK) inhibitor Y-27632, which has weak activity against PKC epsilon. *In vitro* kinase and binding assays showed that two compounds (1.0 and 1.3) are potent and selective against PKC epsilon, with *K*i values less than 20 nM. Both compounds crossed the blood brain barrier, achieved effective concentrations in mouse brain, and acutely reduced ethanol consumption when administered intraperitoneally at 40 mg/kg in wild type but not in *Prkce*$^{-/-}$ mice. Since medications for addiction are dosed repeatedly, here we investigated whether compound 1.0 was effective with repeated administration. Mice were provided with one bottle of water and one bottle of 10% ethanol continuously every day. After baseline drinking was stable, mice received once daily intraperitoneal injections of vehicle or 40 mg/kg compound 1.0 for five consecutive days. In the first cohort of mice (n=5 for vehicle group and n=3 for drug group), compound 1.0 reduced ethanol consumption on all injection days by 30-59 %. One day after the last injection, ethanol drinking returned to baseline. This result is consistent with the 15-hour half-life of compound 1.0 in the mouse brain. Since we also previously found that *Prkce*$^{-/-}$ mice consume less nicotine containing solutions and show decreased conditioned place preference for nicotine, we hypothesized that our PKC epsilon inhibitors would reduce oral nicotine consumption. Using a two-bottle choice voluntary nicotine drinking procedure, we found that intraperitoneal administration of compound 1.0 or 1.3 reduced consumption of 20 µg/ml nicotine by 31% and 24%. These results suggest that inhibitors of PKC epsilon are effective in reducing ethanol consumption when administered repeatedly and are also effective in reducing nicotine intake in mice.

Disclosures: J. Wang: None. **A. Blasio:** None. **M.B. Pomrenze:** None. **H. Wang:** None. **S. McHardy:** None. **R.O. Messing:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); inventor on patent held by UCSF.

Poster

418. Neural Mechanisms of Nicotine Addiction I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 418.12/FFF13

Topic: G.08. Drugs of Abuse and Addiction

Title: Mechanism of action of ITI-333, a novel modulators of serotonin, dopamine and mu opioid receptors for the treatment of opioid use disorder

Authors: *G. L. SNYDER[1], R. E. DAVIS[2], P. LI[2], W. YAO[2], S. CRUZ[2], L. ZHANG[2], J. P. HENDRICK[2], A. FIENBERG[2], K. E. VANOVER[2], S. MATES[2]
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Abstract: New medications are needed to treat opioid use disorder (OUD) by easing the somatic symptoms of drug withdrawal and effectively mitigating the dysphoria and psychiatric comorbidities (e.g., mood and anxiety disorders) that drive opioid use/abuse. Here, we describe the properties of ITI-333, a novel molecule combining high affinity binding (Ki < 50 nM) to three receptors that individually have been associated with treatment of substance use disorders and psychiatric comorbidities: the mu opioid (MOP), serotonin 5-$HT_{2A,}$ and dopamine D_1 receptors. Data from cell-based and *in vivo* assays, demonstrate that ITI-333 functions as a biased MOP receptor ligand with partial agonist activity, acting as an antagonist to block effects of high doses of morphine in both pain and motor activity models while acting alone to provide potent analgesia in models of acute and inflammatory pain. In cell-based assays using human recombinant MOP receptors expressed in CHO cells, ITI-333 alone induces cAMP accumulation (agonism) while blocking the effects of a full MOP agonist, DAMGO (antagonism). Further, ITI-333 displays biased agonism at MOP receptors, acting as an antagonist on beta-arrestin pathways that mediate opioid side effects. In mice, ITI-333, alone (0.01-1mg/kg, SC, 10 mice/group), produces naloxone-sensitive analgesia in the tail flick (TF) assay, while attenuating morphine-induced analgesia in TF (0.1-1mg/kg, SC, 10 mice/group). ITI-333 blocks hyperactivity induced by morphine (32mg/kg, SC, 6 mice/group) without significant effects on spontaneous locomotor activity. In drug abuse assays, ITI-333 (0.3, 1, and 3mg/kg, SC) dose-dependently suppresses the somatic and behavioral signs of opioid withdrawal precipitated by naloxone injection in oxycodone-dependent mice (i.e., oxycodone given for 8 days at increasing daily doses of 9-33 mg/kg b.i.d., 10 mice/group). Chronic (28 day q.d. treatment) of ITI-333 (0.3 or 3mg/kg, SC) does not result in tolerance or physical dependence in rats; acute doses of ITI-333 (0.3 or 3mg/kg, SC, 8 rats/group) do not induce GI or pulmonary side effects. Finally, ITI-333 (0.003-0.01mg/kg, IV, 8/group) is not self-administered by heroin-maintained rats. In summary, evidence supports a role for ITI-333 in mitigating symptoms of opioid withdrawal and supports its potential as a treatment for OUD, associated comorbid mood and dysphoric symptoms, and pain.

Disclosures: **G.L. Snyder:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies Inc. **R.E. Davis:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies Inc. **P. Li:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies Inc. **W. Yao:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies, Inc. **S. Cruz:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies.com. **L. Zhang:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies, Inc. **J.P. Hendrick:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies Inc. **A. Fienberg:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies Inc. **K.E. Vanover:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies Inc. **S. Mates:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies Inc.

Poster

418. Neural Mechanisms of Nicotine Addiction I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 418.13/FFF14

Topic: G.08. Drugs of Abuse and Addiction

Support: American Cancer Society (RSG-16-023-01-CPPB)
NIH S10 RR29577
NIH UL1 TR000001
NIH P30 CA168524

Title: Functional connectivity changes related to self-regulation among moderate to heavy smokers

Authors: *L. MARTIN[1], M. G. BRUCKS[1], D. CATLEY[3], K. P. RICHTER[2], E. F. ELLERBECK[2], V. B. PAPA[1], A. T. FOX[1]
[1]Hoglund Brain Imaging Ctr., [2]Preventive Med. and Publ. Hlth., Univ. of Kansas Med. Ctr., Kansas City, KS; [3]Children's Mercy, Kansas City, MO

Abstract: Resting-state functional connectivity (rsFC) has been shown to predict behavioral and pharmacological treatment outcomes. For example, functional connectivity has been shown to predict treatment outcomes following cognitive behavioral therapy (CBT) for depression. Moreover, functional connectivity between self-regulation and reward regions have been shown to predict smoking cessation following a pharmacological intervention. However, little is known about how functional connectivity changes over the course of health behavior interventions. The current pilot study examined the impact of a health behavior intervention approach on rsFC in moderate to heavy smokers. Smokers (n=23) were recruited and adhered to study procedures. Participants completed a baseline rsFC scan and follow-up scans (2 weeks and 4 weeks), as well as 4 weeks of a CBT intervention to help change health behaviors through scheduled smoking reduction (n=6), scheduled smoking (n=8) or increased fruit and vegetable consumption (n=9). A

whole-brain seed-based functional connectivity analysis was performed to identify changes over time in functional connectivity related to self-regulation using a left dorsolateral prefrontal (dlPFC) seed region. The results showed increased connectivity over time between the left dlPFC and the medial prefrontal cortex, right insula, left middle frontal gyrus, right middle temporal gyrus, and right superior temporal gyrus. Exploratory analyses demonstrated a time by group interaction for connectivity between the dlPFC, middle temporal gyrus, superior temporal gyrus and left middle gyrus ($p < .05$). These results indicate that the group receiving CBT directed at scheduled smoking reduction and CBT directed at increasing fruits and vegetables showed changes in connectivity from baseline to end of treatment. Together these pilot data indicate that self-regulation related functional connectivity changes over the course of 4 weeks of CBT and type of treatment may influence the strength of these connectivity changes. Overall this project will inform future studies aimed at examining neural mechanisms related to treatment changes and identifying the duration of behavioral treatment interventions to see optimal brain changes.

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Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 419.01/DP11/FFF15

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant RO1DA026932

Title: Physiological and psychological cocaine dependence affect neural processing of drug and food stimuli: Correlations with substance use severity and psychopathic traits

Authors: *W. DENOMME[1], M. S. SHANE[2,3]
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Abstract: Recently, we demonstrated at the Society of Biological Psychiatry's annual meeting that 1) individuals with a cocaine-dependence disorder exhibit a neural processing bias towards drug-related rewards relative to non-drug rewards; 2) psychopathic traits are associated with a sensitized neural processing bias among cocaine-dependent individuals (Denomme, Simard, & Shane, 2018). Animal and theoretical models of addiction suggested that biases were due to neuroplastic changes within a corticolimbic circuit that are mediated by chronic substance use and drug withdrawal (Goldstein & Volkow, 2002). Thus, we investigated differences in neural processing biases between participants with a physiological and psychological cocaine dependence within a sample of 101 parolees who viewed drug- and food-related videos while

undergoing an fMRI scan. Physiologically-dependent participants (Phys-D; *n* = 20) exhibited a significantly greater neural processing bias towards drug-related videos compared to both psychologically-dependent (Psyc-D; *n* = 24) and non-dependent (ND; *n* = 57) participants. The Phys-D group exhibited significantly greater drug > food reactivity within the anterior cingulate cortex, dorsomedial prefrontal cortex, and caudate nucleus compared to the Psyc-D group; and greater drug > food reactivity within the anterior cingulate cortex, dorsomedial prefrontal cortex, amygdala, ventral striatum, and caudate nucleus compared to the ND group. There were no significant differences between Psyc-D and ND groups in terms of drug- and food-related neural activity. When assessing correlations between drug > food reactivity with substance use and psychopathic traits, correlations were only observed in Psyc-D participants. Psychopathic traits correlated with increased drug > food reactivity within the dorsomedial prefrontal cortex, whereas substance use correlated with increased activity within the anterior cingulate cortex, ventral striatum and amygdala. Significant psychopathy*substance use interaction effects were observed within the cerebellum and the insula. Our results suggest that previously documented neural processing biases are modulated by whether or not the participant has met diagnostic threshold for cocaine withdrawal and tolerance, and that psychopathic traits and substance use only have statistically significant effects among dependent participants who have not experienced physiological components of dependence. These results have implications for the prevention, diagnosis and treatment of substance use disorders, as well as externalizing behaviors in psychopathic personality disorder.

Disclosures: W. Denomme: None. **M.S. Shane:** None.

Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 419.02/FFF16

Topic: G.08. Drugs of Abuse and Addiction

Support: DA031695

Title: Neural activity of anterior insula during a size-delay task is enhanced after cocaine exposure

Authors: *H. J. PRIBUT[1,2], A. T. BROCKETT[1,2], D. VAZQUEZ[2], E. ALVAREZ[2], M. R. ROESCH[1,2]
[1]Neurosci. and Cognitive Sci., [2]Psychology, Univ. of Maryland, College Park, MD

Abstract: Decision making is a critical aspect of cognitive function that is impaired after exposure to drugs of abuse, causing individuals to pursue drug use even in the face of significant negative consequence. Such behavior most likely arises from impairments of circuitry involved

not just in reward learning, but also in stress and executive control (Keiflin and Janak, 2015; Koob and Volkow, 2016). The insula has recently been examined for its potential role in addiction, with current literature in both human and animal subjects finding activity related to impulsivity (Mechelmans et al., 2017), reward anticipation (Kusumoto-Yoshida et al., 2015), and regret (Jo and Jung, 2016). Given these findings we were interested in whether the insula is responsible for encoding the value of different rewards, and how such encoding may be altered after exposure to drugs of abuse. To explore these questions, we recorded from single neurons in the anterior insula of rats as they performed an odor-guided decision-making task for rewards varying in size and delay of arrival. Preliminary data suggest that subjects who had previously self-administered for cocaine exhibit greater sensitivity to delayed rewards compared to control animals who had previously self-administered sucrose. Single neuron firing in rats from both the cocaine and control group additionally demonstrate a trend for greater activity during blocks of trials where the size of reward is manipulated. These effects are evident especially in cells responsive to reward as opposed to cue onset. Our data suggest the insula encodes both the value of incoming rewards and contextual states, and that such activity is enhanced after exposure to drugs of abuse during anticipation and delivery of delayed reward. This work additionally fits into the greater body of literature suggesting a role for the insula in network of circuitry impaired by addiction.

Disclosures: H.J. Pribut: None. **A.T. Brockett:** None. **D. Vazquez:** None. **E. Alvarez:** None. **M.R. Roesch:** None.

Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

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Program #/Poster #: 419.03/FFF17

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA034696
UMN Viral Vector and Cloning Core

Title: Inhibition of GABA neurons in the prelimbic cortex mimics locomotor activation evoked by cocaine

Authors: *E. MARRON, T. ROSE, H. OBERLE, K. WICKMAN
Pharmacol., Univ. of Minnesota, Minneapolis, MN

Abstract: GABA neurons are the major source of inhibition in the mammalian nervous system. They normally exert their actions in focalized networks; however, recent findings have shown the presence of non-classical long range GABA projections connecting cortical structures (cortico-cortical) or cortical and subcortical structures (cortico-fugal). New technological

advances such as powerful retrograde tracers, opto- and chemogonetics have allowed precise circuit interrogation, including studying the role of these long inhibitory projections. For example, connections between different cortical regions, such as the medial prefrontal cortex and the striatum have been explored demonstrating that these connections can control specific behaviors. Here, we explore the contribution of a corticostriatal GABA projection in the context of drug addiction. Cocaine, and other psychostimulants, produces a pronounced increase in locomotor activity, and previous work from our lab has shown that the excitability of pyramidal cells in the prelimbic cortex (PrLC) controls, at least in part, the magnitude of this response. We started by manipulating the excitability of PrLC GABA neurons by expressing a Designer Receptor Exclusively Activated by Designer Drug (DREADD) virally under the control of the mDlx promoter, a forebrain GABA neuron promoter (Male mice, ~ 50 d at the time of surgery and ~ 70-80 d at the end of behavior). When using the inhibitory DREADD, hM4Di, CNO administration evoked an increase in locomotor activity comparable to that produced by cocaine administration. CNO administration also potentiated locomotion induced by a 15mg/kg dose of cocaine. Electrophysiological recordings confirmed that GABA neuron excitability was reduced in the presence of CNO and that translated in a reduction of $GABA_AR$ responses recorded from pyramidal neurons. Interestingly, direct excitation of PrLC pyramidal neurons using the excitatory DREADD hM3Dq did not have an effect in locomotion, suggesting that the disinhibition of pyramidal neurons might not be the mechanism for the increase in locomotion. We then used a combination of retrograde and anterograde tracing to determine the presence, extension and neurochemical signature of long range GABAergic projections from the PrLC to the nucleus accumbens. Finally, by injecting halorhodopsin in the PrLC cortex under the mDlx promoter and implanting fiber optics in the nucleus accumbens core we manipulated the activity of long range GABA neurons in an open field test confirming our chemogenetic observations. Future efforts will focus on the possible role this projection plays in drug addiction.

Disclosures: E. Marron: None. **T. Rose:** None. **H. Oberle:** None. **K. Wickman:** None.

Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

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Program #/Poster #: 419.04/FFF18

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA IRP

Title: Prior cocaine self-administration increases strength of encoding states with different response rules by DMS cholinergic interneurons

Authors: *L. MUELLER[1,2], A. M. WIKENHEISER[1], M. J. SHARPE[1], D. M. DIETZ[2], T. A. STALNAKER[1], G. SCHOENBAUM[1]
[1]NIH, NIDA IRP, Baltimore, MD; [2]Neurosci., State Univ. of New York at Buffalo, Buffalo, NY

Abstract: When associative rules in effect change abruptly, it is useful to learn about the new situation or context in a manner which does not interfere with the old. To do this, learning in each context is compartmentalized into different "states" which contain the rules appropriate for guiding behavior under those distinct circumstances. Thus, adaptive responding when situations change is dependent upon the ability of an individual to develop and alternate between states. We have previously shown that dorsomedial striatal (DMS) cholinergic interneurons (CINs) encode states with distinct response rules in an odor-guided choice task, consistent with data from Bradfield et al. that the disruption of DMS CIN activity leads to situationally-inappropriate behavior when response-reward contingencies change (2013). Since CINs are activated by cocaine and are implicated in the regulation of extinction learning to cocaine-context associations, cocaine use may disrupt DMS CIN encoding of current state representations and lead to situationally-inappropriate behavior. Here we tested this hypothesis by recording DMS CIN activity in a similar odor task in rats with experience self-administering cocaine. We found that rats that had previously self-administered cocaine were slower to adjust their responding following a state change when compared to controls that had previously self-administered sucrose. Moreover, CINs recorded from these rats display enhanced state encoding across the trial. Interestingly, this effect is particularly robust on suboptimal choices when rats chose the less preferred outcome. These results are consistent with a role for CINs in the reduction of behavioral flexibility and poor decision-making that is observed in individuals following exposure to drugs of abuse.

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Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

Location: SDCC Halls B-H

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Program #/Poster #: 419.05/FFF19

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA035805(YHH)
NIH Grant DA023206(YD)

Title: Melanin-concentrating hormone system in REM sleep-mediated regulation of cocaine craving

Authors: *R. GUO[1], Y. WANG[2], B. CHEN[3], L. CAI[1], Y. LI[2], O. M. SCHLÜTER[2], J. FANG[4], Y. DONG[2], Y. H. HUANG[1]
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Abstract: Sleep abnormalities commonly occur among chronic cocaine users long after withdrawal. The withdrawal-associated sleep problems, including loss of sleep and sleep fragmentation, may increase the risk for cocaine use and relapse. Previously we reported that lengthening rapid eye movement (REM) sleep episode duration by behavioral interventions reduces cue-induced cocaine craving after withdrawal in rats. Furthermore, calcium-permeable AMPA receptors (CP-AMPARs) in the nucleus accumbens (NAc) medium spiny principal neuron synapses are likely a key neuronal substrate that expresses REM sleep-induced regulation of cocaine craving and relapse. In this study, we identify the melanin-concentrating hormone (MCH) neurons in the lateral hypothalamus and zona incerta (LH for short) as a potential REM sleep mechanism in regulating NAc CP-AMPAR accumulation and cocaine craving after withdrawal. Male rats (~ 6-8 weeks old) were trained with cocaine self-administration (overnight followed by 2 hr/day x 5 days). After 21 d withdrawal, REM sleep time and episode duration were inversely correlated with the amount of cocaine intake during training. At this time, in vitro slice recordings from LH MCH neurons from cocaine-trained rats revealed decreased frequency of spontaneous firing and reduced intrinsic membrane excitability, suggesting reduced MCH neural activity after withdrawal. Furthermore, increasing MCH neural activity by either chemogenetic activation (AAV5-MCHp-DREADDs (Gq); clozapine-N-oxide) or optogenetic stimulation (AAV5-MCHp-ChR2-EYFP; 473 nm; 10 Hz) increased REM sleep in rats after cocaine withdrawal. To determine whether MCH receptor signaling in the NAc may regulate CP-AMPARs, we did intra-NAc MCH infusions (1 µg/µl) on withdrawal days 42 and 43 at onset of light phase. Intra-NAc MCH infusions reduced the cocaine-induced CP-AMPAR accumulation in the NAc synapses, and reduced incubation of cocaine craving. In conclusion, our study suggests that LH MCH neural activity is reduced after withdrawal from cocaine; enhancing MCH activity may facilitate REM sleep normalization after cocaine withdrawal; increasing MCH receptor signaling in the NAc may facilitate the attenuation of cocaine craving after withdrawal by down-regulating CP-AMPARs in the NAc.

Disclosures: R. Guo: None. **Y. Wang:** None. **B. Chen:** None. **L. Cai:** None. **Y. Li:** None. **O.M. Schlüter:** None. **J. Fang:** None. **Y. Dong:** None. **Y.H. Huang:** None.

Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 419.06/FFF20

Topic: G.08. Drugs of Abuse and Addiction

Support: CIHR Fellowship 358810

Title: Ventral pallidum Drd3-expressing neurons mediate cocaine-seeking behavior

Authors: *H. PRIBIAG, S. SHIN, E. H. WANG, P. HONMA, V. LILASCHAROEN, B. LIM
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Abstract: Addictive substances hijack the brain's reward system, generating a persistent drive for drug seeking at the expense of natural rewards. Although drug-induced plasticity in the striatum is known to play a critical role in addictive behaviors, persistent drug-induced alterations in other mesolimbic brain structures that integrate and convey reward-related information remain less understood. We identified a population of neurons in the ventral pallidum (VP) that expresses dopamine receptor D3 (Drd3) and that sends projections to several mesolimbic structures implicated in reward and addiction. We explored the role of VP Drd3 signaling in plasticity and drug seeking following withdrawal from cocaine self-administration, and uncovered significantly altered activity of VP Drd3+ neurons during drug seeking. Using viral-mediated tracing, electrophysiology and optogenetics we characterized VP Drd3+ projections to different brains areas, identifying roles for distinct projections in mediating reward-related behavior. Our results provide insight into the role of dopaminergic signaling in the VP and a mechanistic understanding of how VP Drd3+ neurons contribute to persistent drug seeking behavior.

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Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

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Topic: G.08. Drugs of Abuse and Addiction

Support: DA015369
DA012513

Title: Ventral pallidal glutamatergic and enkephalinergic neurons oppositely regulate cocaine seeking

Authors: *J. A. HEINSBROEK, A.-C. BOBADILLA, D. NEUHOFER, T. B. NENTWIG, E. DERESCHEWITZ, P. W. KALIVAS
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Abstract: The ventral pallidum (VP) is a critical structure for driving drug related motivation in addiction, yet precisely how distinct VP circuits mediate drug use and drug seeking during relapse remains unknown. While the VP is thought to be an inhibitory basal ganglia relay nucleus comprised of GABAergic projection neurons (VP^{GABA}) the VP also contains a large number of glutamatergic projection neurons (VP^{Glu}), and a subpopulation of GABAergic projection neurons that co-express the neuropeptide enkephalin (VP^{Penk}). We investigated how these populations of neurons regulate the motivation to seek and take cocaine, mapped their anatomical connectivity and recorded their activity during self-administration.
Virus-based anterograde and retrograde tracing methods, as well as chemogenetics and single cell calcium imaging were used in Vglut2-IRES-Cre, Vgat-IRES-Cre, and PENK-IRES-Cre mice. Animals received indwelling jugular vein catheters, were implanted GRIN lenses, and were trained to self-administer cocaine. After mice reached a stable baseline of responding, the roles of distinct VP neurons during drug taking were tested using a progressive ratio test. Afterwards, mice underwent extinction training and VP neuron contributions to drug seeking were assessed during cue-induced reinstatement.
VP^{Glu}, VP^{GABA} and VP^{Penk} neurons receive differential but overlapping inputs from the nucleus accumbens and other limbic structures, and single cell calcium imaging demonstrated that these populations of VP neurons are differentially engaged during cocaine sensitization and self-administration. Global chemogenetic stimulation of the VP greatly enhanced drug seeking during cue-induced reinstatement, while inhibiting the VP reduced reinstatement. Specific stimulation of VP^{Glu} neurons produced the opposite effect; it abolished the motivation to take cocaine, and reduced cue-induced reinstatement, while inhibiting VP^{Glu} neurons augmented these behaviors. Manipulating VP^{Penk} neuron activity mimicked the effects of global VP manipulations, whereas stimulation of VP^{GABA} neurons produced mixed effects by increasing the motivation to take drugs, while decreasing the motivation to seek drugs during reinstatement.
These results expand our knowledge of the addiction circuitry by showing that VP glutamatergic, GABAergic and enkephalinergic neurons give rise to distinct VP subcircuits and regulate different motivated states underlying drug use and drug seeking.

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Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant R01-DA-032789
NIAAA Training Grant T32-AA-007471

Title: Estrogenic modulation of cocaine response in the medial preoptic area is dependent on biological sex

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Abstract: Behavioral response to cocaine is sex dependent. Which neuroendocrine factors regulate these differences is still unclear. Evidence indicates that the medial preoptic area (mPOA) in the hypothalamus regulates cocaine response, both neural and behavioral activity, as evidenced by conditioned-place preference (CPP) and microdialysis experiments. The hormone estrogen, in particular, acts in the mPOA to modulate response to cocaine. Because the mPOA is a major regulator of sex-specific behavioral differences across various species and for a variety of behaviors, here we investigated whether modulation of cocaine by estrogen in the mPOA is dependent on sex. To this end, male and female rats were divided into groups receiving systemic cocaine or vehicle injections. These were then further subdivided into those receiving estradiol or vehicle microinjections directly into their mPOA, for a total of 8 groups. After receiving cocaine, animals were given a CPP and locomotor test to measure their behavioral response to the drug. Results indicate an important role for estrogen in the mPOA modulating sex differences in the animal's response to the drug. Namely, estradiol microinjections enhanced cocaine-induced CPP in females but not in males. This confirms that cocaine response is sensitive to estrogenic signaling in the mPOA, as previously shown, and moreover, that this influence is dependent on biological sex.

Disclosures: J.R. Martz: None. **C.L. Robison:** None. **J.M. Dominguez:** None.

Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

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Topic: G.08. Drugs of Abuse and Addiction

Support: R01-DA037327
T32-DA-007288

Title: Individual differences in cocaine aversion response correlate with distinct intrinsic and synaptic neuroadaptation in the RMTg to VTA pathway

Authors: *J. PARRILLA-CARRERO[1], Y. S. CHAO[1], M. EID[2], D. PULLMANN[1], H. LI[2], T. C. JHOU[1]
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Abstract: Aside from the strong rewarding effects elicited by drugs of abuse, drugs like cocaine also produce conditioned aversive effects that are most prominent after the rewarding effects dissipate. However, conditioned aversive responses to cocaine vary between individuals, and may influence the predisposition to develop addictive behaviors, but the neural mechanisms involved are not well known. Is well established that Rostromedial Tegmental nucleus (RMTg) efferents to the Ventral Tegmental Area (VTA) modulate the neural response to cocaine, specifically by inducing conditioned place aversions. Thus, we hypothesized that RMTg neurons that project to the VTA (RMTg-VTA) undergo distinct synaptic and intrinsic adaptations that might critically modulate individual differences in cocaine-induced avoidance behaviors. To test this hypothesis, we use a runway operant model that has been extensively used to test the aversive effects of cocaine, and which we found to separate animals into “high cocaine avoiders” and “low cocaine avoiders”, based on latencies to obtain cocaine. After behavioral testing, we used whole cell patch clamp recording techniques to record from RMTg-VTA neurons. We found that RMTg-VTA neurons from high-avoider rats had significantly more current-evoked action-potentials compared to low-avoiders and controls unexposed to cocaine. We also found that high-avoider rats exhibited increased frequency (but not amplitude) of spontaneous excitatory postsynaptic current (sEPSC) activity in RMTg-VTA neurons, consistent with enhanced presynaptic release. Conversely, low-avoider rats showed decreased sEPSC frequency and amplitude compared to drug-naïve controls, which could reflect either pre- or post-synaptic changes. To test for the locus of this synaptic adaptation we measured the eEPSC paired-pulse ratio (PPR) and the AMPA/NMDA ratio recorded at +40mV. High-avoider rats showed a decrease in the eEPSC PPR compared to low-avoider and drug-naive controls, indicating an increase in presynaptic release. The AMPA/NMDA ratio did not differ between high-avoider rats and controls, but was decreased in low-avoiders rats in compared to controls, indicating reduced postsynaptic excitatory transmission specifically in low avoiders. Altogether, these results indicate that distinct neuronal adaptations in the RMTg-VTA pathway are correlated with the aversive phenotype toward cocaine, and suggest that specific phenotypical neural adaptations in the RMTg-VTA pathway may contribute, at least partially, to the development of aversion to cocaine and addiction vulnerability.

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Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA037897
HHMI Gilliam Fellowship

Title: Chemogenetic activation of hindbrain projections to the lateral dorsal tegmental nucleus attenuates cocaine-seeking behavior

Authors: *N. S. HERNANDEZ, V. R. WEIR, H. D. SCHMIDT
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Glucagon-like peptide-1 (GLP-1) is an incretin hormone and neuropeptide that is produced centrally in the nucleus tractus solitarius (NTS). Emerging literature shows that GLP-1 receptor (GLP-1R) agonists reduce cocaine-mediated behaviors in rodents. The lateral dorsal tegmental nucleus (LDTg) is a brain region that plays a critical role in cocaine seeking, expresses GLP-1Rs, and receives direct projections from the NTS. Therefore, the goal of this study was to test if GLP-1R activation in LDTg plays a critical role in the reinstatement of cocaine-seeking behavior, an animal model of relapse. Rats were pretreated with intra-LDTg infusions of the GLP-1R agonist exendin-4 (Ex-4; 0, 0.005 and 0.025 μg) prior to cocaine priming-induced reinstatement test sessions. We show that intra-LDTg Ex-4 attenuated cocaine reinstatement at a dose that does not affect sucrose seeking or *ad libidum* food intake in cocaine-experienced rats. Next, we used a viral-mediated chemogenetic approach to characterize the role of monosynaptic projections from the NTS to the LDTg in cocaine seeking. To selectively activate NTS to LDTg projections, a cre-expressing retrograde canine adenovirus and cre-dependent neural activating DREADD (hM3Dq) and was infused in the LDTg and NTS, respectively. Prior to reinstatement test sessions, rats were pretreated with clozapine-N-oxide (CNO; 0, 0.1, 1 mg/kg) to activate endogenous NTS to LDTg circuits prior to a priming injection of cocaine. CNO pretreatment significant attenuated cocaine seeking, indicating that activation of this hindbrain circuit is sufficient to reduce drug-seeking behavior in rats. To determine if GLP-1-expressing neurons in the NTS were mediating the suppressive effects of CNO on cocaine seeking, we pretreated rats with the GLP-1 receptor antagonist exendin-(9-39) (Ex-9, 10 μg) directly into the LDTg prior to CNO and an acute priming injection of cocaine. We found that pharmacological inhibition of GLP-1 receptors in the LDTg prevented the ability of DREADD activation of the NTS to reduce cocaine seeking. These results highlight a novel role for hindbrain circuits and GLP-1R signaling in cocaine-seeking behavior.

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Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

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Topic: G.08. Drugs of Abuse and Addiction

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NARSAD Award 2017 YI26050

Title: Suppression of operant responding for a natural reinforcer by a cocaine-associated auditory stimulus in rats: Role of locus coeruleus activity and auditory brainstem plasticity

Authors: *M. A. PRESKER, JR[1], D. SOSIDKO[2], S. S. BASKAR[2], K. M. BIESZCZAD[3], G. S. ASTON-JONES[4]
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Abstract: A major obstacle in treating addiction to cocaine is the high propensity for relapse. Stimulus-driven cocaine seeking occurs despite efforts to remain abstinent, and competes with ongoing alternative positive behaviors such as occupational or social activities. Noradrenergic locus coeruleus (LC) neurons project throughout the neuraxis, and modulate sensory processing and attention to orient organisms to sensory stimuli. Previous work has shown that LC activity is altered both acutely and chronically by cocaine and supports a role for LC in mediating the stimulus control of attention by cocaine-cues observed in cocaine addiction. However, few studies have examined whether cocaine-associated stimuli activate LC or exert control over non-drug seeking behaviors. Here we implement a behavioral paradigm specifically designed to examine the effects of cocaine-paired, saline-paired, and unpaired auditory stimuli (tones) on operant responding for a natural reinforcer (water) in rats using a within-subjects paradigm. We found that a cocaine-paired tone suppresses water self-administration compared to saline-paired or unpaired tones (n=12 rats). In a separate group of animals, we performed timed-perfusions to assess Fos expression in LC of rats exposed to cocaine-paired, saline-paired, or unpaired tones, no tones, or homecage controls (n=4 per condition). Imaging and counting of Fos in LC was conducted by an experimenter blind to the treatment conditions. We observed a correlation between expression of Fos in noradrenergic LC neurons and suppression of water self-administration by cocaine- vs. saline-paired tones. These data indicate that LC activity may be involved in modulating the control of behavior by cocaine-associated stimuli. Furthermore, we plan to record auditory brainstem responses to cocaine-paired, saline-paired, or unpaired tones in a separate group of animals (n=8) before, during, and after the conditioning procedure. We will analyze changes in ABR peak latencies to each tone over the course of conditioning to assess potential changes in auditory processing of a cocaine-associated tone compared to control tones. Given the significance of cocaine stimuli for driving relapse, this behavioral regulation by LC may be a target for therapeutic intervention.

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Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

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Topic: G.08. Drugs of Abuse and Addiction

Support: DA015835
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MH091193

Title: Retinoic acid signaling and homeostatic plasticity in nucleus accumbens medium spiny neurons

Authors: *A. M. WUNSCH[1], D. C. CHRISTIAN[1], M. T. STEFANIK[1], M. HINTZE[2], L. CHEN[2], M. E. WOLF[1]
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Abstract: Synaptic plasticity in response to cocaine use leads to a time-dependent heightening in responsiveness to cues during abstinence, a phenomenon termed incubation of cocaine craving. In rodents, profound alterations in glutamate transmission in the nucleus accumbens (NAc) accompany incubation of cocaine craving. In drug naïve rats, calcium (Ca^{2+})-impermeable AMPARs (CI-AMPAR) are responsible for a majority of excitatory postsynaptic AMPAR currents. However, following ~1 month of abstinence from cocaine self-administration, Ca^{2+}-permeable AMPARs (CP-AMPAR) are inserted into excitatory synapses on NAc medium spiny neurons (MSN) and are required for expression of incubation. Some data support the idea that cocaine use leads to reduced activity of MSNs at baseline, which may trigger plasticity leading to incubation. Little is known about signaling cascades that might link reduced activity in the NAc to CP-AMPAR insertion in NAc MSNs. Synaptic scaling is a form of homeostatic plasticity that adjusts synaptic strength to compensate for changes in neuronal activity. One form of inactivity-induced synaptic scaling involves activity-dependent regulation of dendritic protein translation by retinoic acid (RA). In hippocampal neurons, blockade of neuronal activity reduces intracellular Ca^{2+} to disinhibit RA synthesis. This in turn leads to GluA1 translation and synaptic insertion of homomeric GluA1 CP-AMPARs. Thus, RA signaling cascades in MSN may be a critical link between cocaine-induced decreases in NAc activity and CP-AMPAR insertion. Two lines of work are underway to determine if RA contributes to inactivity-induced synaptic scaling and regulates CP-AMPAR insertion in NAc MSNs. First, NAc neurons from postnatal rats are cultured with cortical neurons (to restore excitatory synapses) and transfected with a RA reporter system. We have confirmed that the RA reporter is responsive to changes in intracellular Ca^{2+} levels. Studies are underway to determine how altering excitatory synaptic transmission in the

cultures affects RA signaling and AMPAR subunit composition. Second, whole cell patch clamp recordings are performed on NAc core MSN in brain slices from rats that have undergone incubation of craving leading to CP-AMPAR accumulation. Preliminary data suggest that inhibiting RA synthesis with 4-diethylaminobenzaldehyde (DEAB) may normalize CP-AMPAR levels to those seen in drug naïve rats, suggesting that RA may be an important regulator of CP-AMPARs during abstinence. Ultimately, this work has the potential to define novel targets to reduce drug craving and prolong abstinence in people with substance use disorders.

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Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

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NINDS T32 NS007280-29
NINDS T32 NS007280-30
Stanford Neurosciences Institute NeuroChoice

Title: Formation and refinement of distributed corticostriatal ensembles engaged by cocaine

Authors: *N. R. WALL[1], P. A. NEUMANN[1], K. T. BEIER[1,2], A. K. MOKHTARI[1], L. LUO[2], R. C. MALENKA[1]
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Abstract: Identification of neuronal ensembles mediating various forms of experience-dependent plasticity has been greatly facilitated by genetic strategies that allow labeling of neurons activated by an experience. Identification of more complex, synaptically connected neuronal ensembles mediating behavioral changes has been more challenging, particularly when these ensembles span multiple distinct brain areas. Here we use the Arc-TRAP line of mice, which expresses CreERT2 under the control of the immediate early gene Arc promoter, to identify ensembles of cortical and dorsal striatal neurons activated by a cocaine experience; we then combine this approach with monosynaptic rabies virus tracing to identify their synaptic connectivity. We also used a complementary targeting strategy to gain genetic access to neuronal ensembles that were not activated by cocaine. We find that cocaine-activated corticostriatal neurons, arising from diverse upstream sources including the somatosensory cortices, primary motor cortex, secondary motor areas, and various subdivisions of the prefrontal cortex,

preferentially wired onto co-activated striatal neurons, while more sparsely innervating neighboring non-active neurons. These results suggest that cocaine engages a privileged experience-specific subnetwork linking broadly distributed populations of synaptically connected cocaine-activated neurons in the cortex and dorsal striatum.
Repeated prior cocaine exposure, which elicited locomotor sensitization, further enhanced the connectivity between a select population of cocaine-activated prefrontal cortical neurons and their co-active striatal medium spiny neuron targets. Selective chemogenetic inhibition of the terminals in the dorsal striatum arising from cocaine-activated prefrontal neurons disrupted the expression of cocaine-induced locomotor sensitization, whereas inhibition of terminals arising from non-active prefrontal neurons had no effect. Taken together, these results identify a broadly distributed cocaine-activated corticostriatal neuronal ensemble and reveal portions of the drug-specific subcircuit that may be particularly amenable to targeted therapeutic intervention for drug abuse. In an accompanying abstract (Neumann et al.,) we present results characterizing the properties of corticostriatal synapses made between cocaine-activated versus non-activated cortical and dorsal striatal neuronal populations. The methodology used in these experiments should be applicable to the elucidation of the circuit adaptations mediating a broad range of adaptive and pathological forms of experience-dependent plasticity.

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Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

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Topic: G.08. Drugs of Abuse and Addiction

Support: Stanford Neurosciences Institute NeuroChoice
P50 DA042012
T32 NS007280-30
T32 NS 7280-29

Title: Characterization of corticostriatal cocaine ensemble physiology and behavior

Authors: *P. A. NEUMANN, N. R. WALL, K. T. BEIER, R. C. MALENKA
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Abstract: Genetic strategies to label active neurons during a limited time window have greatly improved our ability to not only visualize neural ensembles, but also to examine how these ensembles may function to mediate various behaviors. This ability to identify experience-specific ensembles is especially relevant to investigating complex experiences mediated by brain regions

with diverse neural connectivity, such as the striatum. In a complementary abstract (Wall et al.), we demonstrate how activity-dependent labeling (using Targeted Recombination of Active Populations, TRAP) can be combined with monosynaptic rabies viral tracing in mice to characterize cocaine-induced changes to corticostriatal synaptic connectivity. These results identify cocaine-induced connectivity changes in motor cortex and orbital frontal cortex inputs to dorsal striatal neurons.
Here, we build upon these connectivity results and examine cocaine-induced physiology changes at cocaine-activated corticostriatal synapses using acute slice physiology. We employ Cre-activation and -inactivation techniques to target channelrhodopsin expression specifically to cocaine-activated or -non-activated neurons, respectively. Our results reveal differences in AMPAR/NMDAR ratios, quantal amplitudes, and paired-pulse ratios at activated vs. non-activated synapses. We then show that chemogenetic inhibition specifically of cocaine-activated ensembles in the orbital frontal cortex inhibits the expression of cocaine locomotor sensitization, whereas inhibition of neighboring non-activated neurons has no apparent effect.
Taken together, these results along with the results shown by Wall et al. demonstrate how a range of techniques, including TRAP labeling, rabies tracing, opto and chemogenetics, and slice electrophysiology, can be combined to analyze circuit connectivity and adaptations that mediate behaviorally-induced plasticity in an unbiased manner.

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Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

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Title: Destabilization of a synaptic engram underlying drug-associated memories

Authors: *W. WRIGHT[1], N. M. GRAZIANE[1], P. A. NEUMANN[1], P. J. HAMILTON[2], H. M. CATES[2], L. E. FUERST[1], Y. SHAHAM[3], O. M. SCHLÜTER[1], E. J. NESTLER[2], Y. DONG[1]

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Abstract: Experiencing drugs of abuse produces drug-associated memories, among which drug-cue associations persistently promote drug seeking and relapse. Like other cue-associative memories, upon cue-induced retrieval, cocaine-associated memories are destabilized for ~6h, followed by reconsolidation. This 6h destabilization window is a therapeutic opportunity to compromise drug-associated memories and reduce drug relapse. Since clear cellular and synaptic mechanisms underlying memory destabilization remain elusive, non-specific amnestic manipulations fail to achieve consistent anti-relapse effects clinically. To explore specific neural substrates underlying drug-associated memories, our previous work shows cocaine experience generates new, immature silent synapses in nucleus accumbens (NAc) medium spiny neurons (MSNs). During withdrawal, these silent synapses functionally mature by recruiting calcium permeable AMPARs (CP-AMPARs), strengthening and consolidating the newly established neurocircuits. Our subsequent results suggest that generation and maturation of silent synapses critically contribute to the acquisition and consolidation of cocaine-cue associative memories. Thus, these cocaine-generated synapses may represent a putative synaptic engram for drug-associated memories. Here, we show that upon cue-induced memory reactivation in male rats, the matured cocaine-generated synapses were re-silenced through CP-AMPAR internalization and destabilized for ~6h, followed by re-insertion of CP-AMPARs and re-maturation. Preventing silent synapse re-maturation during the destabilization phase by disrupting CP-AMPAR re-insertion significantly decreased cue-induced cocaine seeking. Furthermore, the activity of small GTPase, Rac1, acted as a molecular switch to trigger and/or prevent the destabilization window. Taken together, these findings suggest that cocaine-generated silent synapses are key synaptic components of the drug memory engram, through which drug-associated memories can be precisely manipulated for therapeutic benefits.

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Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

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Topic: G.08. Drugs of Abuse and Addiction

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NIDA DA034681

Title: Dynamic neuronal signaling in the nucleus accumbens tracks cocaine experience and contributes to motivated behavior

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Abstract: Exposure to drugs of abuse leads to reorganization of neural circuits and alteration of synapses, which outlive the direct effects of the drug and may contribute to addiction. The nucleus accumbens (NAc) has a significant role in motivation, reward, and reward-related learning, and has been identified as a key area in the development and maintenance of addiction. Furthermore, the prefrontal cortex (PFC) is known to be involved in decision making and impulsive behaviors, and this brain region sends glutamatergic projections to the NAc. However, the contribution of specific NAc neuronal populations and PFC efferents to the NAc in drug reward is still poorly understood. The present study aims to determine how neuronal activity in the NAc is altered in response to acute cocaine experience, and how these changes in activity influence reward and goal-motivated behavior. Neuronal activity was recorded *in vivo* from electrode microarrays bilaterally implanted in the NAc of freely-moving naive male Sprague Dawley rats that were exposed to either cocaine (10mg/kg) or saline. Acute cocaine exposure produced rapid but prolonged increases in activity of a subpopulation of neurons in the NAc, independent of environment or context. We were able to recapitulate similar response profiles in rat primary striatal neurons cultured on multielectrode arrays using the dopamine receptor type 1 (Drd1) agonist SKF38393 (1 μM), suggesting that acute increases in neuronal activity are occurring in Drd1-positive medium spiny neurons. In an additional group of animals, channelrhodopsin (ChR2) was virally expressed in the NAc to determine if photostimulation of neurons in this area is sufficient for reward-related behavior, as measured by real-time place conditioning. Preliminary data show that real-time place preference was generated by ChR2 photostimulation in the NAc core, but not the NAc medial shell. Together with circuit-tracing techniques, ongoing studies aim to combine *in vivo* optogenetic and electrophysiological approaches to investigate how projections from the PFC influence neuronal activity in the NAc, giving rise to motivated behaviors. Overall, these findings suggest that acute cocaine experience significantly increases medium spiny neuron firing in the NAc, and that this activity is sufficient to drive reward-seeking behaviors important in the development of addiction.

Disclosures: M.E. Zipperly: None. **J. O'Malley:** None. **A.C. Brane:** None. **N.A. Simpkins:** None. **D.S. Murray:** None. **K.D. Bunner:** None. **J.J. Day:** None.

Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 419.17/GGG7

Topic: G.08. Drugs of Abuse and Addiction

Title: Contingent versus non-contingent cocaine administration differentially affects the persistence of neuronal intrinsic plasticity of medium spiny neurons in the nucleus accumbens shell

Authors: *A. SEGEV, I. DELINT-RAMIREZ, S. KOURRICH
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Abstract: The ability of cocaine to decrease neuronal intrinsic excitability of medium spiny neurons (MSNs) in the nucleus accumbens shell (NAcSh) has gained prominence in recent years as a novel adaptation contributing to addiction processes. However, it is unclear how the route of administration, contingency of drug administration, and drug regimen affect the persistence of cocaine-induced depression of neuronal intrinsic excitability (i.e., firing rate). To address this question, we used both non-contingent intraperitoneal (i.p.) cocaine (5 d, 15 mg/kg) and contingent cocaine self-administration (SA) (short protocol; 5 d intravenous, i.v.; 0.5 mg/kg) and assessed NAcSh MSNs firing rate at various delays after cessation of drug treatments. We found that non-contingent i.p. cocaine depresses NAcSh MSNs firing rate up to 15 d after the last exposure, consistent with previous studies, and dissipates by 30 d post-treatment. In contrast, contingent cocaine SA depresses NAcSh MSNs firing rate up to 30 d after cessation of drug taking. We also found that cocaine-induced firing rate depression occurs specifically in D1 receptor-expressing (D1R-) MSNs but not in D2R-MSNs. Moreover and similar to i.p. cocaine, blockade of the sigma-1 receptor (σ1) with BD1063, an endoplasmic reticulum chaperone protein, prevents cocaine from depressing NAcSh MSNs firing rate. Importantly, we found that σ1 blockade attenuates cocaine but not food SA, suggesting that BD1063 at the dose used here (40 mg/kg), does not alter basic locomotor behavior or pressing lever for a natural reward. Finally and similar to non-contingent i.p. cocaine, cocaine SA enhances physical interactions between σ1 and Kv1.2 potassium channels and surface Kv1.2. However, in contrast to i.p. cocaine, cocaine SA enhances total protein levels of Kv1.2. In conclusion, the mode of cocaine administration, i.e., non-contingent i.p. versus contingent i.v. cocaine, differentially affects both the persistence and the cellular adaptations underlying cocaine-induced firing rate depression in NAcSh MSNs. To investigate further the persistence of cocaine-induced firing rate depression following cocaine SA, we will also use a drug paradigm that induces incubation of cocaine craving (long protocol: 10 day cocaine SA; Pascoli et al., *Nature*, 2014), and assess its effects on the persistence of MSNs firing rate depression in the NAcSh.

Disclosures: A. Segev: None. **I. Delint-Ramirez:** None. **S. Kourrich:** None.

Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

Location: SDCC Halls B-H

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Topic: G.08. Drugs of Abuse and Addiction

Support: NSFC Grant 31741060
NSFC Grant 91732109
973 Program 2015CB553503

Title: Disinhibition and abnormal activity of parvalbumin interneurons of nucleus accumbens in cocaine-conditioned place preference

Authors: *W. ZHANG[1], H. ZHANG[1], J. SHI[1], L. LU[2]
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Abstract: Drug addiction is a disease which represents a pathological change of the neural mechanisms of learning and memory in brain. Among brain regions affected, Nucleus Accumbens (NAc) is well-known for its role in drug addiction and studies have revealed NAc underwent profound changes in neuronal circuits and neurons functions after drug withdrawal. While the role of excitatory transmission in NAc is well-documented in drug-induced behavior change, less is known for inhibitory GABAergic inputs to MSNs neurons in the same process, nor the functional consequence of it. In NAc, the principle neuron type is medium spiny neurons (MSN) who comprise more than 95 % of all NAc neurons, and are the major output neurons of NAc. Besides these neurons another major neuron type is aspiny interneurons. MSNs are GABAergic projection neurons, they also form connections between each other in NAc. Among interneurons, GABAergic parvalbumin interneurons are important in regulating striatal output, while they only represent a few percent of NAc neurons. Here, we investigated whether drug withdrawal also induced changes in inhibitory transmission of NAc shell in cocaine-conditioned place preference (CPP). We found that following withdrawal from cocaine, both the excitatory and inhibitory inputs to MSNs of C57BL/6J mice endured persistent changes 2 weeks after withdrawal. We observed that cocaine withdrawal led to a reduction of inhibitory transmission. The frequency of mIPSCs significantly reduced in cocaine-treated mice; half-width of mIPSCs also were significantly different between mutant and wild type neurons. On the other hand, the mean peak amplitude of mIPSCs was not altered in MSNs. Using PV-Cre mice, we found activity change in PV+ interneuron activity following withdrawal contributed to disinhibition of MSNs. Our results indicate that both inhibitory and excitatory transmission in NAc endured

persistent change after withdrawal from cocaine, and intervention of the activity of PV+ interneurons might help to alleviate cocaine induced functional change in NAc.

Disclosures: W. Zhang: None. **H. Zhang:** None. **J. Shi:** None. **L. Lu:** None.

Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

Location: SDCC Halls B-H

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Program #/Poster #: 419.19/GGG9

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH DA009621
NIH DA015835
NIH DA036963

Title: NMDA receptors in rat nucleus accumbens are dynamically regulated during withdrawal from cocaine self-administration

Authors: *D. T. CHRISTIAN[1], M. T. STEFANIK[1], A. M. WUNSCH[1], J. R. FUNKE[1], L. A. BEAN[2], C. A. BRIGGS[1], J. LYONS[1], D. NEAL[2], M. MILOVANOVIC[1], G. E. STUTZMANN[1], D. A. NICHOLSON[2], K.-Y. TSENG[3], M. E. WOLF[1]
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Abstract: Cue-induced cocaine craving intensifies or incubates during withdrawal from extended-access cocaine self-administration. After prolonged withdrawal, the expression of incubated cocaine craving is mediated by GluA2-lacking, Ca^{2+}-permeable AMPARs (CP-AMPARs) that accumulate in the nucleus accumbens (NAc). We have focused on this phenomenon in the NAc core. Less is known about NMDAR plasticity during incubation, especially in the NAc core. However, it has been shown that elevated levels of GluN2B-containing NMDARs in the NAc shell during very early withdrawal are important for subsequent incubation (Lee et al., 2013; Ma et al., 2014). Furthermore, incorporation of GluN3- and GluN2B-containing NMDARs accompanies the increase in CP-AMPAR levels in the ventral tegmental area elicited by a single cocaine injection (Yuan et al., 2013). To evaluate NMDAR transmission in NAc core throughout the incubation process, we conducted whole-cell patch clamp recordings in NAc medium spiny neurons (MSN) at different withdrawal times following extended-access cocaine self-administration. By measuring evoked NMDAR-mediated synaptic responses across various membrane holding potentials (-80 to +40) in the presence of subtype selective blockers, we identified an increase in GluN2B-containing receptors beginning on withdrawal day 4-5. During a later period (withdrawal day 13-20), we detected atypical NMDARs not found in saline control animals that are comprised of GluN3 and GluN2B

subunits. These atypical NMDARs persisted in MSN synapses in late withdrawal (withdrawal day 39 and greater). We are presently conducting super-resolution light microscopy immunofluorescence array tomography to characterize the expression pattern of NMDAR subunits in control rats and following incubation of craving. Early results suggest differential expression of NMDAR subunits between treatment groups. Behavioral studies are also in progress to determine if GluN2B- or GluN3-containing NMDARs contribute to the incubation of cocaine craving. To date, our results indicate that alterations in NAc NMDAR function are dynamic and precede the incorporation of CP-AMPARs during withdrawal from extended-access cocaine self-administration, and are then maintained throughout the period when incubated drug seeking is maximal.

Disclosures: D.T. Christian: None. **M.T. Stefanik:** None. **A.M. Wunsch:** None. **J.R. Funke:** None. **L.A. Bean:** None. **C.A. Briggs:** None. **J. Lyons:** None. **D. Neal:** None. **M. Milovanovic:** None. **G.E. Stutzmann:** None. **D.A. Nicholson:** None. **K. Tseng:** None. **M.E. Wolf:** None.

Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 419.20/GGG10

Topic: G.08. Drugs of Abuse and Addiction

Support: NIHNS104705
Nebraska Department of Health and Human Services (DHHS)

Title: Differential role of NMDA receptors GluN2C and GluN2D subunits in cocaine addiction

Authors: *G. P. SHELKAR, R. PAVULURI, J. LIU, P. GANDHI, S. M. DRAVID
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Abstract: Although the N-methyl-D-aspartate receptors (NMDARs) are shown to play an important role in cocaine induced behaviors including place preference, extinction, reinstatement and self-administration, the precise role of NMDARs subtype in these behaviors is not fully explored. This is critical since NMDARs subunits exhibits differential biophysical and pharmacological properties and in particular GluN2C and GluN2D subunits exhibit, lower sensitivity to Mg^{2+}-block and lack desensitization. This allows their activation under basal conditions facilitating firing or oscillatory activity. Therefore, we attempted to address the subunit specific role of NMDARs in cocaine induced behaviors. We studied cocaine (15 mg/kg, intraperitoneal) induced place preference, extinction and reinstatement in the two-chamber bias design conditioned place preference (CPP) apparatus. To study the role of GluN2C subunit, we employed a novel reporter mouse model in which an EGFP cassette is inserted in the intronic region between exons 6 and 7 (C57/B6-Grin2C$^{tm1(EGFP/CreERT2)}$) which also serves as a knockout

model. We also studied the role of GluN2D subunits by using GluN2D WT and KO animals. In the immunohistochemistry, we found enriched expression of GluN2C subunits in reward controlling brain regions including substantia nigra, prefrontal cortex and ventral striatum. The expression of GluN2C in these regions was in distinct cell-types. Additionally, GluN2D subunits expression is also reported in substantia nigra, ventral tegmental area and ventral striatum but in different cell-types compared to GluN2C. GluN2C WT and KO mice were found to have similar level of locomotor sensitization following repeated exposure to cocaine. However, the GluN2D KO animals showed reduced locomotor sensitization to cocaine as compared to WT animals. In CPP, the GluN2C WT and KO animals showed similar responses in conditioning and reinstatement sessions. However, GluN2C KO mice exhibited facilitated extinction compare to WT animals. Moreover, GluN2D KO animals exhibited deficits in extinction and reinstatement of cocaine CPP as compared to WT animals. Thus, the present study for the first time showed subunit specific role of GluN2C and GluN2D in cocaine CPP.

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Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 419.21/GGG11

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH DA009621

Title: AMPA receptor and mGlu1 expression and interactions in the nucleus accumbens core during the incubation of methamphetamine craving

Authors: *C. MURRAY[1], M. E. WOLF[2], M. MILOVANOVIC[2], J. FUNKE[2], A. CACCAMISE[2]
[1]Neurosci., Rosalind Franklin Univ. of Med. and Scien, North Chicago, IL; [2]Neurosci., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: The incubation of cue-induced drug craving in rodents provides a model for persistent vulnerability to relapse in abstinent drug users. After prolonged withdrawal (>30 days) from extended-access cocaine self-administration, we have shown that incubated cocaine craving depends on the strengthening of nucleus accumbens (NAc) core synapses through the accumulation of homomeric GluA1 Ca2+-permeable AMPA receptors (CP-AMPARs) (Conrad et al., 2008). Their accumulation is associated with increased GluA1 translation (Stefanik et al., 2018). Furthermore, we have shown that CP-AMPAR accumulation is preceded and enabled by a downregulation of mGlu1 in the NAc, and that acutely activating mGlu1 removes CP-

AMPARs once they have accumulated (Loweth et al., 2014). In the NAc core of rats that had undergone incubation of methamphetamine (Meth) craving, CP-AMPARs similarly accumulate and are removed by acute mGlu1 activation (Scheyer et al., 2016). The goal of this project was to more thoroughly characterize CP-AMPARs and their relationship to mGlu1 expression during withdrawal from extended-access Meth self-administration. Contrasting with cocaine findings, biotinylation studies and quantitative co-IP studies starting from NAc homogenates failed to reveal increased GluA1 surface expression or an increase in homomeric GluA1 receptors at a Meth withdrawal time when CP-AMPARs can be detected electrophysiologically. Both results suggest local regulation confined to synapses. Studies are underway to assess the rate of GluA1 translation. Also contrasting with cocaine, a time-course study revealed no change in mGlu1 surface expression during Meth withdrawal. Next, we will determine if increasing mGlu1 tone in early withdrawal, via repeated injections of a positive allosteric modulator, prevents CP-AMPAR accumulation during Meth withdrawal. Overall, these results reveal that a similar strengthening of NAc core synapses via CP-AMPAR accumulation occurs during incubation of cocaine and Meth craving, but the mechanisms underlying this plasticity may differ between the two psychostimulants.

Disclosures: C. Murray: None. **M.E. Wolf:** None. **M. Milovanovic:** None. **J. Funke:** None. **A. Caccamise:** None.

Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 419.22/GGG12

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant RO1-DA35821
NIH Grant RO1-NS95809

Title: Modulation of dopamine D2R sensitivity following cocaine exposure

Authors: *S. GONG[1], P. MARCOTT[2], C. FORD[1,2]
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Abstract: The dorsal (Dstr) and nucleus accumbens (NAc) receives dopamine input from separate midbrain neurons, are associated with different behaviors, and are modulated differently by drugs of abuse. How dopamine release is encoded by postsynaptic D2 receptors in each region is poorly understood. To measure D2 receptor activation, we virally overexpressed G protein-coupled inward rectifying potassium (GIRK2) channels in medium spiny neurons (MSNs). Using the resulting outward GIRK current, we found that the sensitivity of D2 receptors

on MSNs was higher in the NAc than the Dstr (EC50: 1.3 vs 5.9 µM). The regional difference in D2 receptor sensitivity resulted from higher levels of $G\alpha_o$ expression in the NAc than Dstr. When coupled to D2-receptors we found that $G\alpha_o$ was more potent at activating downstream signaling cascades than $G\alpha_i$. The regional difference was not seen for other GPCRs as opioid receptors exhibited similar sensitivity across regions and equal sensitivity to leu-enkephalin when coupled to either $G\alpha_o$ or $G\alpha_i$. Similar to the results using GIRK channels, we found that axonal D2 receptors that regulate collateral transmission between MSNs also had higher sensitivity in the NAc than in Dstr (EC50: 2.1 vs 4.8 µM). To examine how exposure to drugs of abuse might alter D2-receptor signaling across regions, we chronically treated animals with cocaine for 7 days (20mg/kg; ip). We found that cocaine reduced the sensitivity of D2-receptors and decreased the expression of $G\alpha_o$ selectively in the NAc. No change was observed in levels of $G\alpha_i$ and only a single day of cocaine treatment had no effect on D2-receptor expression or $G\alpha_o$ expression. These results suggest that chronic cocaine exposure modulates D2 receptors sensitivity in the NAc by altering G-protein coupling.

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

Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 419.23/DP10/GGG13

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant 2R01DA024716-06

Title: Effects of nucleus accumbens core-directed D_2 receptor antagonism on motivation for cocaine following the development of an addicted phenotype

Authors: *T. NESIL, A. BAKHTI-SUROOSH, W. J. LYNCH
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Abstract: The transition from a non-addicted to an addicted stage is accompanied by a diminished role of nucleus accumbens (NAc) dopamine (DA) D_1 receptor (D_1R) signaling in motivating cocaine self-administration. However, it is not yet known whether such a shift also occurs for DA D_2R signaling. In this study, we examined the effects of DA D_2R antagonism in the NAc core on motivation for cocaine following extended access (ExA) cocaine self-administration and 14-days of abstinence, conditions previously shown to induce an addicted phenotype (defined as enhanced motivation for cocaine as compared to short access (ShA) controls). Adult male Sprague-Dawley rats (N=52) were trained to self-administer cocaine (1.5 mg/kg/infusion) under a fixed-ratio 1 (FR1) schedule with a maximum of 20 infusions. Following acquisition, rats were given either ShA (i.e., 3 additional FR1 sessions) or ExA to

cocaine (i.e., 24-hr access under a discrete trial procedure; 4 trials/hr, 1.5 mg/kg/infusion). Motivation for cocaine was then assessed after abstinence using a progressive-ratio (PR) schedule. The effects of NAc infusions of eticlopride (0, 0.3, 1.0, 3.0, and 10.0 µg/side) were tested once a stable PR baseline was established using a within-subject design (baseline was re-established after each dose). Intra-NAc D_2R antagonism produced differential effects on PR responding for cocaine in the ShA versus ExA group, with evidence to suggest an enhanced sensitivity in the ShA group. Specifically, while the low dose of eticlopride robustly decreased PR responding for cocaine in the ShA group (28% decrease), its effect was much less pronounced in the ExA group (16% decrease). Moreover, in the ShA group, higher doses of eticlopride did not further enhance the decrease in PR responding for cocaine indicating that a maximal effect, or ceiling, had been reached at the lowest dose. In contrast, in the ExA group, eticlopride dose-dependently decreased PR responding for cocaine with the greatest decreases observed at the two highest doses. Although a ceiling effect also occurred in the ExA group, it was not evident until higher doses (the 3.0 µg dose). These data consistent with our previous findings for D_1 receptor antagonism (Ramôa et al., 2014), and suggest that the role of NAcc D_2R signaling also becomes diminished with the development of cocaine addiction. However, in contrast to our previous findings with the D_1 receptor antagonism, motivation for cocaine was decreased in the ExA group following both medium and high doses of eticlopride (3.0 and 10.0 µg). These findings indicate that while the role of D_2 receptor in motivating cocaine use decreased with the development of addiction, it is still involved.

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Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

Location: SDCC Halls B-H

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Program #/Poster #: 419.24/GGG14

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R01 DA018326
NIH T32 DA007237
NIH P30 DA013429

Title: SNC80, a delta opioid receptor agonist, reduces cocaine-induced increases in CRF mRNA in female rats

Authors: *K. L. CONNELLY, E. M. UNTERWALD
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Abstract: One of the largest obstacles to effective treatment of drug addiction is the high rates of relapse to further drug use. A major contributor to relapse is the negative affective state produced

by withdrawal. Withdrawal from chronic cocaine activates the hypothalamic-pituitary-adrenal (HPA) axis as well as other extrahypothalmic stress systems, including the extended amygdala. The delta opioid receptor agonist SNC80 attenuates cocaine withdrawal-induced anxiety in rats; however, the mechanism underlying this effect is unknown. This study first determined the time course of gene regulation following withdrawal from cocaine. Male and female adult Sprague Dawley rats (n=5-6/time) were injected with saline or cocaine in a binge-pattern for 14 days. Brains were collected 30 minutes, 24 hours, 48 hours, or 7 days after the last injection. The central amygdala (CeA), bed nucleus of the stria terminalis (BNST), and paraventricular nucleus of the hypothalamus (PVN) were microdissected and expression levels of CRF, CRFR1, and FKBP5 measured by quantitative RT-PCR. Results were analyzed for statistical significance with 2-way ANOVA and Bonferroni post-hoc tests. Following chronic cocaine, CRF mRNA was significantly elevated in the BNST at 30 minutes withdrawal, CeA at 24 hours and PVN at 48 hours. CRF mRNA was elevated at 24 hours in the BNST of females, but not males. FKBP5 mRNA was elevated in the PVN and BNST 30 minutes following cocaine and remained elevated in both regions at 24 hours. FKBP5 mRNA was also elevated in the CeA of females at 30 minutes. To test if SNC80 normalizes expression of stress-related genes, a separate cohort (n=7-8) received SNC80 (10mg/kg) twice after the last injection and brains were collected at 24 hours withdrawal. A significant interaction was found between chronic cocaine and SNC80 administration in the CeA of female rats. Specifically, SNC80 significantly reversed the increase in CRF mRNA produced by cocaine withdrawal. No significant differences were found in males. These studies demonstrate time-dependent alterations in stress-related gene expression during withdrawal from chronic cocaine. These results also provide insight into the anxiolytic effect of SNC80 following chronic cocaine, and suggest that delta opioid receptor agonists may be useful therapeutics for cocaine withdrawal-induced negative affect, particularly in females.

Disclosures: K.L. Connelly: None. **E.M. Unterwald:** None.

Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 419.25/GGG15

Topic: G.08. Drugs of Abuse and Addiction

Support: Irish Research Council

Title: The psychedelic 5-meo-dmt reverses key molecular changes caused by chronic cocaine across multiple brain structures

Authors: *J. O'SULLIVAN[1,2], K. J. MURPHY[2]
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Abstract: Addictive substances activate the reward circuitry in the brain, specifically the mesolimbic dopaminergic neurons originating in the ventral tegmental area projecting to the nucleus accumbens and other limbic structures including the dorsal striatum (DS), hippocampus, amygdala (Amyg) and regions of the prefrontal cortex. Chronic exposure to drugs like cocaine alters gene expression and produces long-term changes in such neural networks to underlie compulsive drug seeking and taking. One of the relatively persistent changes is the accumulation of ΔFosB, a highly stable variant of the FosB protein, in the NAc and connected brain circuitry following chronic drug exposure. ΔFosB, regulates drug-induced synaptic plasticity and is both necessary and sufficient for mediating various addiction-behaviours including the locomotor response to cocaine, conditioned place preference and increased drug self-administration. Targeting the molecular and transcriptional changes underlying alterations in behaviour, mood and cognition associated with persistent drug use may decrease the long-term risk of relapse. Psychedelic drugs produce strong subjective effects including changes in thought, mood, and perception. There is a growing body of evidence supporting the use of psychedelic drugs in treating various affective disorders. Beneficial effects have been reported in clinical trials of various serotonergic (5-HT) psychedelics in the treatment of major depressive disorder, end-of life anxiety and addiction. However, little is understood regarding the mechanism through which these drugs are mediating their benefits. For this study we used the 5-HT targeting psychedelic 5-methoxy-N,N-dimethyltryptamine (5-Meo). 5-Meo is a 5-HT receptor agonist at 5-HT-1A, 5-HT-2A and 5-HT-2C receptors.
The aim of the study was to investigate molecular changes induced by 5-Meo in mesolimbic circuitry following chronic cocaine exposure. Wistar rats were administered cocaine (15 and 20mg/kg i.p.) for 14 days and subsequently received a single administration of 5-Meo. Repeated cocaine induced stable changes previously established within the mesolimbic circuitry, including accumulation ΔFosB. 5-Meo was able to reverse the majority of measured changes induced by the repeated cocaine administration, including restoring expression levels of ΔFosB in the DS and Amyg to levels seen in saline-treated animals. The findings are discussed in the context of the use of psychedelics as treatments for addiction.
Funded by: Irish Research Council

Disclosures: J. O'Sullivan: None. **K.J. Murphy:** None.

Poster

419. Circuit and Molecular Mechanisms of Cocaine Action

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 419.26/GGG16

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

Support: U.S. Public Health Service Grant DA00266

NARSAD Young Investigator Grant (grant # 25360) from the Brain & Behavior Foundation

Title: A novel high affinity cocaine receptor inhibits dopamine reuptake via rapid degradation of the dopamine transporter

Authors: *M. M. HARRAZ, P. GUHA, A. MALLA, I. KANG, E. R. SEMENZA, P. CORTES, S. H. SNYDER
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Abstract: Increased dopamine (DA) concentration in the nucleus accumbens (NAc) mediates cocaine's stimulant effect. Cocaine directly inhibits the DA, serotonin and norepinephrine transporters at high nanomolar to low micromolar levels. Hence, presumably direct inhibition by cocaine of the DA transporter (DAT)-function mediates DA reuptake inhibition and increased dopamine signaling. Potentiation of DA neurotransmission, in turn, mediates cocaine's stimulant actions. However, multiple DA reuptake inhibitors do not produce psychotropic effects like cocaine. This might suggest that inhibition of DAT function is not sufficient to cause the stimulant actions of cocaine. Here, we identify the brain abundant signal protein -1 (BASP1) as a high-affinity cocaine receptor. Cocaine induces autophagy with extremely high potency through BASP1. Autophagy, in turn, selectively targets DAT for degradation resulting in DA reuptake inhibition. In primary cortical cultures, cocaine potently induces autophagy (at sub-nanomoalr levels). Stereotaxic injection of 10 femtomoles cocaine into the mouse NAc rapidly induces autophagy in presynaptic axonal terminals. Autophagy is involved in regulating behavior since blocking autophagy, using three different pharmacologic inhibitors, abolishes cocaine's stimulant effect. Autophagy selectively degrades DAT but not the serotonin or norepinephrine transporters in NAc. We performed anti-cocaine IP from primary cortical cultures. Using Mass spectrometry, we identified BASP1 as a cocaine binding protein. BASP1 is a membrane-associated protein that is enriched in axonal terminals of the forebrain. Using radioligand-binding assays, we demonstrate that BASP1 binds potently to cocaine (Kd 8.1 nM). Stereotaxic injection of a viral vector, capable of retrograde transduction, in NAc to knock down BASP1, inhibits cocaine's stimulant effect in mice. In addition, depletion of BASP1 prevents the potent autophagic actions of cocaine. Taken together, these data suggest a novel mechanism for the behavioral actions of cocaine through BASP1-mediated autophagy. Our findings support a model wherein cocaine acts through BASP1 to induce autophagy, which inhibits DA reuptake by degrading DAT. These findings identify BASP1 and autophagy as novel therapeutic targets for cocaine addiction.

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Poster

420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 420.01/GGG17

Topic: G.08. Drugs of Abuse and Addiction

Support: IRP/NIDA/NIH

Title: Modeling opioid maintenance therapy in rats: Effects of chronic buprenorphine and the biased mu-opioid receptor agonist TRV130 on relapse to oxycodone seeking

Authors: *J. M. BOSSERT[1], J. K. HOOTS[1], S. S. NEGUS[2], B. E. BLOUGH[3], Y. SHAHAM[1]
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Abstract: Background: High relapse rates perpetuate opioid addiction and are a major obstacle in addressing the current U.S. opioid epidemic. Maintenance therapy with opioid agonists (buprenorphine, methadone) is an effective treatment for opioid addiction. Here, we establish an experimental procedure in rats trained to self-administer the prescription opioid oxycodone to compare the efficacy of an established treatment (buprenorphine) with that of a newer biased mu-opioid agonist, TRV130. **Methods:** We trained rats to self-administer oxycodone (0.1 & 0.05 mg/kg/infusion; 7 d/dose, 6-h/d) in Context A where infusions were paired with a discrete tone-light cue. We implanted Alzet osmotic pumps containing vehicle, buprenorphine (3, 6, or 9 mg/kg/d), or TRV130 (3, 6, or 9 mg/kg/d) and performed three tests: (1) responding for drug-paired discrete cues under extinction conditions in a non-drug context (Context B), (2) context-induced reinstatement of oxycodone seeking in Context A after extinction in Context B, and (3) reacquisition of oxycodone self-administration in Context A. **Results:** Chronic buprenorphine decreased responding reinforced by drug-paired discrete cues in Context B and reacquisition of oxycodone self-administration in Context A but did not significantly decrease context-induced reinstatement of oxycodone seeking. The experiment with TRV130 is currently ongoing and results will be reported at the meeting. **Conclusions:** We introduce a rat model to study the effect of agonist-based maintenance therapy on relapse to prescription opioid seeking. We showed that chronic buprenorphine reduced oxycodone seeking provoked by exposure to oxycodone-associated discrete cues and by exposure to oxycodone itself, demonstrating the predictive validity of the model.

Disclosures: J.M. Bossert: None. **J.K. Hoots:** None. **S.S. Negus:** None. **B.E. Blough:** None. **Y. Shaham:** None.

Poster

420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 420.02/GGG18

Topic: G.08. Drugs of Abuse and Addiction

Support: Specialized Center of Research (SCOR) on Sex and Gender Factors Affecting Women's Health, R01 DA033049
Summer Undergraduate Research Program (SURP), Medical University of South Carolina
Honors College Summer Enrichment Grant, College of Charleston

Title: Long-term impact of acute stress on cognition, anxiety, and reinstated heroin seeking in male and female rats

Authors: *J. S. CARTER, A. M. KEARNS, R. A. WEBER, C. M. REICHEL
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Abstract: Withdrawal symptoms following substance use disorders (SUD) and post-traumatic stress disorder (PTSD) reciprocally exacerbate one another. Symptoms induced by cessation of substance use culminate as stressors and lead to persistent and compulsive relapse behavior. In addition, PTSD can be triggered by, and contribute to, withdrawal-induced stress responses further increasing relapse potential. In a model used to mimic PTSD in rodents, restraint stress potentiated cocaine-seeking behavior and resulted in similar neuroplasticity consequences as cocaine. Here, we examined the effects of restraint stress paired with a neutral odor on heroin seeking, anxiety-related behaviors, and social interaction during withdrawal and relapse. Rats assigned to the stress group were restrained in a plastic tube that did not allow for mobility with exposure to a scent (*stress*), while other rats were exposed to the odor in a neutral cage with no restraint (*sham*) for two hours. All animals underwent heroin or saline self-administration (SA), anxiety assessments, and extinction followed by non-cued and cued reinstatement testing. During SA, stress rats acquired heroin faster than sham rats indexed as days to reach criteria. Intake (mg/kg) did not differ between stress conditions, but females had higher intake than males. In subsequent analyses, data were collapsed across sex due to similar patterns of responding. Sham and stress rats did not differ on locomotor activity in a novel environment, object recognition or compartment choice on the elevated plus maze. In a defensive burying test in which the stress-conditioned odor was presented, stress exposure resulted in higher indices of defensive behavior in stress rats. A history of heroin exposure in stress rats resulted in more time interacting with a conspecific during abstinence and greater reinstatement to heroin seeking relative to sham controls. Our results suggest that stress and heroin may be acting on shared mechanisms mediating approach/avoidance and defensive behaviors that increase stress reactivity during drug

abstinence. Additionally, our data support the hypothesis that acute stress exacerbates the risk of increased drug taking and reinstatement of heroin seeking.

Disclosures: J.S. Carter: None. **A.M. Kearns:** None. **R.A. Weber:** None. **C.M. Reichel:** None.

Poster

420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 420.03/GGG19

Topic: G.08. Drugs of Abuse and Addiction

Support: R00DA037344

Title: Reduced opioid intake and stress-induced relapse in fatty acid amide hydrolase (FAAH) knockout rats

Authors: *A. KARNATI[1], J. E. SCHLOSBURG[2]
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Abstract: The number one focus, for both treatment and public policy, is the ongoing opioid epidemic. Overdose rates in the United States have more than tripled over the last decade, with total deaths nearing 70,000 in the past year alone. It has, therefore, become a priority to identify possible agents that could combat excessive opioid use. Fatty acid amide hydrolase (FAAH) is an enzyme responsible for regulating the levels of the endogenous cannabinoid, anandamide (AEA), and inhibition of FAAH has been shown to convey resilience to chronic stressors. Based on the theoretical concept that excessive drug use and withdrawal is a form of chronic stress, we examined whether rats with FAAH genetically inactivated (knockout) are less likely to escalate their intake in extended-access drug self-administration, as well as reduced sensitivity to reinstatement of drug-seeking behavior.
Using long-term self-administration sessions (12 hr) indicates that rats with FAAH genetically inactivated, both males and females, exhibit decreased opioid consumption following temporary periods of withdrawal, resulting in reduced escalation of heroin and oxycodone intake. This is despite the fact that FAAH knockout rats show no differences in seeking for natural reward. We then set out to determine the effects of factors such as stress, drug re-exposure, and drug-associated cues on reinstatement of drug seeking behavior. Yohimbine, an alpha-2 adrenergic antagonist, produced a sympathetic stress response capable of heroin reinstatement, which was significantly reduced in the FAAH knockout rats. Cue-induced reinstatement showed significant increases in responding behavior across both genotypes in comparison to extinction values, while heroin primed reinstatement behavior did not show any significant differences in responding

behavior. Going forward, we hope to examine how the stress response relates to drug withdrawal and the averseness of withdrawal in FAAH knockout rats.

Disclosures: A. Karnati: None. **J.E. Schlosburg:** None.

Poster

420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

Location: SDCC Halls B-H

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Program #/Poster #: 420.04/GGG20

Topic: G.08. Drugs of Abuse and Addiction

Support: PJY-153418 (CIHR)

Title: Chemogenetic activation of prefrontal cortex - thalamic projections in the augmentation of heroin seeking induced by chronic food restriction

Authors: *A. CHISHOLM, E. FORTIN, D. RIZZO, V. MOMAN, R. DAUTH, A. GHEZZO, N. QUTEISHAT, J.-P. MANOLIADIS, A. CASOLA, U. SHALEV
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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. Previous evidence from our laboratory indicates that chemogenetic activation of the paraventricular nucleus of the thalamus (PVT) reduces heroin seeking in food-restricted animals. A major excitatory input to the PVT is from the medial prefrontal cortex (mPFC). Thus, the objective of the current study was to study the effect of chemogenetic activation of the mPFC-PVT neuronal pathway 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 mPFC, and implanted with a guide cannula aimed at the PVT. Next, rats were trained to self-administer heroin over the course of 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 15^{th} day of the withdrawal period, a drug-seeking test was conducted in which rats were intracranially injected with CNO (1.0 mM) into the PVT, to activate the mPFC-PVT 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 results suggest that chemogenetic activation of the mPFC-PVT pathway does not attenuate heroin seeking in food-restricted or sated rats.
These results suggest that the excitatory input from the mPFC to the PVT does not play a role in cue-induced heroin seeking.

Disclosures: A. Chisholm: None. **E. Fortin:** None. **D. Rizzo:** None. **V. Moman:** None. **R. Dauth:** None. **A. Ghezzo:** None. **N. Quteishat:** None. **J. Manoliadis:** None. **A. Casola:** None. **U. Shalev:** None.

Poster

420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

Location: SDCC Halls B-H

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Program #/Poster #: 420.05/GGG21

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA033231
NIH Grant DA025646

Title: Hippocampal mTORC1 and ERK co-regulate drug context-induced heroin-seeking behavior

Authors: *R. A. FUCHS[1], *R. A. FUCHS[1], R. WANG[1], T. A. BROWN[1], S. TAN[1], J. A. HIGGINBOTHAM[1], D. T. LYSLE[2]
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Abstract: The dorsal hippocampus (DH) plays a selective role in reinstatement of drug-seeking behavior elicited by exposure to a drug-paired environmental context. This study examined the roles of mechanistic target of rapamycin (mTOR; an element of mTOR complex 1, mTORC1) and extracellular signal-regulated kinase (ERK) signaling in the DH in drug context-induced heroin-seeking behavior. Sprague-Dawley rats were trained to lever press for in-signaled heroin infusions (diamorphine hydrochloride, 50 µg/infusion then 25 µg/infusion, IV) in a distinct environmental context made up of multimodal sensory stimuli. Next, rats received extinction training sessions in a different context during a minimum of seven daily sessions, until their responding reached the extinction criterion (≤ 25 active lever responses/session during at least two consecutive sessions). At test, rats received bilateral microinfusions of the mTOR inhibitor, rapamycin (0.25 or 0.5 µg/0.5 µl/hemisphere), the ERK inhibitor, U0126 (1 µg/0.5 µl/hemisphere), or vehicle into the DH or the dorsally adjacent trunk region of the

somatosensory cortex (SStr, anatomical control region). The rats were then placed into the previously heroin-paired or the extinction context, where lever presses were assessed without drug reinforcement, or into a novel context, where motor activity was monitored. Exposure to the heroin-paired context reinstated extinguished drug-seeking behavior relative to exposure to the extinction context. Intra-DH, but not intra-SStr, administration of rapamycin or U0126 significantly attenuated heroin-seeking behavior relative to vehicle. The manipulations did not alter motor activity. Together, these findings suggest that mTORC1 and ERK signaling pathways are necessary for the motivational effects of drug-associated contextual stimuli. Based on evidence of ERK-mediated regulation of mTORC1 activity in other systems, ongoing studies explore the possibility that mTORC1 and ERK interact to regulate drug-seeking behavior.

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Poster

420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

Location: SDCC Halls B-H

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Title: Pain-induced negative affect is mediated via recruitment of the nucleus accumbens kappa opioid system

Authors: *N. MASSALY[1], A. R. POE[2], B. A. COPITS[2], L. HIPOLITO[3], T. MARKOVIC[2], S. LIU[4], R. NEVE[5], C. M. CAHILL[4], K. I. SHOGHI[2], R. W. GEREAU, IV[2], J. G. MCCALL[2], R. AL-HASANI[2], M. R. BRUCHAS[2], J. MORON-CONCEPCION[2]
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Abstract: Prolonged negative affect significantly impacts quality of life for patients suffering from pain. These maladaptive emotional states can lead to severe depression, suicide, involuntary opioid overdose, and related neuropsychiatric comorbidities. The nucleus accumbens

(NAc) shell, which integrates both the aversive and rewarding valence of stimuli, exhibits allostatic changes in the presence of pain. In discrete regions of this structure, activation of the kappa opioid receptor (KOR), either by dynorphin, its endogenous agonist, or pharmacological ligands, acutely decreases the reinforcing properties of rewards and induces dysphoria and aversive behaviors. Using a wide range of complementary techniques including pharmacology, optogenetics, chemogenetics, physiology, biochemistry and in vivo positron emission tomography (PET) imaging, our current findings demonstrate that *in vivo* recruitment of NAc shell dynorphin neurons, acting through KOR, is both necessary and sufficient to drive pain-induced negative affect. Furthermore, we reveal that the presence of inflammatory pain impacts patterns of consumption of fentanyl using an intra-venous self-administration paradigm. Those particular patterns, where rats in pain display bursts of consumption interrupted by periods of "rest", could lead to the occurrence of respiratory depression and subsequent involuntary overdose. Taken together, our results provide evidence that adaptations in the kappa opioid system within the NAc shell represent a functional target for therapeutic intervention in pain that could circumvent affective disorders and may prevent life threatening episodes.

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420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA003906

Title: Cellular specificity of matrix metalloproteinase activation on accumbens medium spiny neurons during heroin relapse

Authors: *V. CHIOMA, P. KALIVAS
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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 the 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

extracellular synaptic remodeling both constitutively and transiently. Following chronic cocaine self-administration, cocaine-extinguished rats exhibit enduring increases in MMP-2 activity in NAcore compared to controls, and MMP-9 activity is transiently increased during cued reinstatement. Interestingly, heroin-extinguished rats do not show constitutive MMP activity, however, transient increases were elicited after 15 mins of cued heroin seeking. Although increases in MMP-2,9 fluorescence can be localized to the soma and dendritic processes of medium spiny neurons (MSNs) in the 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 colocalization with MMPs during transient cued heroin seeking, while D2-receptor expressing MSNs express increased colocalization following extinction. We used an AAV cre-dependent mCherry virus to transfect accumbens MSNs in D1 and D2 cre-dependent rats and measured the colocalization of activated MMP-2,9 after FITC-gelatin microinjection under extinguished and reinstated conditions. For D1 MSNs, we observed increased MMP-2,9 colocalization with dendritic surfaces in both extinguished and reinstated animals compared to yoked saline controls. While D2 MSNs showed increased MMP-2,9 colocalization only in heroin-extinguished animals, but MMP-2,9 colocalization after 15 min reinstatement was reduced to yoked saline levels. 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. **P. Kalivas:** None.

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420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

Location: SDCC Halls B-H

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA-026894 (HC)
Davee Foundation (EER)

Title: Operant oral oxycodone self-administration in inbred strains of rats

Authors: X. FAN[1], T. WANG[1], E. REDEI[2], *H. CHEN[1]
[1]Dept Pharmacol, Univ. Tennessee Hlth. Sci. Ctr., Memphis, TN; [2]Psychiatry and Behavioral Sci., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Over two million people in the US are suffering from substance use disorder related to prescription opioid pain relievers in the current opioid abuse crisis. Most of these opioid abusers started with oxycodone. Rapid onset of CNS effects is commonly required for animal models of drug abuse. Thus many current rodent models of oxycodone abuse use intravenous drug self-

administration. However, oxycodone in general is taken orally. We therefore investigated the abuse liability of oral oxycodone using an operant licking procedure in rats. Young adult Lewis rats were used. We also tested the WMI and WLI strains, which were developed as a genetic model of depression. Self-administration was conducted using operant chambers equipped with two lickometers. Licking on the active spout meeting a fixed ratio 5 schedule triggered the delivery of 60 ul oxycodone solution to the tip of the spout. A cue light was triggered to coincide with drug delivery. This was followed by a 20 s timeout, which was signaled by a tone. Rats received five 1-h daily training sessions, which were followed by five 6-h sessions on alternate days. Rats were not deprived of water or food. Water was available during the 6-h sessions. Rats licked significantly more on the active than on the inactive spout ($F_{1,809}=505.4$, $p<2e-16$) across the strains. In Lewis rats trained on 0.025-0.1 mg/ml, the highest dose resulted in the highest intake. This dose difference was no longer significant when access was prolonged to 6-h. Instead, a significant sex difference emerged ($F_{1,23}=4.3$, $p<0.05$, female > male). These rats were then further tested for cross-session dose response. Oxycodone intake (0.70+/- 0.11 - 0.85+/-0.20 mg/kg/session) was stable between 0.1-0.4 ug/ml, indicating self-regulation in drug intake. WMI and WLI strains were trained using 0.1 mg/ml oxycodone. Significant sex difference was found ($F_{1,15}=15.2$, $p=0.001$, female > male). No strain difference was found during the 1-h sessions. There was a significant interaction between strain and sex during the 6-h sessions ($F_{1,16}=15,8$, $p=0.001$). Tukey HSD posthoc showed oxycodone intake was WMI > WLI in males and WLI > WMI in females. Together, these data demonstrated that oral oxycodone is readily self-administered by three inbred strains of rats without prior operant training. Ongoing studies will investigate whether they will develop addiction-like phenotypes with extended drug exposure.

Disclosures: X. Fan: None. **T. Wang:** None. **E. Redei:** None. **H. Chen:** None.

Poster

420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIGMS grant T32 GM081741
NIDA F30 Fellowship F30DA044711

Title: Evidence for the role of biased signaling at the kappa opioid receptor in reducing conditioned place preference to morphine

Authors: *S. KASKI[1], J. GROSS[2], A. SCHROER[2], K. WIX[2], D. P. SIDEROVSKI[2], V. SETOLA[2]

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Abstract: Activation of the kappa opioid receptor (KOR) has been shown to reduce the rewarding properties of opioid drugs in pre-clinical studies. Yet, the dysphoric side-effects of KOR agonists have limited their therapeutic potential. Advancements in KOR ligand pharmacology have identified G protein-biased KOR agonists that elicit a reduced level of aversion in pre-clinical studies. As opioid-induced reward is linked to dopamine release in the nucleus accumbens, and G protein signaling by KOR is known to reduce the release of dopamine in the nucleus accumbens, we hypothesized that G protein-biased KOR agonists may reduce the rewarding properties of co-administered opioid analgesics, while avoiding the therapeutically limiting side-effects of traditional KOR agonists. We tested this hypothesis in C57BL/6J mice using conditioned place preference and conditioned place aversion assays to measure drug-induced reward and aversion, respectively, and open field locomotion to assess for any locomotor suppression. In addition, the hot plate assay was used to assess the possibility that co-administration of a KOR agonist with an opioid analgesic may alter analgesic efficacy. Our results suggest an important role for beta-arrestin signaling in counteracting the rewarding effects of opioid analgesics in mice.

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Poster

420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA037897

Title: Activation of amylin receptors in the nucleus accumbens shell reduces voluntary oxycodone taking in rats

Authors: *Y. ZHANG[1], N. S. HERNANDEZ[2], C. A. TURNER[2], M. KAHNG[2], V. R. WEIR[2], H. D. SCHMIDT[3]
[1]Biobehavioral Hlth. Sci., [2]Univ. of Pennsylvania, Philadelphia, PA; [3]Dept Psychiatry, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA

Abstract: Prescription opioid abuse has reach epidemic-like proportions in the United States. There is a critical need for innovative research aimed at identifying novel mechanisms underlying opioid addiction that could serve as targets for new medications to treat prescription

opioid abuse. Amylin is a peptide hormone co-secreted with insulin from pancreatic β-cells. Amylin crosses the blood brain barrier and activates amylin receptors expressed throughout the brain. Central amylin signaling has been shown to regulate food intake. Specifically, activation of amylin receptors in the mesolimbic dopamine system has been shown to reduce the hedonic value of food. Given that the reinforcing effects of natural rewards and drugs of abuse are regulated by the mesolimbic dopamine system, these findings suggest that central amylin signaling may play an important role in addiction-like behaviors. The goal of these studies was to determine the role of amylin receptors expressed in the nucleus accumbens (NAc) shell in voluntary oxycodone taking behavior. Our results indicate that systemic administration of amylin (5 and 10 µg/kg, i.p.) significantly attenuates oxycodone self-administration under both fixed-ratio 5 and progressive ratio schedules of reinforcement, suggesting that amylin may reduce the reinforcing efficacy of prescription opioids. In addition, administration of amylin (0.4 µg/µl) directly into the NAc shell significantly attenuated oxycodone self-administration in rats, further supporting a novel role for central amylin receptors in opioid addiction. To phenotype amylin receptor-expressing neurons in the ventral striatum, AAV-DIO-YFP was injected directly into the NAc shell of transgenic rats expressing Cre recombinase specifically in medium spiny neurons (MSNs) expressing dopamine type 1 receptors (D1R-expressing MSNs) and dopamine type 2 receptors (D2R-expressing MSNs). Our results show that amylin receptors are expressed by both D1R- and D2R-expressing MSNs in the NAc shell. Taken together, these findings demonstrate an important role for central amylin receptors in preclinical models of prescription opioid addiction.

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Poster

420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

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Program #/Poster #: 420.11/GGG27

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA IRP

Title: Economic choice in squirrel monkeys for studying opiate reward

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Abstract: There is a growing need to develop translational animal models to better understand choice behavior and other complex phenotypes relevant to addiction. The objective of the present

study was to develop a touchscreen approach to assess economic choice in squirrel monkeys. Using a touchscreen to present behavioral tasks allows for the presentation of diverse stimuli and recording of responses. In this study, 14 male squirrel monkeys (*Saimiri sciureus*) were trained daily, using response shaping, to target a stimulus on the touchscreen for delivery of a highly-palatable milk reward (30% sweetened condensed milk). Animals were then trained to perform a choice task in which two stimuli were simultaneously presented, with the number of symbols in each stimulus representing relative milk reward volumes. Twelve subjects learned to discriminate and consistently chose the larger milk reward on at least 85% of trials of the choice task, across 34 sessions. Subsequently, drug-naïve animals will be outfitted with intravenous catheters and trained with a separate set of stimuli to intravenously self-administer remifentanil, a short-acting μ-opioid receptor agonist. A choice distribution between drug and non-drug reward will be established which will be a primary metric for evaluating candidate treatments and investigating the neurocircuitry involved in this reward-seeking behavior.

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Poster

420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

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Support: NIH Grant T32DA16176
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Title: Fentanyl demand decreases after activation or blockade of μ opioid receptors in male rats

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Abstract: Individuals exhibiting high levels of economic demand for abused illicit opioids, such as fentanyl, in the month preceding treatment are more likely to use illicit opioids during medication-assisted therapy (MAT; Worley et al., 2015, Drug Alcohol Depend, 148:62-68). Thus, lower demand prior to MAT is associated with better treatment outcomes. Because MAT directly affects the reinforcing properties of opioids, it is possible that those who fail to achieve abstinence from illicit opioids will nevertheless eventually reduce their economic demand for opioids, and thus be more likely to achieve abstinence with continued treatment. In the current study, we examined demand for the opioid fentanyl in rats following s.c. pre-treatment with buprenorphine (0, 0.3, 1.0, and 3.0 mg/kg), naltrexone (0, 0.1, 0.3, and 1.0 mg/kg), or morphine

(0, 0.3, 1.0 and 3.0 mg/kg). Using adult male Sprague-Dawley rats (n=8), we obtained estimates of two measures of economic demand, elasticity and demand intensity, using a threshold procedure. After rats learned to earn 5 µg/kg fentanyl on an FR1 schedule we transitioned to the threshold procedure on an FR3, where in each session they earned progressively smaller quantities of drug for each infusion across eleven 10-min intervals. Thus, during the course of a session the price for 1.0 µg/kg of fentanyl increased from 0.6 to 190 responses. Rats received 14 sessions with the threshold procedure before the first drug pretreatment test. The design was within-subjects, with two retraining sessions between tests, and each rat received each drug/dose combination according to a Latin-square design. Although the drugs tested have different effects on µ opioid receptors, we found that each of the drugs dose-dependently increased elasticity. Morphine and naltrexone also decreased demand intensity. Buprenorphine did not affect demand intensity during the test session, but went on to dose-dependently decrease demand intensity for at least 48 hours following the test session. Taken together, the results suggest that fentanyl demand is highly sensitive to activation or blockade of µ opioid receptors. The protracted effects of buprenorphine on demand suggest that patients who do not achieve full abstinence from illicit opiates may still benefit from medication-assisted therapy.

Disclosures: L.R. Hammerslag: None. **J. Beckmann:** None. **R.S. Hofford:** None. **M.T. Bardo:** None.

Poster

420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 420.13/HHH2

Topic: G.08. Drugs of Abuse and Addiction

Support: W81XWH-17-1-0004
W81XWH-13-2-0075

Title: Effects of morphine abstinence on oxycodone self-administration in male and female rats

Authors: *T. LINTZ, M. MAVRIKAKI, B. ESAYEAS, S. PAGE, E. CHARTOFF
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Abstract: Opioid abuse is a major public health concern, and abuse often begins after withdrawal from a prescribed regimen of opioid painkillers. Opioid withdrawal can include intense, short-lived physical symptoms and protracted stress-like psychological symptoms, which are thought to contribute to addictive behavior through negative reinforcement mechanisms. Accumulating evidence indicates that women are more likely than men to abuse opioids to self-treat negative affect and pain, suggesting that withdrawal-induced negative affective states would increase abuse risk more in women than in men. An unmet clinical need is

a way to predict vulnerability to opioid abuse in patients. One possibility is the use of distress intolerance—defined as the perceived inability to tolerate negative physical (e.g. pain) and emotional states. Opioid abuse is associated with heightened levels of distress intolerance, raising the possibility that distress intolerance measures can be used to predict the likelihood of initiating prescription opioid abuse. Here we used acoustic startle, warm water tail flick latency, and somatic withdrawal signs as proxies for distress intolerance in adult male and female Sprague Dawley rats after a non-contingent regimen of escalating dose chronic morphine (5 - 30 mg/kg, 2xd for 12d; N=15-18/group). After 2-weeks of withdrawal from morphine (or vehicle), rats were implanted with jugular vein catheters and allowed to self-administer oxycodone (0.06 mg/kg/inf). We found that morphine withdrawal was associated with decreased tail flick latencies in male, but not female, rats—indicative of hyperalgesia. In addition, somatic withdrawal signs were increased in both sexes, whereas no change in acoustic startle amplitudes was observed in either sex. We found that male, but not female, morphine-withdrawn rats initially self-administered more oxycodone than vehicle-withdrawn rats, suggesting that morphine withdrawal increases vulnerability to initiate prescription opioid abuse specifically in males. However, after extended exposure to 1-hr/d self-administration sessions, intake normalized across groups. Furthermore, subsequent dose response functions indicated that morphine-withdrawn male and female rats were less sensitive to the reinforcing efficacy of oxycodone. Individual distress intolerance measures (e.g., acoustic startle, tail flick latency) were correlated with acquisition of oxycodone self-administration behavior, supporting our hypothesis that distress intolerance may be useful in predicting vulnerability to initiating prescription opioid abuse.

Disclosures: T. Lintz: None. **M. Mavrikaki:** None. **B. Esayeas:** None. **S. Page:** None. **E. Chartoff:** None.

Poster

420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 420.14/HHH3

Topic: G.08. Drugs of Abuse and Addiction

Support: UNH SURF Grant
UNH REAP Grant
NIH GM113131

Title: Individual vulnerability to traumatic stress predicts increased demand for heroin and cue-induced reinstatement: A novel preclinical approach to study individual differences underlying traumatic stress and heroin use comorbidity

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Abstract: Heroin use is a prevalent epidemic in the U.S. Long-term consequences of stress and high rates of comorbidity between substance abuse disorders and mood disorders are critical risk factors in use, relapse, and overdose. Individuals with stress-related disorders are 3-5 fold more likely to develop substance use disorders. High prevalence rates coupled with poor treatment outcomes indicate a need for improved understanding of how stress reactivity contributes to the mechanisms of substance abuse. Preclinical models of traumatic stress have provided key insights into the effects of stress on self-administration of drugs of abuse. However, there is a significant gap in our understanding of how ethologically relevant stressor exposure contributes to heroin self-administration. Moreover, there are no studies investigating individual differences underlying reactivity to stress and subsequent stress-induced heroin self-administration. We hypothesized that greater individual vulnerability to stress would predict higher demand for heroin self-administration in a rodent model of heroin use. To this end, rats were first exposed to an inescapable swim stress and stress reactivity was assessed via a panel of behavioral tests of anxiety (open field, social anxiety) and depression (forced swim). Individual demand for heroin was assessed using behavioral economics approach. Subsequently, all rats underwent extinction and reinstatement tests with stress, non-contingent cues, or yohimbine as triggers. For the first time, our results demonstrate that individual reactivity to an ethologically relevant traumatic stressor predicted subsequent demand for heroin. Specifically, rats most vulnerable to traumatic stress had higher demand for heroin. Furthermore, our results demonstrate that responding in extinction was more persistent among rats with the lowest demand for heroin. Additionally, we show that non-contingent cue presentation reinstated heroin seeking and was significantly related with individual reactivity to traumatic stress. More studies are warranted to better understand individual vulnerability to stress and how stress relates to substance use and abuse if the goal is to develop effective individualized prevention and treatment approaches.

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Poster

420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 420.15/HHH4

Topic: G.08. Drugs of Abuse and Addiction

Support: DHP STTR-W81XWH-17-C-0031
DHP STTR-W81XWH-17-C-0032

Title: Morphine experience and expectations alter 50-55 kHz ultrasonic vocalization counts and acoustic characteristics

Authors: *C. L. DUVAUCHELLE[1], C. CERVANTES[1], J. BAILEY[2], A. MARTINEZ[3], H. TRINH[4], N. MITTAL[5], N. THAKORE[1], W. MADDOX[6]
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Abstract: Intense craving for the drug is a critical feature of opiate addiction and a strong trigger for drug use and relapse. Though positive affective states in rodents can be monitored in real-time through ultrasonic vocalization (USV) emissions, few animal studies have determined the role of emotional status as a motivational factor for opiate abuse. Existing USV reports on this topic have yielded conflicting findings on USV emission rate through varied methodologies involving short duration studies and experimenter-administered opioid intake. Our laboratory has developed a reliable, high-speed analysis technique, called WAAVES, that extracts USV calls and quantifies a number of acoustic characteristic (specifically, mean frequency, duration, bandwidth, and power) associated with each call. WAAVES allows us to examine all data generated from long duration, multiple-session experiments and to utilize advanced statistical techniques to examine acoustic characteristic differences across experimental conditions. In the present study, we examined the influence of the timing and delivery of morphine availability during a multi-session morphine self-administration experiment. In this study, we recorded USVs from two groups (Chronic and Intermittent) male Wistar rats prior to (e.g. “Anticipation”) and during 10 morphine self-administration sessions (e.g. “Session”). Both groups received the same number of self-administration sessions, but Chronic animals received daily sessions (5 days/2wks) while morphine access for the Intermittent group was on a variable schedule (e.g., 5 days/3wks). To determine if the USV acoustic characteristics from animals in the Chronic group can be distinguished from animals in the Intermittent group we used discriminant analysis to determine if a linear combination of the acoustic characteristics can successfully distinguish animals in each group. Results revealed significant differences in acoustic characteristics between animals in the Chronic and Intermittent group with 80% accuracy. As 50-55 kHz USV emissions are mediated through mesolimbic dopaminergic activation these data indicate that expectations of impending opiate drug use can significantly alter the pharmacological effects of morphine. These findings further suggest that drug expectation and drug experience may be important factors in opiate addiction and accidental overdose.

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Poster

420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 420.16/HHH5

Topic: G.08. Drugs of Abuse and Addiction

Title: Sex differences in oxycodone preference choice task

Authors: *J. GERSON[1,2]
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Abstract: Aim
Due to the continuing rise in opioid addiction, it is critical to discover the underlying factors that play a role in drug abuse. Sex differences are a relatively new focus of study in the field of opioid addiction and it is essential to add to this growing body of research.
Methods
To add to the growing body of research on the role of sex in addiction vulnerability, we examined differences in oxycodone (0.03 mg/kg/infusion, IV) and food (45 mg banana pellets) reinforcement between male (n=7) and female (n=8) Sprague Dawley, rats. Each rat was implanted with a jugular catheter before undergoing behavioral testing using a two-lever operant chamber model of drug self-administration. Rats underwent both single-reinforcer progressive ratio sessions for both oxycodone and food separately, followed by discrete choice sessions where both reinforcers were concurrently available.
Results
During choice sessions, males and females selected food more frequently than oxycodone, showing a significant preference for food over oxycodone ($F(3,26)=24.35$, $p<0.0001$). There were no significant differences in food vs. oxycodone preferences between sexes when both oxycodone and food were concurrently available. Furthermore no rats showed a preference for oxycodone (selecting drug on over 50% of trials). In contrast, when only oxycodone was available under progressive ratio, female subjects displayed significantly higher breakpoints for oxycodone than males ($U=8.0$, $p<0.05$).
Conclusions
These results indicate females exhibit higher motivation for oxycodone than their male counterparts but, at this dose, there are no sex differences in the choice model of addiction. These results are in agreement with previous work which demonstrated a higher breakpoint for female rats self-administering heroin and morphine on a PR schedule (Cicero et al., 2003) as well as with female rodents showing a greater propensity to display higher intake, seeking, and reinstatement for drug reinforcement (Becker & Koob, 2016).

Disclosures: J. Gerson: None.

Poster

420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 420.17/HHH6

Topic: G.08. Drugs of Abuse and Addiction

Support: USPHS Grant R01 DA035281

Title: The reinforcing properties of heroin vapor self-administration

Authors: *A. GUTIERREZ[1], M. A. TAFFE[2]
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Abstract: Abuse of opioid drugs has reached epidemic proportions. Although many research studies address the reinforcing properties of opioid drugs, there is a gap in understanding route-specific effects. Smoking is a common route of heroin administration and of those who inject the drug, a large percentage initially began using by smoking it. E-cigarette technology has been steadily increasing in popularity, especially among young people, and reports exist of this technology being used to self-administer illicit drugs. The novelty of this technology means that few data exist to address the reinforcing effects of vapor delivery of illicit substances, including heroin. The aim of this study was to assess whether self-administration was supported by vapor delivery of heroin to rats using e-cigarette-type technology. Operant vapor chambers made of transparent acrylic boxes with response levers on each side were used. Each lever had a stimulus light directly over it which illuminated immediately after the reward lever was press, indicating a 20 second time-out interval. Air was routed through a commercially available vapor canister, triggered by computer to deliver reinforcers, directed though an air inlet in the front of the box and a vacuum cleared the chambers through an outlet located on the far end of the box. Heroin was prepared in propylene glycol at concentrations ranging from 1.0 mg/mL to 50 mg/mL. Adult female Wistar rats were given 1 h sessions in operant vapor chambers where they could lever-press for a 1 second puff of the vaporized heroin under a Fixed Ratio (FR) 1 reinforcement schedule for 5 sessions. The animals were then trained using a 50 mg/mL heroin solution. The 50 mg/mL concentration was found to be efficacious at increasing and maintaining heroin self-administration. Experiments using a progressive-ratio schedule and drug-substitution testing were then used to further probe the efficacy of vaporized heroin as a reinforcer. The findings of this study will help establish a model of heroin vapor self-administration and will provide insight into the harm potential of e-cigarette technology misuse.

Disclosures: A. Gutierrez: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); The vapor delivery technique used in this study was developed with the help of La Jolla Alcohol Research, Inc. **M.A. Taffe:** 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.; La Jolla Alcohol Research, Inc..

Poster

420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 420.18/HHH7

Topic: G.08. Drugs of Abuse and Addiction

Title: Polyaminergic agents modulate extinction and reinstatement of conditioned place preference in mice

Authors: *M. A. RUBIN[1], B. A. GIRARDI[2], S. FABRIN[2], A. L. WENDEL[2], F. R. MELLO[2], C. F. MELLO[2]
[1]Federal Univ. of Santa Maria (UFSM), Santa Maria, Brazil; [2]Federal Univ. of Santa Maria, Santa Maria, Brazil

Abstract: Morphine addiction is a chronic disease that involves biological, cognitive and behavioral changes developed after the repeated and compulsive use of the drug. Drug addicts constantly relapse to drug seeking after recall of memories linked to the drug experience. The glutamatergic system is critically involved in specific processes of memory, including the modulation of dopaminergic neurons for the formation and maintenance of drug-induced drug-related memories. Polyamines are endogenous modulators of the NMDA receptor and it is not known whether polyaminergic agents modulate the extinction and reinstatement of morphine-induced conditioned place preference (CPP). In the current study we determined whether spermidine, a polyamine ligands, modulate the morphine CPP extinction and reinstatement. Adult male albino Swiss mice received saline (0.9 % NaCl, intraperitoneally (i.p.)) or morphine (5 mg/kg, i.p.) and were respectively confined to a black or a white compartment for 30 min for four consecutive days for CPP induction. The effect of spermidine (10-30 mg/kg, i.p.) or ifenprodil (0.1-1 mg/kg, i.p.), antagonist GluN2B NMDAr and agonist of the polyamine-binding site at the NMDA receptor, on the extinction and reinstatement of morphine CPP was studied. Spermidine and ifenprodil facilitated the extinction of morphine CPP *per se*. The treatment with spermidine during the extinction period impairs the reinstatement induced by a noneffective dose of morphine (1.25 mg/kg). Ifenprodil (0.1 mg/kg) prevented the effect of spermidine on the extinction of morphine CPP but did not prevent the reinstatement of CPP induced by morphine. These results suggest that spermidine facilitated the extinction and prevented the reinstatement of morphine CPP by modulating the polyamine-binding site at the NMDA receptor.

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Poster

420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 420.19/HHH8

Topic: G.08. Drugs of Abuse and Addiction

Support: This work was supported by NIDA/NIH

Title: Incubation of oxycodone craving after conflict-induced voluntary abstinence

Authors: *I. FREDRIKSSON, A. MINIER-TORIBIO, J. M. BOSSERT, Y. SHAHAM
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Abstract: A major problem of the current U.S. opioid addiction epidemic is high relapse rates. To date, preclinical studies on relapse to prescription opioids like oxycodone are limited, and in these studies, relapse was determined after experimenter-imposed or forced abstinence periods. In humans, however, abstinence is often self-imposed and drug-seeking episodes typically involve a conflict situation where addicts choose between the desire to experience the drug's rewarding effects and the adverse consequences of drug seeking. Here, we introduce a rat model of relapse to oxycodone seeking after conflict-induced voluntary abstinence. We trained male and female (n=12-14/sex) rats to self-administer oxycodone (0.1 mg/kg/infusion, 6-h/d) for 14 days. Next, we tested the rats for relapse to oxycodone seeking under extinction conditions on abstinence day 1. We then introduced an electric barrier near the drug-paired lever and increased the barrier shock intensity daily from 0.1 to 0.4 mA over days until the rats achieved abstinence (conflict phase). We tested the rats for relapse in the absence of shock or drug on abstinence day 15 or 30. The rats showed a time-dependent increase in drug seeking after conflict-induced voluntary abstinence indicated by higher lever presses on abstinence day 30 compared to day 1 or day 15 (incubation of oxycodone craving). There were no sex differences in either oxycodone self-administration or incubation of oxycodone craving. We introduce a conflict-induced voluntary abstinence procedure to study mechanisms of incubation of opioid craving after cessation of drug taking by adverse consequences associated with drug use.

Disclosures: I. Fredriksson: None. **A. Minier-Toribio:** None. **J.M. Bossert:** None. **Y. Shaham:** None.

Poster

420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 420.20/HHH9

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH intramural funds (Yavin Shaham)

Title: Relapse to fentanyl seeking after choice-based voluntary abstinence

Authors: *D. J. REINER[1], O. M. LOFARO[1], M. VENNIRO[2], Y. SHAHAM[3]
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[3]IRP/NIDA/NIH, Baltimore, MD

Abstract: Background: The current prescription opioid crisis is a major public health problem. A high relapse rate is a core feature of addiction to prescription opioids like fentanyl. To date, there are few preclinical studies of prescription opioid relapse, and these studies have used animal models in which the abstinence period prior to relapse is experimenter-imposed or forced. However, in humans, abstinence is often self-imposed, because the former drug user chooses nonaddictive alternative rewards while the drug is still available. We recently developed a rat model of choice-based voluntary abstinence and here we used the model to study relapse to fentanyl seeking.
Methods: We first trained male and female rats (n=14/sex) to self-administer palatable food pellets for 6 days (6-h/day) and fentanyl (2.5 microgram/kg/infusion, i.v.) for 12 days (6-h/day). We then assessed relapse to fentanyl seeking in extinction tests, after 1 and 14 abstinence days. Between tests, rats underwent voluntary abstinence, which was achieved through a discrete choice procedure between drug and palatable food (20 trials/day).
Results: We found no sex differences in fentanyl self-administration or in the preference for palatable food over fentanyl during discrete choice trials. In both sexes, there was no difference in fentanyl seeking during the relapse tests, indicating an absence of a time-dependent increase in fentanyl seeking (incubation of drug craving) following food choice-based voluntary abstinence.
Conclusions: Our data extend previous findings that food-choice voluntary abstinence prevents incubation of heroin craving. We currently explore brain mechanisms of relapse to fentanyl seeking after voluntary abstinence.

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Poster

420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 420.21/HHH10

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA R21 DA037728
Minneapolis Medical Research Foundation #07637
Minneapolis Medical Research Foundation #07434
T32 DA007097

Title: Confirmatory factor analysis of drug self-administration in rats

Authors: *Y. SWAIN[1,2], M. GADES[1], P. MUELKEN[2], M. G. LESAGE[2,1], M. MCGUE[1], J. G. GEWIRTZ[1], A. C. HARRIS[2,1]
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Abstract: Individual differences in susceptibility to addiction in humans have been widely studied through confirmatory factor analysis (CFA), a statistical method that identifies “latent” variables (variables that are not measured directly) that reflect the common variance among a larger number of related measures. Despite its widespread application in clinical research, CFA has been virtually unused in preclinical addiction models. The current study used CFA to examine the latent factor structure between six measures of i.v. morphine self-administration (SA) in rats (e.g., acquisition, demand elasticity, reinstatement). Because individual differences in multiple measures of abuse liability in humans have been shown to be best accounted for by one single latent factor, a one-factor model was fitted to the data. Results from a chi-squared test of overall model fit indicated that the one-factor model did not fit the data well. Standardized factor loadings for demand intensity (0.64) and morphine-induced reinstatement (0.74) were the highest, suggesting that these measures are the strongest indicators of the latent factor. However, extinction and stress-induced reinstatement loadings did not significantly differ from 0, suggesting that they are not related to the latent factor. These results contrast with human studies, which have indicated a common drug addiction factor underlying multiple measures of addiction propensity, and instead suggest specific factors contributing to some SA measures. Additional analyses are needed to explore and identify multi-factor models of drug SA measures. Further establishing CFA approaches in preclinical neuropsychopathology will provide more reliable core measures of disease vulnerability in animal models for further cellular, molecular, and genetic analysis, as well as a quantitative approach for studying predictors of individual differences in addiction vulnerability.

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Poster

420. Drugs of Abuse and Addiction: Opioid Reinforcement, Seeking, and Reinstatement

Location: SDCC Halls B-H

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Program #/Poster #: 420.22/HHH11

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA024044
NIH Grant DA039168
UCSB Academic Senate

Title: Genetic variance in prescription opioid abuse: A comparison between 129 mouse substrains

Authors: *K. K. SZUMLINSKI, M. A. COELHO, S. FERDOUSIAN, N. STAILEY, S. M. JIMENEZ, E. K. F. FULTZ, 93106-9660
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Abstract: Genetic factors are theorized to contribute to the substantial inter-individual variability in opioid abuse/addiction. With intense concern over the high rate of prescription opioid abuse and related deaths, we have sought to advance the behavioral genetics of prescription opioid abuse by comparing oxycodone and/or fentanyl self-administration between inter-related 129 mouse substrains. When provided with the opportunity to consume low-dose solutions under both home-cage drinking and operant-conditioning procedures, our earlier studies identified the 129S1/SvlmJ (S1) and 129P3/J (P3) mouse substrains, respectively, as low and high opioid-taking. However, in follow-up studies, S1 mice failed to acquire sucrose self-administration under various operant-conditioning procedures, suggesting a motivational or operant-learning deficit in this substrain. Subsequent place-conditioning studies indicate robust oxycodone-induced place-preference in P3, but no conditioning in S1, mice. However, the lack of conditioning exhibited by S1 mice reflected a pronounced immobility during testing, which confounded data interpretation. Although S1 mice exhibited lower sucrose consumption in the home-cage, compared to P3 mice, both substrains exhibited an escalation of sucrose intake with repeated opportunities to drink. Intriguingly, S1 and P3 mice initially exhibited equivalent oxycodone and fentanyl consumption in the home-cage, however opioid intake escalated only in P3 mice with repeated opioid experience. No sex differences were observed for any of our measures. These data provide additional evidence for robust differences in opioid addiction-related behaviors between P3 and S1 substrains, suggesting that P3 mice harbor allelic variance that not only augments opioid reward/reinforcement but that promotes a loss of control over

prescription opioid intake. These findings argue further the utility of 129 mice for the study of the behavioral genetics of prescription opioid addiction.

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Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.01/HHH12

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R21DA040773
NIH R01AA13650

Title: Distinct neural mechanisms of monetary and drug reward seeking decisions under uncertainty

Authors: C. HUTSLAR[1], R. POLK[1], E. ATKINSON[1], J. MACY[2], L. CHASSIN[4], C. PRESSON[4], P. FINN[1], *J. W. BROWN[3]
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Abstract: A handful of studies have explored the neural effects of addictive drug administration during fMRI. Still, little is known about the neural mechanisms of choice and contingent drug reward with actual drug reward delivery during scanning. To explore this question, we used human fMRI to directly compare gambling under uncertainty for monetary reward vs. drug reward. Each subject was a heavy smoker who had abstained from nicotine prior to the scan session, as verified by exhaled carbon monoxide measures. The same subjects gambled for drug reward on one day and monetary reward on another day, with the order of days counterbalanced across subjects. They were presented on each trial with a gambling task, in which they had to make a two alternative forced choice between a risky decision with uncertain reward vs. a safe decision with a certain reward amount. The gambles varied independently in expected value, probability of failing to win reward, and the level of risk (i.e. variance of the gamble outcome distribution). Drug reward was administered via a novel MR-compatible electronic cigarette with a valve to control access and an airflow monitor to measure vapor intake. Greater drug reward was administered by opening the airflow valve for a longer period of time after each trial. Results: Greater variance of a gamble for nicotine was associated with greater activation in the dorsal anterior cingulate cortex (BA32), while gamble variance for monetary reward did not show a significant correlation in the same region. Furthermore, there was a striking reversal of error effects in money vs. nicotine reward: losing minus winning a monetary gamble was

associated with greater dorsal cingulate and bilateral anterior insula activation, but the effect was reversed for nicotine reward, such that winning was associated with greater activity than losing in the same regions. All results survived whole brain cluster correction. The results challenge the notion that monetary and drug reward are processed similarly in the brain and highlight the importance of studying drug use decisions directly with neuroimaging rather than using monetary reward as a proxy.

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Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.02/HHH13

Topic: G.08. Drugs of Abuse and Addiction

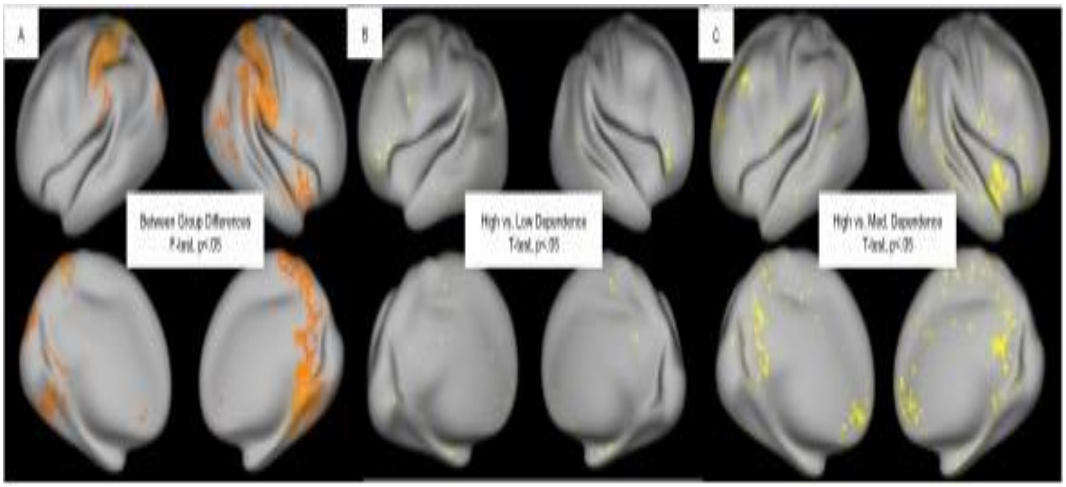
Support: NIDA, FDA P50DA036114
NIGMS P20GM103644

Title: Characterizing the neurobiology of nicotine dependence using multimodal human neuroimaging

Authors: *P. SPECHLER[1], H. GARAVAN[2], A. IVANCIU[2], B. CHAARANI[2], S. ADISE[2], S. HIGGINS[2]
[1]Psychiatry, Psychological Sci., [2]Psychiatry, Univ. of Vermont, Burlington, VT

Abstract: The brain features of nicotine dependence have yet to be studied in a large sample of individuals using high-resolution MRI. Uncovering brain profiles of nicotine dependence will inform the nature of addiction and treatment. All participants (N=110) were daily smokers and categorized into three levels of nicotine dependence (mild, moderate, high) based on criteria established from a large representative study (Schnoll et al, 2013). Following overnight smoking abstinence, participants received an MRI assessing morphology, blood perfusion, and activation to three cognitive tasks (stop-signal, monetary incentive, and cue reactivity). The cue reactivity task required participants to respond to smoking and neutral images.MRI data were processed using pipelines from the Human Connectome Project (Glasser et al., 2013). Differences in reaction time and brain activations for smoking vs. neutral images were calculated. Behavioral data were analyzed using a one-way ANOVA with dependence as a between-subjects factor. Brain data were submitted to non-parametric permutation tests using a general linear model with significance set using a whole-brain correction $p<.05$ from 5000 permutations. Results indicated the dependence levels failed to differ on behavioral performance. However, brain activity to

smoking vs. neutral images differed across all levels with activations in visual, parietal, prefrontal, and insular cortices (Fig1A). Post-hoc tests indicated the high vs. low dependence groups differed in activations in bilateral inferior frontal cortices. Robust differences were evident in the high vs. medium dependence groups in bilateral orbitofrontal and right insular cortex (Fig1B&C). Analyses suggest that nicotine dependence modulates brain activity to smoking cues. Results point to a linear trend, such that the highest level of dependence is characterized by the highest activations in key regulatory and somatosensory regions. Future studies are needed on how treatments and tobacco regulation strategies targeting nicotine dependence may attenuate brain differences.



Disclosures: P. Spechler: None. **H. Garavan:** None. **A. Ivanciu:** None. **B. Chaarani:** None. **S. Adise:** None. **S. Higgins:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.03/HHH14

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R01DA030394 [Franklin]
NIH Grant R01DA029845 [Franklin]
NIH Grant K23AA023894 [Wetherill]

Title: Obese smokers show altered reward-related functional connectivity associated with level of nicotine dependence

Authors: *A. V. ELY[1], K. JAGANNATHAN[2], K. A. KETCHERSIDE[2], C. NUTOR[2], T. FRANKLIN[2], R. WETHERILL[2]
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Abstract: Background: Obesity and cigarette smoking are two of the leading causes of chronic illness and preventable death in the United States. Obese smokers are thus at particularly high risk for lower quality of life and shorter life span. Prior findings show obese smokers have altered smoking cue-potentiated brain responses compared to lean smokers; however, whether differences exist in functional connectivity that may contribute to these comorbid conditions remains unstudied.
Methods: This study used arterial spin-labeled (ASL) perfusion fMRI to compare resting state connectivity between 26 lean and 25 obese treatment-seeking individuals with nicotine use disorder (NUDs). Data were preprocessed in DPARSF toolbox, which is based on SPM8 within a MATLAB environment. *A priori* functionally-defined seed regions were used to identify differences in reward-related (ventral striatum, VS/orbitofrontal cortex, OFC) and inhibitory-related (middle frontal gyrus, MFG) connectivity. Self-report measures of nicotine dependence and craving were acquired.
Results: Controlling for age and sex, MFG connectivity did not differ significantly between groups. In contrast, obese NUDs showed significantly reduced VS/OFC connectivity with the bilateral anterior insula compared to lean NUDs. Among obese NUDs, greater connectivity between VS/OFC and left insula was related to a longer smoking history and higher scores on the Fagerstrom Test for Nicotine Dependence. Correlations were not seen among lean participants.
Conclusions: This differential pattern of functional connectivity is consistent with our research showing that obesity uniquely impacts neurobiology in NUDs. Results suggest obese individuals may fail to appropriately integrate interoceptive or motivational states with reward response. This may contribute to severity and maintenance of nicotine dependence. Future studies are necessary to elucidate the interaction between obesity and cigarette smoking, and impact on treatment outcome.

Disclosures: A.V. Ely: None. **K. Jagannathan:** None. **K.A. Ketcherside:** None. **C. Nutor:** None. **T. Franklin:** None. **R. Wetherill:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.04/HHH15

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH/NIDA K01-DA037819. This research was supported by the Intramural Research Program of the National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD, USA.

Title: Functional connectivity of the human ventral striatum during smoking abstinence and pharmacologic administration

Authors: *R. POUDEL[1], M. J. TOBIA[1], M. C. RIEDEL[1], A. R. LAIRD[1], T. J. ROSS[2], B. SALMERON[2], E. A. STEIN[2], M. T. SUTHERLAND[1]
[1]Florida Intl. Univ., Miami, FL; [2]Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Extensive animal and human research implicates the ventral striatum (VS) and its interconnected circuitry in reward processing and addiction. Dysregulated VS resting-state functional connectivity (rsFC) has been linked with nicotine use and a failure to maintain abstinence. However, differential rsFC of striatal subregions remains to be more fully characterized as a function of a smoking trait (smoker vs. nonsmoker) and pharmacological state (nicotine withdrawal vs. administration). As such, we performed seed-based rsFC assessments on fMRI data (8 min) collected from overnight abstinent smokers (n=24; 12 females; age=36 ±10) and nonsmokers (n=20; 10 females; age=30±7) following administration of nicotine (via patch), varenicline (via pill), both, or neither (placebo-controlled). We employed two bilateral VS seeds (right and left superior VS seeds [RVSs, LVSs] and right and left inferior VS seeds [RVSi, LVSi]) and assessed group effects (smoker vs. nonsmoker collapsed across drug sessions in independent samples t-tests) and drug effects (e.g., nicotine vs. placebo in a linear whole-brain mixed effects ANOVA using AFNI's 3dLME). Regarding group effects, smokers (relative to nonsmokers) showed significantly greater rsFC between the RVSi seed and the medial prefrontal cortex, parahippocampal gyrus, and anterior cingulate cortex, whereas the LVSs seed showed greater rsFC with the superior frontal gyrus (Fig. 1A.). Regarding drug effects, we observed that among smokers, nicotine (vs. placebo) administration significantly reduced the rsFC between the RVSs seed and a cluster encompassing the insula, putamen, and parahippocampal gyrus ($P_{voxelwise}<0.005$, cluster extent: 213 voxels, $P_{corrected}<0.05$) (Fig. 1B.). No effect of Varenicline was observed. Taken together, these outcomes indicate that the rsFC of VS subregions are differentially impacted as a function of a smoking trait (smokers vs. nonsmokers) and the current pharmacological state of a smoker (nicotine vs. placebo).

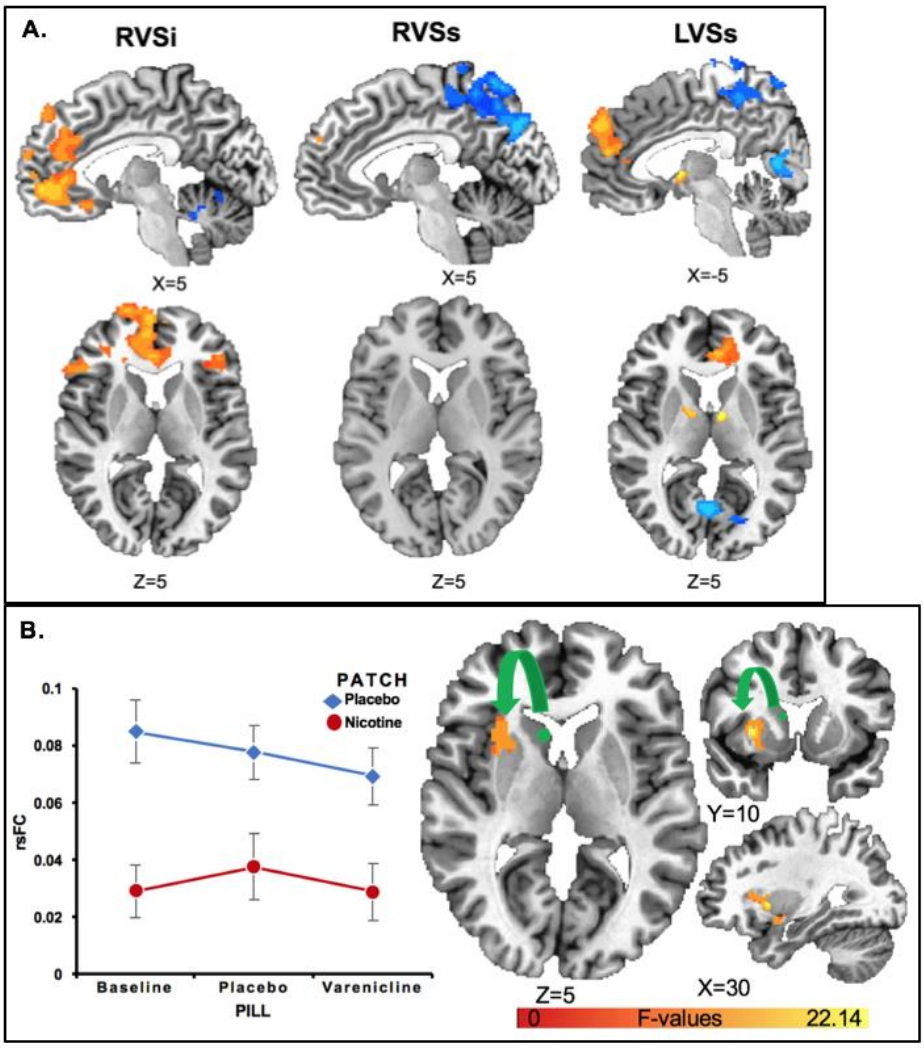

Fig. 1: Group difference and drug effects on rsFC of ventral striatum subregions. A. Group effect (smoker vs. nonsmoker): Significant whole-brain rsFC difference map for ventral striatal seeds: right ventral striatum inferior (RVSi), right ventral striatum superior (RVSs), left ventral striatum superior (LVSs). Orange (smokers > nonsmokers), blue (nonsmokers > smokers). Cluster corrected using ETAC. **B.** Drug effect (nicotine vs. placebo): Significant cluster showing effect of patch on RVSs (green circle) rsFC.

Disclosures: R. Poudel: None. **M.J. Tobia:** None. **M.C. Riedel:** None. **A.R. Laird:** None. **T.J. Ross:** None. **B. Salmeron:** None. **E.A. Stein:** None. **M.T. Sutherland:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.05/HHH16

Topic: G.08. Drugs of Abuse and Addiction

Support: China Precision Medicine Initiative (2016YFC0906300)

Title: WGBS reveals genome-wide differential methylation of occupational exposure to coal mining and tobacco smoking

Authors: *M. WANG[1], M. D. LI[2]
[1]Zhejiang Univ., Zhejiang Province, China; [2]Inst. NeuroImmune Pharmacol., Seton Hall Univ., South Orange, NJ

Abstract: DNA methylation is a crucial epigenetic modification that plays essential roles in multiple biological processes and is susceptible to many environmental factors, such as air pollution and smoking. Here, we conducted WGBS (whole genome bisulfite sequencing) on 120 samples, including 36 underground miners, 36 ground miners and 48 non-miners. Through the comparison of underground miners with non-miners, we identified 300 significant ($P < 0.1$, 1-step Šidák multiple-testing correction) differentially methylated regions (DMRs). Strikingly, we found more than expected (73.3%) significant DMRs showing gradient methylation levels among the three groups, i.e., the DMR methylation levels of ground miners were intermediate between the two other groups. Subsequent GO analysis of 176 genes associated with DMRs with gradient methylation levels revealed 21 significantly enriched biological process ($P < 0.05$, Benjamini-Hochberg correction), primarily related to oxidative stress (mitochondrion regulation, glutathione metabolic process and sulfur amino acid metabolic process) and immune response (interferon-gamma production and antigen receptor-mediated signaling). Considering the fact that the most enriched terms group was related to mitochondrion regulation, we investigated whether coal mining have an influence on mitochondrial DNA copy number (MtDNAcn), defined as the ratio of WGBS coverage of mitochondrial genome to nuclear genome. We found that MtDNAcn of underground miners and ground miners were significant more than non-miners ($P = 0.01$ and $P = 0.007$); however, there was no significant difference between underground miners and ground miners. In sum, our results showed the effects of detrimental occupational exposure on DNA methylation and revealed potential implicated biological processes.

Disclosures: M. Wang: None. **M.D. Li:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.06/HHH17

Topic: G.08. Drugs of Abuse and Addiction

Title: Implication of critical role of GluN3A in nicotine dependence by both genetic association and molecular functional studies

Authors: *C. J. LI[1], M. D. LI[2]
[1]Zhejiang Univ., Zhejiang, China; [2]Inst. NeuroImmune Pharmacol., Seton Hall Univ., South Orange, NJ

Abstract: Nicotine dependence (ND) is a chronic brain disease. The glutamate receptor gene, ionotropic N-methyl-D-aspartate 3A (*GluN3A*), is a crucial subunit of N-methyl-D-aspartate receptors (NMDARs), which plays an essential role at synaptic plasticity in the brain by regulating ion flow across membranes in response to glutamate signaling. Although both common and rare variants of *GluN3A* have been associated with ND in the European-American (EA) and African-American (AA) samples, no reported study has investigated the association between *GluN3A* and ND in Chinese smokers. We thus performed an association study of 16 single nucleotide polymorphisms (SNPs) in *GluN3A* with ND in 2,616 Chinese individuals. Individual SNP-based association analysis indicated that SNP rs1323423 was significantly associated with FTND score (P = 0.0026). Haplotype-based association analysis revealed that two major haplotypes, formed by rs1323423-rs10989591, were significantly associated with FTND score (P = 0.0183). Considering the critical role of *GluN3A* in regulating neurotransmission and its association with ND in human study, we used CRISPR/Cas9 technique to edit *GluN3A* in HEK293T cells, which showed that *GluN3A* expression level affects expression of other NMDA subunit receptors. Moreover, we demonstrated that nicotine at a concentration of 100 μM decreased expression of *GluN3A* in both SH-SY5Y and HEK293T cells at both the RNA and protein levels, respectively. Taken together, by using a combined approach of both genetic association and molecular studies, we provided novel evidence for the involvement of *GluN3A* in developing ND, suggesting that this gene has a great effect on the molecular mechanisms of ND-related behaviors.

Disclosures: C.J. Li: None. **M.D. Li:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.07/HHH18

Topic: G.08. Drugs of Abuse and Addiction

Support: Emergency Medicine, School of Medicine, University of California, Start Up Funds

Title: Role of a 3'utr polymorphism (rs2304297) in the human alpha 6 nicotinic receptor subunit gene in adolescent substance use

Authors: *A. CARDENAS[1], S. LOTFIPOUR[2]
[1]Pharmacol. Sci., [2]Emergency Medicine, Pharmacol. Sci., Univ. of California, Irvine, Irvine, CA

Abstract: The alpha 6 (<**α**;6>) nicotinic acetylcholine receptor (nAChR) subunit is expressed in the dorsal and ventral striatum as well as the ventral tegmental area and substantia nigra. <**α**;6> nAChRs exhibit peak expression during adolescence, which coincides with the initiation and use of nicotine and other drugs of abuse. Evidence has demonstrated that in adolescent humans, a single nucleotide polymorphism, rs2304297, in the 3-untranslated region (3'UTR) of the <**α**;6> nAChR gene, $Chrna6^{C123G}$, is associated with enhanced substance use and structural enlargement of the striatum. Given the localization of <**α**;6> nAChRs in reward circuitry and the developmental peak expression during adolescence, modifications in subunit quantity and/or function may significantly influence drug seeking behavior. The mechanisms underlying the functional role of the $Chrna6^{C123G}$ genetic variant are unknown. One hypothesis may be a reduction in mRNA stability via disruption of microRNA (miRNA) binding at the site of the $Chrna6^{C123G}$ polymorphism. To test this hypothesis *in vitro*, we used a 3'UTR-reporter assay containing the $Chrna6^{C123G}$ genetic variants fused to a luciferase reporter gene. We identified potential miRNAs that are predicted to bind to the $Chrna6^{C123G}$ genetic variants using *in silico* analyses. Our data illustrate that the $Chrna6^{C123G}$ genetic variant did not influence miRNA binding, suggesting that an alternative mechanism of action independent of miRNAs could be involved. To further investigate the mechanisms, our lab generated a viable 3'UTR humanized mutant rat line via CRISPR/Cas9 genomic engineering. Using our animal model, our study aims to understand the genetic, environmental, and neurobiological mechanisms mediating the effects of the $Chrna6^{C123G}$ genetic variant on adolescent substance use. Our findings could have a important impact in the scientific and public health communities which may contribute to the development of improved prevention and intervention strategies for addiction.

Disclosures: A. Cardenas: None. **S. Lotfipour:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.08/HHH19

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R21DA041600

Title: Understanding the molecular basis of nicotine addiction by integrative functional genomic analyses in a rat model and hiPSC-derived DA neurons

Authors: *A. KOZLOVA[1,2], S. ZHANG[1,2], T. UJAS[2], M. STREIT[1], H. ZHANG[1], A. SANDERS[1,2], Z. PANG[3], P. GEJMAN[1,2], P. VEZINA[2], J. DUAN[1,2]
[1]Northshore Univ. Healthsystem, Evanston, IL; [2]Dept. of Psychiatry and Behavioral Neurosciences, Univ. of Chicago, Chicago, IL; [3]Dept. of Neurosci. and Cell Biol. and Child Hlth. Inst. of New Jersey, Rutgers Univ., New Brunswick, NJ

Abstract: Cigarette smoking is the largest preventable risk factor for mortality and a primary risk factor for many chronic diseases. Tobacco consists of more than 4,800 compounds, among which nicotine (NIC) is responsible for the addictive nature of smoking. Repeated exposure to NIC leads to NIC sensitization, which enhances the self-administration (SA) of NIC and other stimulants and is thus a key process in the development of NIC-related addictive behaviors in rats. NIC sensitization and SA in rats correlate with neurobiological changes in multiple brain regions, however, the molecular basis of these changes has yet to be determined. We aim to identify translatome profiles (RNA transcripts bound to ribosomes and actively involved in protein synthesis) in NIC addiction-relevant brain regions following NIC sensitization and in dopaminergic (DA) neurons derived from human induced pluripotent stem cells (hiPSCs). We are using F1 progeny of two inbred rat strains [Fischer-344 (F344) and Brown Norway (BN)] to analyze allele-specific NIC sensitization associated translatome changes. To this end, we have generated F1 F344/BN hybrid rats from F344/NHsd and BN/RijHsd breeding pairs using reciprocal crosses to account for confounding parental imprinting effects. We found that both male and female F1s show a dose-dependent increase in NIC-induced locomotion; however, only males exhibited NIC sensitization. We are in the process of performing the translatome profiling of F1 F344/BN hybrid rats in major brain regions relevant to NIC addiction, the ventral tegmental area (VTA) and nucleus accumbens (NAc). To determine whether the translatome profiles of NIC sensitization observed in rats are similar to those observed in hiPSC-derived NIC sensitized midbrain DA neurons, we evaluated, as a proof of concept, the transcriptomic similarity of these hiPSC-DA neurons to human postmortem brains from Genotype-Tissue Expression (GTEx) and BrainSpan projects. We found a strong expression correlation between iPSC-DA neurons and brain regions relevant to addiction. Interestingly, substantia nigra, a major

DA neuron enriched area, showed the highest correlation with iPSC-DA neurons for NIC/DA-related genes, supporting the validity of hiPSC-derived DA neurons in the transcriptomic study of NIC addiction. We are currently subjecting hiPSC-DA neurons to NIC sensitization. The NIC-induced translatome changes in iPSC-DA neurons will be assayed and compared to those observed in rat brain tissues. Identifying novel gene targets relevant to NIC sensitization will increase understanding of the neurobiology of human NIC abuse and inform the development of more effective therapeutics.

Disclosures: A. Kozlova: None. **S. Zhang:** None. **T. Ujas:** None. **M. Streit:** None. **H. Zhang:** None. **A. Sanders:** None. **Z. Pang:** None. **P. Gejman:** None. **P. Vezina:** None. **J. Duan:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.09/HHH20

Topic: G.08. Drugs of Abuse and Addiction

Support: UST Young Scientist Research Program (2017YS03)
Korea Ministry of Education (2015R1D1A1A01058556)

Title: Striatal cholinergic interneurons control behavioral sensitivity to nicotine

Authors: *B. KIM[1,2], J. WOO[1,2], C. LEE[1,2], H.-I. IM[1,2]
[1]Brain Sci. Inst. (BSI), Korea Inst. of Sci. and Technol. (KIST), Seoul, Korea, Republic of;
[2]KIST School, Korea Univ. of Sci. and Technol. (UST), Seoul, Korea, Republic of

Abstract: The effect of nicotine is driven in large part by striatal nicotinic acetylcholine receptors, but the role of striatal cholinergic interneurons (ChIs) in the behavioral sensitivity to nicotine remains unclear. Here, we demonstrate that RNAi-mediated inhibition of striatal ChIs leads to effective mitigation of nicotine preference and withdrawal signs in mice. Data mining through biological databases predicted a type of brain-enriched and evolutionarily conserved non-coding RNA (designated as ncRNA1) as a potential inhibitor of ion channel subtypes expressed in striatal ChIs. In hemizygous ChAT-Cre mice, AAV-mediated selective overexpression of ncRNA1 in striatal ChIs ($ChI^{ncRNA1\text{-}OE}$) resulted in ion channel knock-down and action potential threshold elevation, consequently lowering the action potential generation capacity of ChIs. In a comprehensive battery of behavioral tests, $ChI^{ncRNA1\text{-}OE}$ mice displayed diminished nicotine preference and somatic withdrawal signs without affecting general locomotion, anxiety, motor coordination, and long-term spatial memory. Interestingly, a neurotransmitter-wide LC-MS analysis revealed that $ChI^{ncRNA1\text{-}OE}$ led to paradoxical changes in striatal acetylcholine level after nicotine administration. Overall, our data indicate that RNAi can be utilized to inactivate neurons, and that striatal ChI inhibition can alleviate behavioral

sensitivity to nicotine in an unconventional manner. Further study is required with respect to the mechanism underlying $ChI^{ncRNA\text{-}OE}$-dependent changes in striatal acetylcholine level and the effect of $ChI^{ncRNA1\text{-}OE}$ in nicotine self-administration.

Disclosures: B. Kim: None. **J. Woo:** None. **C. Lee:** None. **H. Im:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.10/HHH21

Topic: G.08. Drugs of Abuse and Addiction

Title: Diversity in midbrain dopaminergic circuitry in response to drugs of abuse

Authors: *C. NGUYEN[1,2], S. TOLU[2], S. MONDOLONI[2], R. DURAND-DE CUTTOLI[2], F. MARTI[2], P. FAURE[2]
[1]Inst. de biologie Paris Seine, Univ. Paris Sorbonne, Paris, France; [2]Neurosci. Paris Seine, CNRS UMR8246, INSERM U1130, Paris, France

Abstract: Dopaminergic (DA) neurons of the ventral tegmental area (VTA) are at the core of the reward circuit. They play a crucial role in motivation, motor control, learning processes and reinforcement. Drugs of abuse target this system triggering maladaptive neurological modifications that may lead to addiction. Acute administration of nicotine, the main psychoactive compound of tobacco, increases DA cell firing and DA release in the VTA and in target structures such as nucleus accumbens (NAc), thereby initiating reinforcement and drug addiction.Contrasting with this classical view that suggests an activation of VTA DA cells, we recently identified a subpopulation of DA neurons, located in the medial part of the VTA, that are inhibited by nicotine. Yet, the mechanism by which nicotine inhibits DA neurons, and the consequences of such inhibition at the circuit and behavioral levels are unknown.
We first showed that despite different basic mechanisms of action at molecular and cellular levels, both nicotine and ethanol inhibit a subpopulation of VTA DA cells. The DA neurons that were activated by nicotine were also activated by ethanol and similarly most of DA neurons that were inhibited by nicotine were also inhibited by ethanol. Furthermore, using in vivo juxtacellular electrophysiological recordings combined with retrograde tracers (retrobeads), we also investigated the projection profiles of DA cells depending on their responses to nicotine.Thus, we demonstrated that NAc-projecting neurons are exclusively activated by nicotine, while inhibited neurons are mostly amygdala-projecting neurons.
Taken together, these results highlight the diversity of DA circuits in response to drugs of abuse and suggest a mechanism where opposing effects on two distinct neuronal populations triggers the nicotine effects.

Disclosures: C. Nguyen: None. **S. Tolu:** None. **S. Mondoloni:** None. **R. Durand-De Cuttoli:** None. **F. Marti:** None. **P. Faure:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.11/HHH22

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R01-DA021274
NIH Grant R15-DA040130

Title: Insulin normalizes the decrements in dopamine transmission observed in diabetic rats

Authors: *B. CRUZ[1], L. M. CARCOBA[1], R. J. FLORES[1], E. J. ESPINOZA[1], A. NAZARIAN[2], L. E. O'DELL[1]
[1]Psychology, Univ. of Texas at El Paso, El Paso, TX; [2]Western Univ. of Hlth. Sci., Pomona, CA

Abstract: Introduction: Pre-clinical studies in our laboratory have shown that the rewarding effects of nicotine are enhanced in diabetic rats. The neurochemical mechanisms that modulate nicotine reward involve dopamine release in the nucleus accumbens (NAc), a terminal region of the mesolimbic reward pathway. Given that the etiology of diabetes reflects a lack of insulin signaling, an important question is whether the neurochemical effects of nicotine are altered in diabetic rats in an insulin-dependent manner. To address this issue, the present study examined whether insulin supplementation normalizes nicotine-induced increases in the NAc of diabetic and healthy control rats. **Methods:** Rats first received vehicle or streptozotocin (STZ; 45 mg/kg), a drug that is toxic to pancreatic insulin-producing cells and increases plasma glucose levels. STZ-treated rats were then implanted with an insulin pellet or received a sham surgery. Two-weeks later, microdialysis probes were implanted in the NAc to measure dopamine release during baseline and following systemic administration of escalating doses of nicotine (0.3, 0.6, & 0.9 mg/kg). **Results:** Healthy control rats displayed an increase in NAc dopamine release following nicotine administration, and this effect was blunted in diabetic rats. Interestingly, the suppression of dopamine release observed in STZ-treated rats was normalized to control levels following insulin supplementation. **Conclusion:** These data suggest that insulin systems play an essential role in modulating the strong rewarding effects of nicotine observed in diabetic rats.

Disclosures: B. Cruz: None. **L.M. Carcoba:** None. **R.J. Flores:** None. **E.J. Espinoza:** None. **A. Nazarian:** None. **L.E. O'Dell:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.12/HHH23

Topic: G.08. Drugs of Abuse and Addiction

Title: Enhanced GABA release by projections from the nucleus accumbens to the ventral pallidum reduces nicotine self-administration in rats

Authors: A. L. SMITH, 37614[1], R. T. CHAPMAN[4], C. A. BRADLEY[2], H. W. SHELTON[3], *M. I. PALMATIER[2]
[1]Psychology, [3]Dept. of Biomed. Sci., [2]East Tennessee State Univ., Johnson City, TN; [4]Biomed. Sci., East Tennessee State Univerisy, Johnson City, TN

Abstract: Medium Spiny GABA neurons (MSN) project from the Nucleus Accumbens (NAc) to the Ventral Pallidum (VP) and play critical role in incentive motivation and reward. Rewards and reward-associated cues are thought to dynamically alter this pathway by suppressing GABA release to the VP. However, the role of the NAc to VP projection has never been investigated in nicotine self-administration. We hypothesized that increasing GABAergic signaling from the NAc to the VP would decrease the reinforcing effects of nicotine. To selectively increase GABA release in this pathway, a retrograde AAV was injected into the anterior (1.5 AP, ±1.8 ML, -7.9 DV)) and posterior (-0.5 AP, ±2.6 ML, -8.2 DV) VP to express CRE recombinase (AAV pmSyn1-EBFP-Cre, ADDGENE) of male CD rats (Charles River). After two weeks, a CRE dependent excitatory DREADD (pAAv-hSyn-DIO-hM3D(Gq)-mCherry, ADDGENE, n=14) or a control vector (pAAV-hSyn-DIO-mCherry, ADDGENE, n=5) was injected into the NAc (1.6 AP, ±1.3 ML, -7.3 DV) and rats were instrumented for intravenous nicotine self-administration. After recovery, the rats injected with the DREADD self-administered nicotine (30 ug/kg/infusion, DREADD+NIC, n=7) or saline (DREADD+SAL, n=7). The rats injected with the control AAV also self-administered nicotine (30 ug/kg/infusion). After responding for nicotine stabilized, clozapine was administered IP at doses below the threshold for activation of dopamine D2 receptors (0.03-0.75 mg/kg) during behavioral testing. Following testing, brains were collected and prepared for immunohistochemistry (IHC). Clozapine selectively reduced responding in rats injected with the DREADD, responding was not changed in rats injected with the control vector. Significant reductions in responding for nicotine and saline infusions were observed, but clozapine did not reduce responding on the inactive levers. This pattern suggests that the MSN projections from the NAc to the VP generally reduced reinforcement, although not specifically nicotine reinforcement. IHC confirmed DREADD expression in the NAc core and shell. Additional studies using microdialysis are being conducted to confirm that clozapine increases extracellular GABA in this model.

Disclosures: A.L. Smith: None. **R.T. Chapman:** None. **C.A. Bradley:** None. **H.W. Shelton:** None. **M.I. Palmatier:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

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Program #/Poster #: 421.13/HHH24

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R01-DA009411-19
NIH Grant T32-GM07517

Title: Acute nicotine exposure alters ventral tegmental area inhibitory transmission and promotes diazepam consumption

Authors: *R. WITTENBERG, A. OSTROUMOV, B. A. KIMMEY, M. B. TAORMINA, W. M. HOLDEN, J. A. DANI
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Nicotine is the main addictive component of tobacco and e-cigarettes and its abuse continues to pose a serious public health problem. Not only is smoking the leading cause of preventable death worldwide, but exposure to nicotine also represents a prominent risk factor for further drug use. Recently, we found that an acute nicotine injection increased subsequent ethanol self-administration through a change in ethanol-induced GABA signaling in the ventral tegmental area (VTA). In this study, we show that this alteration in inhibitory signaling arises from a downregulation in the function of the potassium-chloride cotransporter, KCC2, in VTA GABA neurons. Impaired KCC2 function results in intracellular chloride accumulation and depolarizes the $GABA_A$ receptor reversal potential (E_{GABA}) in GABA neurons of nicotine-treated rats. Depolarized E_{GABA} switches diazepam-induced inhibition of VTA GABA neurons to paradoxical excitation. Increased activity of VTA GABA neurons leads to increased GABA release onto VTA DA neurons and blunts DA neuron firing. At the behavioral level, acute nicotine injection increased diazepam intake without altering saccharin consumption. Together these results demonstrate that nicotine may be a risk factor for subsequent diazepam abuse mediated by downregulation of KCC2 function in VTA GABA neurons. KCC2-targeted therapies, therefore, may present a novel treatment mechanism for disrupting nicotine-drug interactions which facilitate drug abuse.

Disclosures: R. Wittenberg: None. **A. Ostroumov:** None. **B.A. Kimmey:** None. **M.B. Taormina:** None. **W.M. Holden:** None. **J.A. Dani:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.14/HHH25

Topic: G.08. Drugs of Abuse and Addiction

Support: DA038843-01-A1

Title: Extracellular dopamine release induced by co-presentation of nicotine and flavor conditioned reinforcers: Effect of nicotine dose

Authors: C. S. BAILEY[1], *G. A. DEEHAN, JR[2], C. A. BRADLEY[3], A. SMITH[4], M. I. PALMATIER[3]
[1]Psychology, East Tennessee State Univ., Watauga, TN; [2]Psychology, [3]East Tennessee State Univ., Johnson City, TN; [4]Psychology, East Tennessee State Univ., Elizabethton, TN

Abstract: Nicotine is a primary reinforcer that increases operant responding and increases dopamine release in the nucleus accumbens (NAc), an important terminal region in the mesocorticolimbic incentive motivation system. However, nicotine also serves as a reinforcement enhancer meaning that it increases responding for other reinforcers, including conditioned reinforcers (CRs). We have shown that tobacco flavor additives (e.g. licorice) increase nicotine self-administration in rats after they are established as CRs. However, no previous studies have investigated whether enhanced responding is associated with changes in extracellular dopamine in the NAc. To investigate this hypothesis, male Sprague-Dawley rats were randomly assigned to one of two groups: CR (n=12) or Neutral (n=11). For the CR group, 1% licorice root extract (LRE, v/v) was established as a CR via pairing with 20% sucrose. The Neutral group was exposed to 1% licorice root extract (unsweetened) but received 20% sucrose with grape kool-aid to equate conditioning. Rats in both the groups were then instrumented for nicotine self-administration. During self-administration contacts at a sipper tube were recorded and meeting the schedule of reinforcement on the active sipper resulted in oral delivery 1% unsweetened LRE solution and a iv delivery of 0.2 ml/kg/infusion of 20 or 40 ug/kg nicotine. Rats were tested under escalating fixed ratio (FR) schedules that increased from FR2 to FR10 across sessions. Subsequently all rats were tested under a progressive ratio (PR) schedule, in which each reinforcer earned incremented the schedule requirement for the next reinforcer according to an exponential formula. After behavioral manipulations, cannulas were implanted into the nucleus accumbens shell (AcbSh; AP+1.7, ML+2.4, DV-5.4). After recovery from surgery, microdialysis samples were collected according to the following schedule: washout (90 m), baseline (60 m), oral LRE (60 m), IV NIC + oral LRE (140 m). High pressure liquid chromatography of DA samples during this interval revealed an increase in extracellular DA for the CR rats during the LRE interval; no such increase was observed in the Neutral rats. Further

elevation in extracellular DA was detected during co-presentation of NIC and LRE for CR rats, but not for Neutral rats. The enhancement of extracellular DA release by flavor CRs suggest that they play a critical role in the maintaining tobacco use and may promote the transition from use to dependence.

Disclosures: C.S. Bailey: None. **G.A. Deehan:** None. **C.A. Bradley:** None. **A. Smith:** None. **M.I. Palmatier:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.15/HHH26

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R00 DA036569 and -S1 (CDG)

Title: Nuclear factor kappa B signaling in the nucleus accumbens core mediates cue-induced nicotine seeking and modulates glutamate transporter 1 expression

Authors: *M. D. NAMBA, C. D. GIPSON
Dept. of Psychology, Arizona State Univ., Tempe, AZ

Abstract: Withdrawal from chronic nicotine self-administration is associated with enduring alterations in glutamatergic plasticity within the nucleus accumbens core (NAcore), including basal potentiation of dendritic spines on medium spiny neurons and dysregulation of glial glutamate transport. The nuclear factor kappa B (NF-κB) pathway, which is activated by IκB kinase (IKK) and mediates drug-induced neuroinflammation, is a key regulator of synaptic plasticity and may be a critical regulator of cue-induced neurobehavioral plasticity. Notably little is known about NF- κB's role in cue-induced nicotine seeking. Here, we assessed whether 1) NF-κB mediates cue-induced nicotine reinstatement and 2) if NF-κB signaling underlies the attenuating effects of the antioxidant and glutamatergic agent *N*-acetylcysteine (NAC) on cued nicotine seeking. Rats underwent nicotine self-administration (0.02 mg/kg/infusion) for a minimum of 10 days prior to 14 days of extinction training. On day 10 of extinction, rats received intra-NAcore microinjections of a herpes virus expressing constitutively active IKK (IKKca), a dominant negative mutant of IKK (IKKdn), or eGFP, as well as NAC (100 mg/kg/i.p.) or saline injections between days 10-14 of extinction. Following extinction, rats underwent cue-induced reinstatement (2hr) and were immediately sacrificed for NAcore tissue collection. IKKdn blocked cue-induced nicotine reinstatement and IKKca impaired NAC from attenuating reinstatement. Interestingly, IKKdn alone did not restore GLT-1 expression, while IKKca blocked NAC-mediated restoration of glutamate transporter 1 (GLT-1). These results indicate that NF-κB is regulates cued nicotine seeking behavior and is a key mechanism

underlying the therapeutic efficacy of NAC and its ability to restore GLT-1. These results also highlight a novel target for the development of new pharmacotherapeutics that have anti-inflammatory activity and suppress nicotine relapse.

Disclosures: M.D. Namba: None. **C.D. Gipson:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

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Program #/Poster #: 421.16/HHH27

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA039658

Title: Extracellular vesicle integration in the rodent brain

Authors: *A. F. AHMED, V. LALLAI, C. D. FOWLER
Univ. of California Irvine, Irvine, CA

Abstract: Extracellular RNA-containing vesicles have recently been implicated in human disease. Our prior studies have found the presence of extracellular vesicles in high density within the cerebrospinal fluid (CSF) of the brain, potentially indicating that extracellular factors play a role in regulating neuronal signaling. The medial habenula, which contains cholinergic cells, is involved in mediating the aversive properties of nicotine and borders the dorsal third ventricle. Therefore, we hypothesized that CSF-derived extracellular vesicles from nicotine self-administering rats would integrate into habenular cholinergic cells *in vivo*. To visualize the integration of extracellular vesicles into neurons, CSF was extracted from a donor subject via the cisterna magna, and vesicles were isolated with ExoQuick-TC and labeled with ExoGreen. Thereafter, labeled vesicles were stereotaxically injected into the ventricles of rats and mice. Brain tissue was processed and examined with confocal microscopy. We document the presence of labelled vesicles in various regions of the brain, including the cholinergic region of the medial habenula. Together, these studies suggest that extracellular vesicles integrate into the neuronal parenchyma and may thereby alter neural function through the release of signaling factors.

Disclosures: A.F. Ahmed: None. **V. Lallai:** None. **C.D. Fowler:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.17/HHH28

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH DA039658 to CDF

Title: Extracellular vesicle release from the choroid plexus and small rna content modulated by nicotine

Authors: *V. LALLAI, A. F. AHMED, C. D. FOWLER
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Abstract: Cerebrospinal fluid (CSF), produced by the choroid plexus in the brain, contains extracellular vesicles carrying signaling molecules, including various RNA species. In previous studies, we have demonstrated functionally active cholinergic signaling mechanisms in the choroid plexus, in which nicotine differentially alters the expression of microRNAs both in the choroid plexus tissue and in the CSF. In this study, we extend these findings by examining whether pharmacological antagonism of cholinergic receptors prevents the release of miRNAs both *in vivo* and *in vitro*. For *in vivo* studies, subjects were administered the general nicotinic acetylcholine receptor antagonist, mecamylamine, prior to intravenous nicotine self-administration, and CSF was collected thereafter. To further validate these results, *in vitro* studies were conducted with primary cells of the choroid plexus from rats, and cell culture serum was collected following drug exposure. The expression of miRNAs in the CSF and serum were assessed with Taqman microRNA assays with qRT-PCR.
Our findings demonstrate the specific activity of nicotine on cholinergic receptor function in the choroid plexus and further implicate nicotine's actions in mediating extracellular vesicle signaling in the brain.

Disclosures: V. Lallai: None. **A.F. Ahmed:** None. **C.D. Fowler:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.18/HHH29

Topic: G.08. Drugs of Abuse and Addiction

Title: Participation of metabotropic and endocytic mechanisms in nicotine-induced upregulation of α7 nicotinic acetylcholine receptors (nAChRs) in xenopus oocytes

Authors: *J. PANCHAL[1], M. ISLAM[3], K. DEBOEUF[4], J. B. ANDERSON[5], I. MCFATRIDGE[2], J. FARLEY[6]
[1]Neurosci., [2]Indiana Univ., Bloomington, IN; [3]Neurosci., [4]Psychology and Neurosci., [5]Psychological and Brain Sci., [6]Indiana Univ. Bloomington, Bloomington, IN

Abstract: The α7 nAChR is widely expressed throughout peripheral and central nervous systems and is implicated in many neuropathologies/syndromes (e.g., Alzheimer's Disease, inflammation, stroke, cancer, schizophrenia) and nicotine addiction. Functional/numerical upregulation of α7 Rs by many compounds has been reported and several different mechanisms have been suggested to participate. Prolonged nicotine exposure may upregulate α7 and contribute to nicotine addiction. We've previously shown ~ 2-fold functional and numerical upregulation of α7 Rs in oocytes following 12 hr of 100 μM nicotine and extensive washout. Nic-upregulation was dependent upon intracellular Ca2+ (being blocked by BAPTA-AM), but independent of Ca2+- influx from extracellular media. We further identified several Ca2+-signaling pathways involved in nic-upregulation. Because α7 R (like another Cys-loop LGIC, Gly R1) contains a canonical G-protein binding cluster (GPBC) in the M3-M4 intracellular loop, whose mutation abolishes GPCR-Ca2+-signaling of α7 Rs (King & Kabbani, 2015), we tested the involvement of this cluster in nic-upregulation. Mutation of GPBC (RMKR to AAAA), preventing interaction of endogenous Gαq/ Gβγ with GPBC and disrupting Gαq-PLC-IP3-Ca2+ release, abolished nic-upregulation of α7 Rs. Furthermore, when Gαq was exogenously co-expressed with wt α7 Rs, we saw ~2X upregulation similar to nicotine treatment. We also found that a calcineurin inhibitor cyclosporine A (CsA) produced 2X-upregulation of wt α7 Rs and occluded nic-upregulation. Endocytic inhibitors (Dynasore, PitStop 2) produced ~2X upregulation of both wt and mutant α7 Rs and occluded nic-upregulation. Brefeldin A (BFA, inhibitor of protein transport from ER to Golgi) failed to affect nic-upregulation. Interestingly, prolonged exposure to the membrane-permeable competitive antagonist methyllycaconitine (MLA) upregulated α7 Rs in a calcium-independent manner, and inhibition of MLA-induced upregulation by BFA supported a chaperone-role for MLA. Our results suggest that nicotine and MLA upregulate α7 Rs by different mechanisms. Nic-upregulation of α7 arises from sustained GPCR-Ca2+-signaling by α7 Rs, inhibiting basal/constitutive endocytosis of α7 Rs and not via stimulation of ER-Golgi trafficking (unlike MLA). Although GPCR-Ca2+ signaling is blocked in mutant α7 Rs channels, the downstream effects of calcineurin- and endocytic-inhibition are still produced pharmacologically in these mutant channels, reproducing the 2X nic-upregulation.

Disclosures: J. Panchal: None. **M. Islam:** None. **K. DeBoeuf:** None. **J.B. Anderson:** None. **I. McFatridge:** None. **J. Farley:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.19/HHH30

Topic: G.08. Drugs of Abuse and Addiction

Support: DePauw University

Title: Nicotine-induced behavior and gene expression in larval zebrafish

Authors: *H. SCHNEIDER, A. PEARSON, D. HARRIS
Dept. of Biol., Depauw Univ., Greencastle, IN

Abstract: Dependence on nicotine-containing products is a major cause of preventable diseases in the U.S. and worldwide. Current smoking cessation therapies with varenicline and bupropion have still a high rate of relapse. Behavioral tests that measure the response to nicotine following pretreatment with a chemical are used in animal models and could lead to the discovery and development more effective pharmacotherapeutics. Larval zebrafish represent an excellent model for the screening of chemicals that reduce the neurobehavioral response to nicotine. In addition, comparing gene expression dynamics before and after pretreatments and before and after nicotine application could provide new insight into the function of potential pharmacotherapeutics. When exposed to nicotine in the water, larval zebrafish respond with an increased movement activity. Previously, we have identified chemicals in the serotonin system that reduce the acute response to nicotine. Pretreatment with serotonin receptor agonists for serotonin receptors of type 2 (htr2cl1) and agonists and antagonists for the serotonin receptor type 2a (htr2aa) reduce the acute nicotine response significantly. Reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) methods were used to explore gene expression profiles associated with the acute nicotine response including the htr2c and htr2a mediated change of nicotine responses. First experiments confirm a significant increase in cfos gene expression after 30 min of nicotine exposure. In contrast, htr2cl1 and htr2aa receptor gene expression appears to remain unchanged. The cfos gene activity returns to background levels within 2 hours after the start of nicotine exposure. No significant change in htr2cl1 and htr2aa receptor gene expression seems to occur within 24 hours of nicotine exposure. Higher levels of htr2cl1 gene activity have been measured compared to the htr2aa gene. The results indicate that nicotine itself does not appear to change gene expression levels of serotonin receptors htr2cl1 and htr2aa within 24 hours of nicotine exposure. In the future, experiments will explore gene expression profiles following pretreatment with serotonin receptor agonists and antagonists, that reduce the acute nicotine response.

Disclosures: H. Schneider: None. **A. Pearson:** None. **D. Harris:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.20/HHH31

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R01-DA021274
NIH Grant R24-DA029989
NIH Grant R25-DA033613

Title: Overexpression of a stress peptide in the nucleus accumbens selectively increases nicotine self-administration in female versus male rats

Authors: *K. P. URIBE, R. J. FLORES, V. CORRERA, B. CRUZ, L. E. O'DELL
Psychology, The Univ. of Texas at El Paso, El Paso, TX

Abstract: Previous studies have shown that nicotine withdrawal produces a larger increase in the expression of the stress-associated gene, corticotrophin-releasing factor (CRF) in the nucleus accumbens (NAc) of female versus male rats. The present study expands this work by assessing sex differences in the rewarding effects of nicotine following over-expression of CRF in the NAc of female and male rats. A group of ovariectomized (OVX) females were also included to examine whether our behavioral effects are ovarian-hormone mediated. Extended access (23-hour) to nicotine self-administration was compared in female, OVX female, and male rats that received intra-NAc administration of an adeno-associated vector (AAV-CRF) that over-expressed CRF in this region. Control rats received intra-NAc administration of a control vector (AAV-GFP). The rats were then surgically prepared with a jugular catheter for subsequent self-administration of escalating doses of nicotine (0.015, 0.03, 0.06 mg/kg). After the final session, the rats were sacrificed and the NAc was dissected to verify over-expression of CRF using qRT-PCR methods. Our results revealed that over-expression of CRF in the NAc produced a selective increase in nicotine self-administration in intact female versus male rats. The effects of CRF-overexpression in the NAc appeared to be ovarian-hormone dependent, as OVX females did not display an increase in nicotine intake relative to intact females. Over-expression of CRF in the NAc produced a similar increase in CRF in the NAc of female and male rats relative to their respective GFP controls. However, over-expression of CRF produced a larger increase in the expression of CRFr1 and CRFr2 in females versus male and OVX female rats. In conclusion, these findings suggest that stress systems in the NAc play a key role in modulating sex differences in the reinforcing effects of nicotine

Disclosures: K.P. Uribe: None. **R.J. Flores:** None. **V. Correra:** None. **B. Cruz:** None. **L.E. O'Dell:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.21/HHH32

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA032681
SPARC Grant, Univ. of South Carolina
SmartState Program at Univ. of South Carolina

Title: Disruption of hippocampal NRG3-ERBB4 signaling ablates nicotine withdrawal-induced anxiety-like behaviors

Authors: *M. FISHER[1], E. ANDERSON[1], J. L. TWISS[2], J. R. TURNER[1]
[1]Col. of Pharm., [2]Biol. Sci., Univ. of South Carolina, Columbia, SC

Abstract: Addiction to nicotine and the ability to quit smoking are influenced by genetic factors. Identifying altered gene networks and how those networks contribute to nicotine dependence and withdrawal will only accelerate therapeutic development of new smoking cessation aids. Previous work from our lab and that of our collaborators demonstrate that SNPs across the Neuregulin 3 (NRG3) gene and its cognate receptor, ERBB4, are associated with smoking cessation outcomes. These genes are critical in the maintenance of synaptic connectivity and plasticity during development and adulthood. Our studies show that during nicotine withdrawal both mRNA and protein levels of NRG3 and ErbB4 are upregulated in the hippocampus, a region that mediates affective withdrawal symptoms in mice, suggesting that aberrant hippocampal NRG3 signaling may underlie select withdrawal behaviors. Current studies aim to interrogate the functionality of this signaling pathway in the hippocampus during nicotine and withdrawal, and examine how nicotine-induced changes in NRG3 and ErbB4 may contribute to withdrawal-induced phenotypes in genetically modified mice. To do this we disrupted the pathway via conditional hippocampal ErbB4 deletion in ErbB4-floxed mice and evaluated nicotine withdrawal anxiety-like behaviors using the novelty-induced hypophagia test and the open field exploration task. We found that ErbB4 deletion on GABAergic interneurons results in the ablation of withdrawal-induced anxiety-like behavior, demonstrating a potential role of this signaling pathway in nicotine dependence. Ongoing studies are utilizing single molecule fluorescence in situ hybridization coupled with immunofluorescence to identify the underlying cell type and circuit-specific modulation of NRG3 signaling by nicotine within the hippocampus of these animals. Collectively, these data will provide insight into NRG3-ErbB4 dependent mechanisms underlying nicotine withdrawal-induced phenotypes.

Disclosures: M. Fisher: None. **E. Anderson:** None. **J.L. Twiss:** None. **J.R. Turner:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.22/HHH33

Topic: G.08. Drugs of Abuse and Addiction

Support: TRDRP award 26IP-0043

Title: Sex-dependent behavioral effects of adolescent exposure to a cannabinoid agonist and nicotine in adult mice

Authors: *A. EUGENE[1], A. N. PUSHKIN[1], A. TORRES MENDOZA[1], C. D. FOWLER[2]
[1]Univ. of California, Irvine, Irvine, CA; [2]Neurobio. and Behavior, Univ. of California Irvine, Irvine, CA

Abstract: Recent studies suggest that adolescent exposure to substances of abuse, including nicotine or cannabis, may alter neuromaturation and neurocognitive function during adulthood. Nicotine acts in the brain via neuronal nicotinic acetylcholine receptors, whereas the main psychoactive component in cannabis, THC, acts on cannabinoid receptors. Here, we examined the effects of adolescent exposure to nicotine, a cannabinoid receptor agonist (WIN55-212,2), or co-exposure to both nicotine and WIN55-212,2 on operant learning, generalized locomotion, and anxiety- and reward-related behaviors in male and female mice. Our findings reveal differential effects of adolescent drug exposure on later anxiety and reward-related behaviors between males and females. However, significant differences were not found in operant learning or generalized locomotor/exploratory behaviors. Together, these data provide evidence that adolescent co-exposure to nicotine and cannabinoids can alter later affective and reward-related behaviors in a sex-dependent manner during adulthood.

Disclosures: A. Eugene: None. **A.N. Pushkin:** None. **A. Torres Mendoza:** None. **C.D. Fowler:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.23/HHH34

Topic: G.08. Drugs of Abuse and Addiction

Support: Department of Psychiatry and Behavioral Neurosciences (SAP)
Fund for Anesthesiology Research (FG)

Title: Traumatic stress enhances nicotine-induced locomotor sensitization in a rat model of PTSD

Authors: *F. GHODDOUSSI[1], T. GORE[2], M. J. LISIESKI[2], K. KARAVIDHA[2], D. K. KNOX[3], S. A. PERRINE[2]
[1]Anesthesiol. & Magnetic Resonance Core, Wayne State University, Sch. of Med., Detroit, MI; [2]Psychiatry & Behavioral Neurosciences, Wayne State University-School of Med., Detroit, MI; [3]Psychological and Brain Sci., Univ. of Delaware, Newark, DE

Abstract: **Introduction**: Post-traumatic stress disorder (PTSD) is a debilitating psychiatric disorder characterized by anxiety, avoidant behavior, and hyper-reactivity to trauma-associated stimuli that affects nearly 25 million people in United States. Smoking is the leading preventable cause of death and nicotine dependence has a life time prevalence of 22.5% of the US population. Nicotine dependence is more prevalent in mental health disorders, including PTSD, than in non-clinical populations. Furthermore, clinical studies have linked enhanced symptoms in PTSD with smoking rates in PTSD. Trauma exposure, even in the absence of clinically diagnosed PTSD, is also associated with increased smoking rates and with physiological/behavioral associated outcomes. These reports suggest a link between trauma exposure and nicotine dependence, and raise the strong possibility that there are neurobiological mechanisms via which traumatic stress exposure leads to susceptibility to nicotine dependence. However, the mechanisms behind this comorbidity are not currently understood. In spite of this, there have been few studies that have studied this relationship. Proton-Magnetic Resonance Spectroscopy (1H-MRS) is a non-invasive clinically-relevant technique that we have used to show decreased glutamate signaling in the prefrontal cortex in a rat model of PTSD. Here we expand of these findings to study the mechanisms underlying the relationship between trauma and nicotine exposure. **Methods:** Single Prolonged Stress (SPS), a multimodal series of stressors, was used in rats to model PTSD. Sprague-Dawley rats were divided in 4 groups CTRL-Saline(n=6), CTRL-Nicotine(n=8) , SPS-Saline (n=8) , SPS-Nicotine (n=8). Effects of nicotine on behavior was measured by nicotine-induced locomotor sensitization. Brains were removed following behavioral testing and High Resolution Magic Angle Spinning (HR-MAS) 1H-MRS was performed on the cortical and striatal tissues. **Results:** Locomotion activity increased over the 5-day period in rats who received both SPS and nicotine and was significantly different from rats receiving SPS and saline ($p=0.0125$). Analysis of cortical and striatal, MRS measurable glutamate and GABA levels is currently underway. **Discussion:** Our preliminary results indicate that psychological trauma enhances nicotine induced locomotion sensitization. We hypothesize that decreased cortical glutamate is associated with enhanced nicotine sensitization in trauma-exposed animals.

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Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 421.24/HHH35

Topic: G.08. Drugs of Abuse and Addiction

Support: 5T32DA017637-14

Title: The effects of nicotine and withdrawal on sleep latency in C57BL/6J mice

Authors: *H. L. MATHEWS[1,2], V. IYER[3], J. A. STITZEL[4]
[1]Psychology & Neuroscience, IBG, Univ. of Colorado Boulder Dept. of Psychology and Neurosci., Boulder, CO; [2]Inst. for Behavioral Genet., Boulder, CO; [3]Univ. of Colorado - Boulder, Boulder, CO; [4]Inst. Behav Genet., Univ. of Colorado Boulder, Boulder, CO

Abstract: Increased sleep latency, a primary symptom of insomnia, is observed and reported during periods of nicotine consumption and withdrawal. Yet, there is no established animal model to study the effects of chronic nicotine use and withdrawal on this insomnia related phenotype. The present study used mice to characterize inactive phase sleep latency during periods of nicotine administration and withdrawal and to measure inactive phase corticosterone following a series of sleep latency trials. In experiment one (n=6), mice were implanted with EEG/EMG recording devices. During the pre-nicotine baseline condition, mice had free access to food and .2% saccharin water. To generate nicotine dependence, 200µg/ml of nicotine was added to the .2% saccharin water. After 14 days of nicotine exposure, withdrawal was induced by excluding the nicotine from the water. Multiple sleep latency testing (MSLT) consisted of three consecutive trials spaced one hour apart occurring under three conditions: baseline (BL), nicotine exposure day 8 (N8), and withdrawal day 1 (WD1). MSLT procedures were initiated at ZT 2 (n=3) or ZT 2.5 (n=3). During each trial, mice were kept awake for 5 minutes by means of gentle handling. Sleep latency was measured as the time (in seconds) it took to enter the first bout of sleep. Sleep/wake quantity and architecture was assessed for each 55min period following each trial and averaged. Relative to BL, sleep latency was not altered on N8. No difference was observed for measures of sleep/wake quantity and architecture between BL and N8. Compared to BL, sleep latency proceeding trial one was significantly increased on WD1, suggesting difficulty falling asleep. A significant decrease in total sleep, explained by a reduction in NREM sleep, was also observed during the WD1 condition. In the second experiment (n=4), an ELISA was used to assess plasma corticosterone immediately following a third sleep latency trial during BL, N8, and WD1 conditions. No main effect of condition was observed, however multiple comparisons tests revealed a significant decrease in corticosterone during WD1, relative to BL. In summary, these data suggest an impairment in the HPA axis during withdrawal which might contribute to the increased latency to sleep during WD1.

Disclosures: H.L. Mathews: None. **V. Iyer:** None. **J.A. Stitzel:** None.

Poster

421. Neural Mechanisms of Nicotine Addiction II

Location: SDCC Halls B-H

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Program #/Poster #: 421.25/HHH36

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

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an ATS Foundation K to R Transition Award to L.C.A.

Title: Effects of chronic inhalation of electronic cigarette vapor containing nicotine on neurotransmitters in the frontal cortex and striatum of C57BL/6 mice

Authors: *F. ALASMARI[1,2], L. E. CROTTY ALEXANDER[2], A. M. HAMMAD[1], C. M. BOJANOWSKI[2], A. MOSHENSKY[2], Y. SARI[1]
[1]Pharmacol. and Exptl. Therapeut., Univ. of Toledo, Toledo, OH; [2]Dept. of Medicine, Div. of Pulmonary and Critical Care, Univ. of California at San Diego (UCSD), La Jolla, CA

Abstract: Electronic (E)-cigarettes are the latest form of nicotine delivery device, and are highly popular in the general population. It is currently unknown whether vaping E-cigarettes leads to nicotine addiction. Alterations in the levels of the neurotransmitters in the mesocorticolimbic areas have been reported to mediate the initiation and development of nicotine dependence. Therefore, to determine whether E-cigarettes activate the same addiction pathways as conventional cigarettes, we investigated the effects of daily inhalation of E-cigarette vapor-containing nicotine for six-months on the concentrations of these neurotransmitters in the frontal cortex (FC) and striatum (STR) of male C57BL/6 mice. We found that chronic inhalation of E-cigarette vapor-containing nicotine reduced dopamine concentration only in the STR. There were no changes in serotonin concentrations in the FC or STR. Chronic E-cigarette exposure also increased glutamate concentration in the FC alone, while glutamine concentrations were increased in both the FC and STR. We found that E-cigarette exposure also decreased GABA concentration only in the FC. These data suggest that chronic E-cigarette use alters homeostasis of several neurotransmitters in the mesocorticolimbic areas, which may result in the development of nicotine dependence in E-cigarettes users.

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Poster

422. Invertebrate Learning and Memory

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 422.01/HHH37

Topic: H.01. Animal Cognition and Behavior

Support: NIMH Grant 1R15MH107892-01

Title: Transcriptional changes extend far beyond forgetting of a long-term sensitization memory in *Aplysia* californica

Authors: *R. CALIN-JAGEMAN[1], U. PATEL[2], L. PEREZ[2], S. FARRELL[2], D. STECK[2], I. CALIN-JAGEMAN[3]
[1]Psychology, [2]Neurosci. Program, [3]Biol., Dominican Univ., River Forest, IL

Abstract: Most long-term memories are forgotten, becoming progressively less likely to be recalled. Still, some memory fragments may persist beyond forgetting, as savings memory (easier relearning) can persist long after recall has become impossible. What happens to a memory trace during forgetting that makes it inaccessible for recall and yet still effective to spark easier re-learning? We are addressing this question by tracking the transcriptional changes that accompany learning and then forgetting of a long-term sensitization memory in the tail-elicited siphon withdrawal reflex of *Aplysia californica*. First, we tracked savings memory. We found that even though recall of sensitization fades completely within 1 week of training, savings memory is still robustly expressed at 1 week and 2 weeks post training. Next, we used microarray to identify transcriptional changes that persist beyond the decay of recall; we identified 11 transcripts strongly regulated 1 week after training that validated with qPCR in an independent set of samples. Finally, we tracked these time-course of regulation of these 11 'savings-related' transcripts at 1 hour, 1 day, 5 days, and 2 weeks after the induction of sensitization. Some are regulated rapidly after induction and then persist for up to 1 week; others show a delayed but persistent regulation. Remarkably, 2 transcripts still show strong regulation of expression 2 weeks after training. Our results provide the first evidence of transcriptional fragments of a learning experience that persist far beyond the decay of recall.

Disclosures: R. Calin-Jageman: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIMH Grant 1R15MH107892-01. **U. Patel:** None. **L. Perez:** None. **S. Farrell:** None. **D. Steck:** None. **I. Calin-Jageman:** B. Contracted Research/Research Grant (principal

investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIMH Grant 1R15MH107892-01.

Poster

422. Invertebrate Learning and Memory

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 422.02/HHH38

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant SC3GM111188

Title: Differential contribution of protein kinase G to short-term and long-term behavioral plasticity induced by sensitization training in *Aplysia*

Authors: *R. MOZZACHIODI, R. CHATTERJI, E. SALAS, M. WAINWRIGHT
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Abstract: In the mollusk *Aplysia*, exposure to aversive stimuli causes a learned enhancement of defensive responses, known as sensitization, and a concurrent suppression of feeding. Sensitization and feeding suppression are co-expressed across different training protocols and share common temporal domains (Acheampong et al. 2012; Shields-Johnson et al. 2013). In particular, a training protocol, consisting of a single trial of electric shocks to the body wall, induces short-term (15 min) concomitant sensitization of the tail-induced siphon withdrawal reflex (TSWR) and feeding suppression. An extended training protocol, consisting of four trials, spaced 30 min apart, induces more prolonged behavioral changes, with both sensitization and feeding suppression lasting at least 24 h. The strong relation in the temporal dynamics of sensitization and feeding suppression suggests that these two processes may share common signaling pathways.
In this study, we investigated the role of the protein kinase G (PKG) in the concomitant learning-dependent behavioral modifications in defensive and non-defensive responses induced by short-term and long-term sensitization training. The selective PKG inhibitor KT5823 (henceforth KT) was used (Michel et al. 2011). Animals were injected *in vivo* with 1 mL per 100 g of body weight of either KT (6.5 µM in 1% DMSO) or vehicle solution (1% DMSO; Michel et al. 2011). In both the single-trial and the four-trial protocols, four groups of animals were used: trained/injected with KT, untrained/injected with KT, trained/injected with vehicle, and untrained/injected with vehicle. TSWR duration and feeding were measured prior to (pre-test) and at given post-test time points (15 min or 24 h) after training.
We first examined the role of PKG in the short-term behavioral changes induced by the single-trial protocol. KT did not prevent the expression of either sensitization or feeding suppression 15 min after training, suggesting that PKG does not contribute to the short-term behavioral plasticity

induced by sensitization training. Next, we investigated whether PKG plays a role in the long-term behavioral changes induced by the four-trial protocol. KT prevented the expression of both sensitization and feeding suppression 24 h after training, suggesting that PKG contributes to the long-term behavioral modifications induced by sensitization training.
Overall, these findings suggest that a PKG signaling pathway is necessary for the induction of both long-term sensitization and feeding suppression, but not for the induction of short-term behavioral changes.

Disclosures: R. Chatterji: None. **E. Salas:** None. **M. Wainwright:** None.

Poster

422. Invertebrate Learning and Memory

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 422.03/HHH39

Topic: H.01. Animal Cognition and Behavior

Support: Program of Russian Academy of Sciences
RFRF Grant 17-00-00216

Title: Regulation of context memory maintenance during reconsolidation with serotonin precursor and epigenetic regulators

Authors: *P. M. BALABAN, A. VINARSKAYA, A. ZUZINA
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Abstract: In the present study we tested possible ways of modification of the long-term context memory using the reconsolidation process as a tool for memory labilization. Recently it was shown that the reinforcing neurotransmitter serotonin is necessary for successful repeated reconsolidation of context memory in terrestrial snails, and we used injection of the serotonin precursor 5-HTP for reinstatement of memory after its impairment during reconsolidation with a protein synthesis blocker or with a specific inhibitor (ZIP) of atypical proteinkinase PkMzeta shown to be involved in memory maintenance. It was observed that application of 5-HTP known to increase the serotonin concentration or just reminding did not restore the context memory, while combination of 5-HTP+reminder effectively reinstated the impaired context memory. Application of an epigenetic regulator, histone deacetylase inhibitor sodium butyrate (NaB), was not as effective as serotonin, and the reminder+NaB reinstated memory only partially after impairment with ZIP, while additional session of training under NaB effectively reinstated the impaired memory. Application of an inhibitor of DNA methyltransferases RG108 erased context memory, and the memory was not reinstated by reminder or additional training session, while NaB+reminder and NaB+training partially reinstated the memory. Obtained data confirmed the assumption that serotonin/reinforcing transmitter is necessary for successful reconsolidation,

demonstrated possible ways of memory regulation during the reconsolidation process by the epigenetic factors.

Disclosures: P.M. Balaban: None. **A. Vinarskaya:** None. **A. Zuzina:** None.

Poster

422. Invertebrate Learning and Memory

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 422.04/HHH40

Topic: H.01. Animal Cognition and Behavior

Support: BBSRC Grant BB/P00766X/1

Title: Learning and circuit-dependent replacement of memory

Authors: M. CROSSLEY, G. KEMENES, *P. R. BENJAMIN, I. KEMENES
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Abstract: Temporary lapses in memory recall during consolidation of long-term memory have been observed in many species, including humans, raising questions about their function in the phenomenon of memory lability. These lapses could represent windows of opportunity for one memory to be replaced by another. To examine this hypothesis, we induced a memory and then examined the effects of the formation of a second memory using the model molluscan system *Lymnaea*. When the second training was applied at a lapse-point, the original memory was replaced by the new memory. However, when applied at a non-lapse point, both memories were retained in the case of appetitive followed by aversive training whereas only the first memory was consolidated if two different appetitive paradigms were used. We further demonstrate that aversive and appetitive conditioning induce training-specific cellular changes. We provide evidence that the fate of the second memory is dependent on whether the two memories are encoded within the same or different memory circuits.

Disclosures: M. Crossley: None. **G. Kemenes:** None. **P.R. Benjamin:** None. **I. Kemenes:** None.

Poster

422. Invertebrate Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Support: Israel Science Foundation Grant 1379/12
NIH Grant NS047073
NSF Grant DMS-1010434
NSF Grant IIS-1065489

Title: Successful and unsuccessful attempts to swallow regulate *aplysia* feeding responses in a reduced *in vitro* preparation

Authors: *A. J. SUSSWEIN[1], J. M. MCMANUS[2], H. J. CHIEL[3]
[1]Bar-Ilan Univ., Ramat-Gan, Israel; [2]Biol., Case Western Reserve Univ., Cleveland, OH; [3]Case Western Res. Univ., Cleveland, OH

Abstract: Cyclic behaviors in behaving animals are modulated by feedback from effectors and by learning and memory. We examined modulation of cyclic *Aplysia* feeding activity in a reduced preparation in which the buccal and cerebral ganglia are attached to the buccal muscles effecting feeding. We challenged this system with either edible or inedible foods, loads causing learned changes in behavior. We examined how the preparation responds to the loads, and whether such loads produce learned changes in response. Repetitive feeding responses were induced by bathing the cerebral ganglion in the cholinomimetic carbachol (CCh). Comparing the effects of CCh in preparations with and without the buccal musculature showed that presence of the muscles speeded up the maximal response rate, and also produced a mixture of feeding responses more like that in intact animals, presumably via feedback from the effectors. Repetition of the CCh exposure after 1 h produced a decrease in responses. Challenging the system with edible strips of food produced an increase in the maximal response rate during the first CCh exposure, and on repetition of the procedure after 1 h memory was expressed as a lack of decrease in the maximal response rate that would have been caused by the CCh alone. Challenging the system with inedible food had no effect on any of the parameters of feeding responses during the initial exposure to CCh+inedible food, or during the repetition of this procedure 1 h later. All preparations were exposed a third time to CCh alone, 1 h after the 2nd test of CCh effects. During this test, there were no differences in response parameters between preparations previously challenged with CCh alone or with CCh+edible food, indicating that memory was dependent on a repetition of the presence of the edible food. However, there was a decrease in response to CCh alone in preparations previously challenged with CCh+inedible food. This decrease suggests that memory arises in part via a post-synaptic decrease in response to CCh in cerebral ganglion neurons excited by cholinergic taste afferents. Since no changes in response were present during training, these findings also indicate that changes in response during learning with inedible food do not appear to determine memory formation.

Disclosures: J.M. McManus: None. **H.J. Chiel:** None.

Poster

422. Invertebrate Learning and Memory

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 422.06/HHH42

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant NS088835

Title: Sleep deprivation inhibits early steps in the induction of associative memory

Authors: *L. C. LYONS[1], H. C. KRISHNAN[1], E. J. NOAKES[1], L. M. CANCIO[2]
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Abstract: Acute sleep deprivation and chronic sleep loss constitute a rising public health problem in teenagers and adults. Sleep deprivation causes significant impairments in memory and performance resulting in substantial risks to health and society through increased occupational errors and traffic accidents. However, our understanding of the molecular impacts of sleep deprivation remains limited. The marine mollusk *Aplysia californica* is an excellent model for studies of sleep and represents a well-established model for non-associative and associative memory. Recently, we demonstrated that acute and chronic sleep deprivation inhibits the induction of short and long-term operant memory using the learning that food is inedible paradigm (LFI). In the current research, we tested the hypothesis that sleep deprivation prior to training disrupts early steps in memory formation. We investigated the effects of sleep deprivation on the immediate induction of MAPK activity following LFI training, a necessary shared step in the formation of both short and long-term memory. We found that sleep deprivation suppressed the activation of training-induced MAPK activity. Moreover, the induction of MAPK activity following training was still significantly inhibited even when training occurred 24 hours after the period of sleep deprivation, suggesting a molecular mechanism through which sleep deprivation persistently blocks memory formation. To identify the underlying molecular changes caused by sleep deprivation, we examined the effects of sleep deprivation on protein synthesis and protein degradation. Using the SUnSET assay, we found that acute sleep deprivation significantly decreases protein synthesis. Interestingly, we also found that sleep deprivation increases protein ubiquitination specifically in the buccal ganglia, which has previously been shown to be the site of training-induced MAPK signaling necessary for LFI memory. Furthermore, we found that pharmacological inhibition of proteasome activity reverses the effects of sleep deprivation on short and long-term memory, although inhibition of proteasome activity in the absence of sleep deprivation blocks long-term memory. These results suggest that the effects of sleep deprivation on protein synthesis and protein degradation impair the induction of associative memory and that these molecular impacts of acute sleep deprivation persist for at least 24 hours.

Disclosures: L.C. Lyons: None. **H.C. Krishnan:** None. **E.J. Noakes:** None. **L.M. Cancio:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant NS019895

Title: Ribosomal S6 kinase (RSK) is essential for long-term synaptic facilitation (LTF) at sensorimotor (SN-MN) synapses of *Aplysia*

Authors: *R.-Y. LIU, Y. ZHANG, L. J. CLEARY, J. H. BYRNE
Dept. of Neurobio. and Anat.,, McGovern Med. Sch. of UTHSC At Houston, Houston, TX

Abstract: RSKs are a family of serine-threonine kinases that are activated by mitogen-activated protein kinase (MAPK) and consequently phosphorylate and activate cAMP response element-binding protein (CREB). Although the RSK2 gene has been identified in *Aplysia* (NCBI Reference Sequence: XP_005094788.1), and a recent study indicates activation of RSK via the ERK (a MAP kinase isoform) pathway (Philips et al. 2013), the complete signaling pathways and their roles in long-term synaptic facilitation (LTF) have not been fully examined. In the present study, we used immunocytochemistry to measure the phosphorylation of RSK induced by serotonin (5-HT), a neurotransmitter mediating sensitization in *Aplysia*, and found that a standard LTF-inducing treatment with 5-HT leads to a complex dynamics of RSK phosphorylation observed over 24 h in isolated sensory neurons (SNs) (See companion poster). This effect depended upon the MEK-ERK pathway as evidenced by the attenuated phosphorylation produced by the MEK inhibitor U0126 (U0). Moreover, inhibiting either the activation of ERK by U0 or the downstream effects of RSK by its specific inhibitor BI-D 1870 (BID), attenuated 5-HT-induced phosphorylation of CREB1. These results suggest an important role for ERK/RSK in the activation of CREB1. To elucidate the roles of RSK in synaptic plasticity, we employed siRNA knockdown to inhibit the expression of RSK protein, and then measured CREB1 phosphorylation and LTF. 5-HT-induced increases in phosphorylated CREB1 were reduced by RSK siRNA injection. Moreover, either injection of RSK siRNA into the SN from SN-MN co-culture or inhibition of RSK using BID resulted in a significant reduction of LTF. These data suggest that RSK is required for the induction of LTF. The reduction in RSK and LTF after RSK siRNA injection suggests that knock down of RSK by siRNA may constitute a single-cell analogue of Coffin-Lowry syndrome (CLS), a cognitive disorder that is associated with deficits in learning and memory caused by mutations in the RSK2 gene. Interestingly, the impairment in both phosphorylation of CREB1 and LTF can be restored by a computationally designed

Enhanced Protocol, which was previously demonstrated to augment normal LTF and long-term memory (LTM) (Zhang et al. 2012). These results reveal that *Aplysia* RSK is a critical upstream kinase for CREB1 and required for LTF. Moreover, this study indicates that a computationally-designed Enhanced protocol not only improves normal LTF and LTM, but also rescues deficits in LTF in a single-cell CLS analogue.

Disclosures: R. Liu: None. **Y. Zhang:** None. **L.J. Cleary:** None. **J.H. Byrne:** None.

Poster

422. Invertebrate Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01 NS019895

Title: Biphasic regulation of MAPK pathways contributes to dynamics of CREB1 and CREB2 after serotonin treatment

Authors: *Y. ZHANG, R.-Y. LIU, L. J. CLEARY, J. H. BYRNE
McGovern Med. Sch. of UTHSC at Houston, Houston, TX

Abstract: Serotonin (5-HT)-induced long-term synaptic facilitation (LTF) of the *Aplysia* sensorimotor synapse depends on the activation of transcription activators such as CREB1 and inactivation of transcription repressors such as CREB2 (Bartsch et al., 1995,1998). Levels of CREB1 and CREB2 mRNA and proteins and phosphorylation of CREB1 exhibit complex dynamics after induction of LTF (Liu et al., 2008, 2011). Processes that underlie these dynamics are not well characterized, however. To address this issue, we examined three elements of the MAP kinase cascade that are critical for activation of CREB1 and CREB2: extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (p38 MAPK) and p90 ribosomal S6 kinase (RSK). LTF requires activation of the ERK MAPK isoform that relieves CREB2-mediated repression of CREB1 activity and activates CREB1 via RSK (See companion poster). Activation of the p38 MAPK isoform inhibits LTF by activating CREB2 and suppressing ERK (Guan et al., 2002, 2003; Zhang et al., 2017a). We treated isolated *Aplysia* sensory neurons with five 5-min pulses of 5-HT and examined the phosphorylation of ERK, p38 MAPK and RSK at the same time points used previously to investigate CREB1 and CREB2 dynamics (Liu et al., 2008, 2011). Compared to vehicle control measurements at the same time points, 5-HT treatment induced a rapid increase in phosphorylated p38 MAPK at 1 h after treatment (+19.6 ± 7.2%, n = 9), which returned to basal levels at 2 h (-9.8 ± 8.3%, n = 9), but subsequently increased a second time at 5 h (+16.2 ± 5.0%, n = 10), eventually returning to basal level at 24 h (+3.6 ± 6.4%, n = 9) (Zhang et al. 2017b). Complex dynamics of RSK phosphorylation were also observed over 24

h. Phosphorylated RSK (pRSK) was elevated by +21.1 ± 8.9% (n = 10) at 1 h after treatment, followed by a decrease to -4.6 ± 9.8% (n = 8) at 2 h. A second increase in pRSK levels was evident at 5 h (+19.1± 8.1%, n = 11), and pRSK levels returned to basal levels at 24 h (+7.1% ± 7.6%, n = 8). In contrast, 5-HT induced a significant increase of phosphorylated ERK (pERK) immediately after treatment (+57.3% ± 9.9%, n =10), which gradually declined thereafter but remained significantly elevated at 2 h after treatment (+31.9% ± 11.7%, n = 11). Levels further decreased to +15.0% ± 7.3% at 5 h (n = 6), and returned to the basal level at 24 h (-3.6% ± 9.8%, n = 9). These data suggest that complex regulation of MAPK pathways contribute to the dynamics of CREB1 and CREB2, which in turn are important to understanding the efficacy of training protocols to form LTF. Further investigation is needed to investigate the interaction between these kinases and quantify the relationship between MAPK signaling and CREB1/CREB2 dynamics.

Disclosures: Y. Zhang: None. **R. Liu:** None. **L.J. Cleary:** None. **J.H. Byrne:** None.

Poster

422. Invertebrate Learning and Memory

Location: SDCC Halls B-H

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Program #/Poster #: 422.09/HHH45

Topic: H.01. Animal Cognition and Behavior

Support: NSERC Discovery Grant #122216-2013
NSERC CGS Master's Scholarship

Title: Differentiating the molecular mechanisms underlying short-term behavioural plasticity

Authors: *A. J. YU[1], E. L. ARDIEL[1], C. H. RANKIN[2]
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Abstract: Non-associative learning, including sensitization and habituation, is ubiquitously observed in a wide range of species following presentation of stimuli of certain patterns and properties, that allows organisms to modulate their responses to subsequent stimuli. The experience-dependent behavioural plasticity can be expressed in forms of increase or decrease in response, corresponding to sensitization and habituation. Several other processes can also mediate short-term behavioural plasticity in conjunction or interaction with non-associative learning. For example, in dishabituation, a novel strong stimulus can produce recovery (dishabituation) or facilitation (dishabituation and sensitization) of the habituated response. The serotonergic signalling pathway underlying sensitization in Aplysia has been delineated for decades; however, other mechanisms mediating these behavioural plasticities remain poorly understood at cellular and molecular levels.

In this study, we analyzed different forms of short-term behavioural plasticity in the tap withdrawal circuit of *Caenorhabditis elegans*. The tap withdrawal circuit is well annotated at the synaptic level, and genes expressed in these neurons are known. Through a mutant screen and our optogenetic behavioural assays, we found that several peptidergic signalling pathways play a substantial role in mediating various forms of short-term behavioural plasticity. We identified the cellular and molecular components in at least one peptidergic signalling pathway underlying tap-induced sensitization that is not involved in dishabituation. Similarly, another peptidergic signalling pathway has been found to mediate only dishabituation and not sensitization. Additionally, we showed that different training paradigms can differentially produce sensitization or dishabituation. Thus, two forms of behavioural plasticity that both increase responsiveness in the same response can be differentiated at cellular and molecular levels. Several theoretical implications can be extrapolated from our findings. The findings both support, and provide a new interpretation of the classic Dual-Process Theory of non-associative learning. The results also highlight the importance of extra-synaptic signals in plasticity in the nervous system.

Disclosures: A.J. Yu: None. **E.L. Ardiel:** None. **C.H. Rankin:** None.

Poster

422. Invertebrate Learning and Memory

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Program #/Poster #: 422.10/HHH46

Topic: H.01. Animal Cognition and Behavior

Support: CIHR MOP 130287

Title: High-throughput phenomic characterization of ASD-associated genes reveals a functional gene network underlying hypersensitivity and impaired habituation

Authors: *C. H. RANKIN[1], T. MCDIARMID[3], M. BELMADANI[4], K. HAAS[5], P. PAVLIDIS[2]
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Abstract: A primary challenge facing Autism Spectrum Disorder (ASD) genetics is the large and growing number of genes and gene variants of unknown functional significance. Here, we used *Caenorhabditis elegans* to systematically functionally characterize ASD-associated genes *in vivo*. Using our custom machine vision system we characterized 26 quantitative phenotypes spanning morphology, locomotion, mechanosensory responding, and habituation learning in 87 strains of *C. elegans* each carrying a mutation in an ortholog of an ASD-associated gene. This research has generated a large number of novel genotype to phenotype relationships that range from severe developmental delays and uncoordinated movement to subtle deficits in sensory and

learning behaviours. Clustering based on multi-parametric phenomic profiles revealed a set of 12 genes that all result in a strikingly similar profile characterized by hypersensitivity and impaired habituation learning. Current epistasis experiments are aimed at determining whether the phenomic similarity among members of this cluster results from previously undiscovered functional interactions. One of the genes in this cluster is the sole *C. elegans* ortholog of neuroligins, *nlg-1*. Transgenic pan-neuronal expression of human *NLGN3* in *nlg-1* mutant *C. elegans* rescued their sensory and learning impairments; confirming functional conservation. The wealth of *in vivo* phenomic functional data generated in this work will inform more targeted studies in vertebrates and offers novel positive and negative pathway components as therapeutic targets for ameliorating the effects of ASD.

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Poster

422. Invertebrate Learning and Memory

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Program #/Poster #: 422.11/HHH47

Topic: H.01. Animal Cognition and Behavior

Title: Investigation into the role of motor response performance in associative learning in *C. elegans*

Authors: M. R. PRIBIC[1], J. T. BYLIN[1], L. C. LAWRENCE[2], M. N. BISHOP[2], *J. K. ROSE[3]
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Abstract: *Caenorhabditis elegans* can detect and respond to stimuli in their environment and form associations (e.g., worms will migrate towards a chemical or temperature previously associated with the presence of food). To test the potential role activation of motor circuitry has on learning, an associative conditioning protocol previously described by our lab was employed. Briefly, this assay utilizes the combined activation of two distinct sensory circuits that typically trigger opposing locomotor responses when presented alone (reversal for one stimulus and forward motion for the other). In wild type *C. elegans*, after five stimulus pairings delivered at a 60 s interstimulus interval, an altered motor response is seen at test when a single stimulus is presented; worms pause (a cessation in motion) more frequently at test stimulus onset compared to naive worms ($p<0.01$). This change in response lasts for at least 10 minutes post-conditioning. To determine if motor response performance is necessary for this alteration in response following stimulus pairing, transgenic strains expressing channelrhodopsin in cholinergic motor neurons ($_p$unc-17::ChR2), GABAergic motor neurons ($_p$unc-47::ChR2) or muscle ($_p$myo-3::ChR2) underwent the same stimulus-pairing procedure. In these worms, exposure to blue light alone was sufficient to generate the previously reported channelrhodopsin-mediated motor

responses; $_{p}$unc-17::ChR2 and $_{p}$myo-3::ChR2-expressing worms showed shortening of the body due to light-activation of the cholinergic motor neurons or body wall muscle resulting in contraction. $_{p}$unc-47::ChR2 worms displayed an overall cessation of movement. Initial conditioning trials with blue light to simultaneously activate channelrhodopsin during stimulus pairings indicate $_{p}$unc-47::ChR2 do not show the same altered motor response as seen with wild-type. Results from this study will elucidate if motor circuit activation influences signalling at upstream neural circuits involved in learning.

Disclosures: M.R. Pribic: None. **J.T. Bylin:** None. **L.C. Lawrence:** None. **M.N. Bishop:** None. **J.K. Rose:** None.

Poster

422. Invertebrate Learning and Memory

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 422.12/HHH48

Topic: H.01. Animal Cognition and Behavior

Support: The Salt Science Foundation

Title: Exploring the role of the chloride channel CLH-1 in salt chemotaxis learning in the nematode *C. elegans*

Authors: *C. PARK[1], Y. SAKURAI[3], Y. IINO[2], H. KUNITOMO[4]
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Abstract: *Caenorhabditis elegans* is an excellent model organism to study the neural substrate of behavior thanks to its well documented and simple nervous system as well as amenability to genetic manipulations. Also, *C. elegans* shows a behavioral plasticity in NaCl chemotaxis, which is regulated by food availability. Animals are attracted to the salt concentration at which they have been previously fed, while avoid the salt concentrations they have been starved at (hereinafter referred to as salt chemotaxis learning). The insulin/PI3-kinase signaling in the ASER salt-sensing neuron regulates starvation-induced behavioral change (Tomioka et al. 2006, Ohno et al. 2014). However, PI3-kinase pathway mutants showed no discernable defect in food-associated salt-concentration preference, suggesting that the mechanism of food-associated salt chemotaxis learning is at least partially different from that of starvation-induced salt chemotaxis learning (Kunitomo et al. 2013). Recent studies show that anion channels play important roles in synaptic transmission by regulating the excitability of neurons. ClC chloride channels are conserved across various animal species, and they are expressed in most tissues, including neurons. ClC channels have been the subject of many studies, due to their malfunction being

implicated in various diseases in humans. Roles of ClC channels in learning and memory, however, are not fully understood. Here, we show that ClC chloride channel CLH-1 acts in food-associated salt chemotaxis learning of *C. elegnas*. We screened for mutants that show defects in food-associated learning but not in starvation-induced learning, and obtained two mutants JN572 and JN577. Both mutants had a missense mutation in *clh-1* that encodes a ClC chloride channel. CLH-1 is known to function as a bicarbonate transporter in pH homeostasis of glial cell in *C. elegans* (Grant et al. 2015). Interestingly, *clh-1* null mutants did not show behavioral defects in food-associated learning. This result suggests that the missense mutations are neomorphic. Tissue-specific rescue experiments indicated that CLH-1 acts in ASER.

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Poster

422. Invertebrate Learning and Memory

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Program #/Poster #: 422.13/HHH49

Topic: H.01. Animal Cognition and Behavior

Support: PICT 2013-2474
CONICET postdoctoral grant

Title: Honey bees form separated memory traces after experiences containing appetitive and aversive consequences

Authors: *M. KLAPPENBACH[1], F. F. LOCATELLI[2]
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Abstract: In natural situations animals must be able to acquire information from experiences that combine appetitive and aversive consequences. How learning derived from those experiences is stored and retrieved to produce adaptive behavior is a major question in neurobiology. Honey bees have been lengthily used to study olfactory learning and memory processes triggered upon olfactory conditioning of the proboscis extension response. In its appetitive version, the odor is presented with sugar solution and the bees learn to extend the proboscis in response to the odor. In the aversive version, the odor is presented with a salty or a bitter solution, and the behavior observed is the withdrawal of the proboscis in response to the odor. In the present study we performed a series of experiments aimed at evaluating in which extend these two forms of learning interact when appetitive and aversive stimuli take part in the same training protocol. First of all, we established a testing protocol focused on measuring the occurrence and duration of the aversive olfactory-gustatory memory. During the training session, bees receive paired presentations of the odor and a quinine bitter solution. Aversive learning

becomes evident just during the testing phase, in which the same odor is paired with sugar solution and the bees show deficit in appetitive conditioning. We found that multiple trials of aversive conditioning endow a memory that lasts 24 but not 48 h. Afterwards, we demonstrate that honey bees are able to recognize the aversive conditioned odor when during the test it is presented as component of a binary mixture. Then, we performed a differential conditioning protocol, alternating presentations of two odors that were paired with the appetitive and the aversive reinforcements respectively. When the memory test was performed separately for each odor, we observed that bees responded accordingly to the learned appetitive or aversive valences. Finally, the bees that had undergone differential conditioning were challenged in a test session in which the appetitive and the aversive odors were combined in a mixture. We found that confronted to this decision bees behaved either according to the appetitive or the aversive odor depending on their satiation level. Starved bees responded as detecting the appetitive trained odor, while fed bees responded as if they detect the aversive odor. In summary, bees are able to learn and remember the association between different odors and different reinforcements acquired during the same experience, and use this information according to the motivational state during retrieval.

Disclosures: M. Klappenbach: None. **F.F. Locatelli:** None.

Poster

422. Invertebrate Learning and Memory

Location: SDCC Halls B-H

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Program #/Poster #: 422.14/HHH50

Topic: H.01. Animal Cognition and Behavior

Support: NSF INSPIRE 1548121
NSF IOS EDGE 1645219
NSG IOS 1457162
NSF IOS 1557923

Title: Sequencing of entire brains at the single-cell resolution: Principles of molecular classification (neurosystematics) and periodic system of neurons

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Abstract: There is more than one way to develop cell and tissue complexity, and animals frequently use different molecular toolkits to achieve similar functional outcomes (=convergent evolution). However, the genomic bases of convergent evolution are largely unknown. Here, we used a combination of microfluidic and massive-parallel single-cell RNA-seq (scRNA-seq) to

capture more than one million cells in representatives of 13 animal phyla. These species also represent major transitions in the formation of mesoderm, muscle and neural organizations as well as origins of complex organ systems (from comb jellies to cephalopods). First, we revisited the animal phylogeny and start to develop a metric to quantitate each cell's type transcriptional relationships as well as criteria for cell-/neuronal homologies. Second, we showed that for many species tested, virtually all neurons are unique in their RNA modifications, non-coding RNAs, and secretory molecules, providing the foundation for the natural neuronal classification or NEUROSYSTEMATICS. The discovered molecular complexity of the numerically "simpler" neural systems and the emerging single-cell data suggest the hypothesis: neurons are different not only because they have different functions, but also because neurons and circuits have different genealogies, and perhaps independent origins at the broadest evolutionary scale from ctenophores and cnidarians to mollusks and chordates. Origins of neurons (and synapses) from different types of ancestral secretory cells might have occurred at least three times during Metazoa evolution. It appeared that mesoderm and muscles, including striated muscles, evolved independently. Also, our reconstructions suggest 9-12 independent events of nervous system centralizations from a common bilaterian/cnidarian ancestor with diffuse-like neural systems. Thus, using examples of convergent evolution, we set up a foundation toward natural genealogical classification of neuronal classes across phyla. Perhaps, there is a type of the Periodic System of Neurons, which might be an analog of the Periodic System of chemical elements, with a predictive power for cellular phenotypes.

Disclosures: L.L. Moroz: None. **A. Kohn:** None.

Poster

422. Invertebrate Learning and Memory

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 422.15/HHH51

Topic: H.01. Animal Cognition and Behavior

Title: Reprogramming of feeding behavior by diet

Authors: *T. PARDO, C. MAY, A. VAZIRI, M. DUS
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Abstract: While we understand how changes in the environment such as temperature and light direct animal behavior by acting acutely on neural circuits, we know less about how the environment can lead to persistent changes in brain and behavior. Tackling this question has been challenging because it requires having a circuit-based understanding of the behavior and a mechanistic way to study how neural connections are changed by the environment. The reshaping of circuits that regulate food intake by a hyper-caloric diet in *Drosophila melanogaster* provides an attractive model for studying this question because the circuits are mapped, the

behavior is easily quantifiable, and the environmental variables are simple to measure. We found that animals fed a Western style high-calorie diet show profound deregulation of feeding states: they incorrectly process the nutritional value of food, eat more, and become obese. We will present data showing how these behaviors are mediated by the metabolic-transcriptional reprogramming of distinct feeding circuits by diet and how their effect is persistent even after animals are returned to the control diet.

Disclosures: C. May: None. **A. Vaziri:** None. **M. Dus:** None.

Poster

422. Invertebrate Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Support: NIH 5DP1MH110234-02 Pioneer Grant
HFSP Long term Postdoctoral Fellowship

Title: Mother knows the threat: Neural mechanisms of wasp-induced oviposition depression in *Drosophila*

Authors: *M. K. SADANANDAPPA, G. BOSCO
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Abstract: In addition to mounting an immunological response after endoparasitoid wasp infection, fruit flies have developed various behavioral adaptations to protect themselves as well as their progeny from the impact of infection. In presence of larval endoparasitoid wasp, *Drosophila* females either reduce their egg lay or prefers to lay their eggs in food containing toxic levels of alcohol. This preferential alteration in ethanol-seeking behavior self-medicates the larvae against wasp infection, thereby prevents wasp adults emerging from fly pupae. Despite its importance, the neuronal and molecular mechanisms that underlie wasp-induced physiological changes in female fruit flies remains to be elusive.
To address the above question, we have combined the behavior assays with the neurogenetics and immunohistochemistry approaches. When adult flies (5 females and 2 males, 12-24 replicates) are exposed to wasps for 24hrs, the flies decrease their mean egg lay compared to naïve flies. Interestingly, either in the absence of visual (nina B^1 mutants) or olfactory inputs ($Orco^1$ mutants), female flies fail to depress their eggs upon exposure to wasps. Strikingly, this observed oviposition depression is specific to larval endoparasitoid, but not to pupal endoparasitoid wasps. Together, these findings point that the adult fruit flies have developed a behavioral immunity specific to larval endoparasitoid based on the search images that distinguish between the larval endoparasitoid from pupal endoparasitoid.

Furthermore, as the neuropeptide F (NPF) and its receptor mutants (NPFR) display defects in wasp-induced oviposition response, we are currently dissecting the neural circuits that are upstream and downstream of the NPF neurons. Additionally, given that the decreased egg lay is associated with transient retention of matured eggs in wasp-exposed females, we are also investigating how do the neuronal inputs modulate the female germline physiology.

Disclosures: G. Bosco: None.

Poster

422. Invertebrate Learning and Memory

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 422.17/DP13/HHH53

Topic: H.01. Animal Cognition and Behavior

Support: German Research Council SFB 889/B4

Title: Associative olfactory learning in *Drosophila* induces synaptic de-correlation of axonal Kenyon cell boutons

Authors: *A. FIALA[1], B. GEURTEN[2], F. BILZ[1]
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Abstract: Plastic changes in synaptic transmission represent a neuronal substrate underlying learning and memory formation. Since sensory stimuli are typically encoded as sparsely distributed activity across assemblies of many neurons, it is challenging to determine which and how individual synaptic connections change to acquire a stimulus-specific memory. Here we used *in vivo* calcium imaging in *Drosophila* to monitor learning-induced synaptic plasticity. Fruit flies can learn to avoid an odor stimulus that is temporally paired with a punitive electric shock. We trained fruit flies positioned under a two-photon microscope using this classical aversive olfactory conditioning regime, and monitored odor-evoked calcium activity through a window cut in the head capsule. One odor (CS+) was presented in coincidence with a punitive electric shock. A second odor (CS-) was subsequently presented without punishment. Control animals received the same odorant stimulation, but without the electric shock. The MARCM technique was used to express the calcium sensor GCaMP in single γ-lobe Kenyon cells of the mushroom body, a brain region to which the acquisition of associative olfactory short-term memory could be localized. We measured odor-evoked activity in synaptic boutons along individual axons and across many neurons. Using a subsequent immunohistochemical staining we could assign axonal boutons to specific γ-lobe sub-compartments. A comparison of calcium activity before and after associative learning revealed that albeit associative learning induced bi-directional changes in synaptic bouton activity, their overall calcium activity across all boutons remained constant.

However, odor-evoked synaptic bouton activity within and across single Kenyon cells de-correlated as a result of associative learning, and specifically for the CS+. No de-correlation between boutons was observed for the calcium activity evoked by the CS- or the control odor. This reveals a novel principle of how associative memories can be differentially encoded across assemblies of neurons and axonal compartments.

Disclosures: A. Fiala: None. **B. Geurten:** None. **F. Bilz:** None.

Poster

422. Invertebrate Learning and Memory

Location: SDCC Halls B-H

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Program #/Poster #: 422.18/HHH54

Topic: H.01. Animal Cognition and Behavior

Title: Distinctive roles of pre- and post-synaptic DD2Rs in *Drosophila* larval olfactory learning

Authors: C. QI[1], *D. LEE[2]
[1]Biol., [2]Ohio Univ., Athens, OH

Abstract: Dopamine (DA) plays a critical role in associative learning by activating excitatory D1- or inhibitory D2-like receptors. In Drosophila larvae, postsynaptic D1 receptors (dDA1) are known to mediate olfactory learning. However, whether Drosophila D2 (DD2R) plays a crucial role in olfactory learning hasn't been fully investigated. DD2Rs are comprised of pre- and postsynaptic receptors, which are likely involved in larval olfactory associative learning. In olfactory learning, the presynaptic neurons are dopaminergic neurons (DAN), and the postsynaptic neurons are in mushroom body (MB), the key structure for fly learning. Therefore, if DD2Rs are expressed in MB neurons or DANs, it strongly indicates their important role in larval learning. Former studies showed that DANs in DL1 clusters innervating the vertical lobes of MB are necessary for aversive olfactory learning, while those in the pPAM clusters innervating the medial lobes of MB are important for appetitive learning. In this study, we aimed to examine whether DD2R are expressed in MB and different DAN clusters, and whether these DD2Rs are involved in *Drosophila* olfactory learnings. By using a GFP-tagged DD2R strain, expression patterns of DD2R were explored in both MB neurons and DANs. In pPAM cluster, DD2R exists in all four DANs. In DL1 cluster, DD2R is expressed in 5 out of 7 DANs. As distinct DANs innervate different compartments on MB lobes, this result indicated DD2Rs in distinct DANs may have unique roles in specific learning tasks. To investigate this, we drove expression of DD2R-RNAi under tissue-specific drivers. The olfactory learning assay showed both appetitive and aversive learning are completely impaired in larvae with DD2R-RNAi expression under 30Y-GAL4 or 201Y-GAL4 driver, showing DD2Rs in MB neurons are necessary for both kinds of larval learning tasks. As to DD2Rs in DANs, aversive learning is completely impaired in larvae with DD2R knockdown under a DA-specific driver TH-GAL4

(including DL1, not pPAM), while the appetitive learning is partially impaired. This is consistent with our results of DAN-to-MB GRASP using TH-GAL4. Intensive GRASP signals were observed in the vertical lobes showing functional synapses formed between DL1 and vertical lobes of MB. These results demonstrate DD2Rs in the DL1 have an important role in larval aversive learning. In contrast, knockdown of DD2R under a pPAM-specific driver (R58E02-GAL4) completely impaired appetitive learning, which indicates DD2Rs in pPAM clusters have an important role in appetitive learning. Our findings revealed DD2R in different brain structures have distinct functions in *Drosophila* larval olfactory learning.

Disclosures: C. Qi: None. **D. Lee:** None.

Poster

422. Invertebrate Learning and Memory

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Program #/Poster #: 422.19/HHH55

Topic: H.01. Animal Cognition and Behavior

Title: Discovery of long-lasting context-dependent olfactory memory in *Drosophila*

Authors: *B. ZHAO, X. ZHANG, Q. LI, Y. ZHONG
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Abstract: How much memory is recollected depends largely on the circumstance. People always retrieve memory better in the context where they have ever learnt, termed as 'Context Effect'. However, we still lack a biologically mechanistic understanding of how context facilitates memory retrieval. Here we find that a single aversive olfactory conditioning in *Drosophila*, which was believed produce memory lasting for only one day, can yield context-dependent memory lasting for more than two weeks. Such context-dependent memory is resistant to protein synthesis inhibitor and cold shock treatment. Moreover, we found that silencing neurons of mushroom body, a classical center for olfactory learning and memory, did not affect context-dependent memory retrieval. Instead, outputs of several sensory input brain regions were required. Correspondingly, we found that context-dependent memory can be retrieved only when all external conditions are consistent with learning situation, since any alteration of perceptible context condition, such as temperature and light, led to failure of this memory retrieval. Taken together, our data indicate that behaviorally observed memory may not be actually formed memory for such memory recall can be improved by providing learning context again.

Disclosures: X. Zhang: None. **Q. Li:** None. **Y. Zhong:** None.

Poster

422. Invertebrate Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Support: Lundbeckfonden grant no. DANDRITE-R248-2016-2518

Title: Fruit flies integrate reward history into foraging decisions

Authors: *S. E. SEIDENBECHER[1], J. SANDERS[2], D. KVITSIANI[3]
[1]Aarhus Universitet, Aarhus C, Denmark; [2]Sanworks LLC, Stony Brook, NY; [3]DANDRITE, Aarhus Univ., Aarhus C, Denmark

Abstract: Foraging, or searching for food, is a fundamental behavior exhibited by all moving animals throughout evolution. Every behavior, including foraging, is fundamentally steered by decision-making processes. One particularly interesting type of foraging decision is the decision to return to a spot in the environment that was previously experienced as profitable. We are interested in the basic principles underlying this type of decision. To break down the decision behavior and gain access to these foraging decision principles, we use mathematical models that objectively describe the behavior. These models can give us a set of parameters that can be related to single genes driving this behavior. If all moving animals make foraging decisions according to these principles then we can hypothesize that certain behaviors of foraging and their genetic basis are conserved among different species. We developed an experimental setup to study foraging decisions in male fruit flies using optogenetic rewards and investigated the animal's behavior for different reward probabilities. In particular, we are interested in behavioral changes to varying uncertainty in the environment. Our results so far suggest that, on a population level, the flies base their decisions to return to a rewarded spot more strongly on previous rewards, when the reward probability is low while they become less sensitive to the reward history for higher probabilities. In the long run, our aim is to find computational models for fly foraging decisions and to uncover the key molecular players, by relating single genes and behavior through these models.

Disclosures: S.E. Seidenbecher: None. **J. Sanders:** None. **D. Kvitsiani:** None.

Poster

422. Invertebrate Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Support: EPSRC Grant EP/P006094/1
ARC Grant 140100452
ARC Grant 150101172
Royal Society International Exchanges Grant

Title: A computational model for a spatial map in the fan-shaped body of *Drosophila* based on neuroanatomical evidence mined from the FlyCircuit database

Authors: *A. J. COPE[1], A. B. BARRON[2], J. A. R. MARSHALL[3]
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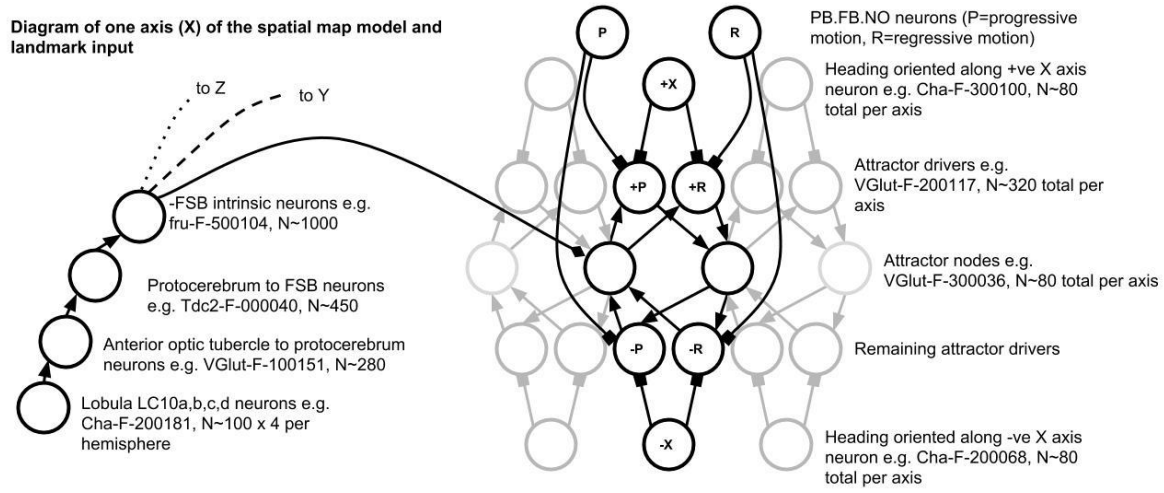
Abstract: In invertebrates the central complex (CX) is an ancient, highly preserved, structure implicated in a wide range of behaviours (e.g. mating, locomotion, pattern recognition). Intriguingly it is also unique in spanning the brain midline. Despite this its role in the brain is poorly understood. Here we present an investigation of the role of the CX using the rich data sets available for the Drosophila brain. We performed a systematic review of the FlyCircuit.tw database for the CX fan-shaped body (FSB) using the NAT R package. This review involved an initial search to return all neurons with arborisation in the FSB over a fixed threshold. These were categorised based on morphology and large sets of heavily stereotyped neurons were identified. This morphological categorisation was validated using searches constructed to extract each large set. We hypothesised that these sets formed the parts of one circuit. Connectivity was inferred from arborisation overlap and neuron count extrapolated from the number in each set, assuming even sampling in the database. Following this bottom-up review a top-down approach was used to identify potential roles of these neurons. This approach strongly identified a potential role for the FSB in part as a 3D Cartesian spatial map. A computational model of these circuits was constructed based on three orthogonal linear attractors (see Figure). Activity through the attractor links is gated by progressive and regressive motion signals via the noduli, and by the heading of the fly via neurons which map to four heading quadrants. Landmark information is associated onto this network through a pathway via the anterior optic tubercle, the superior protocerebrum, and a separate layer of the FSB. Associative connections to the nodes of the linear attractors are via a very large set of interneurons. Our computational model is capable of driving movement towards a remembered location in a virtual environment. A spatial map

indicates a role for the CX for representing invertebrates' place in the world, providing a site for context across disparate fixed and learned behaviours.



Disclosures: A.J. Cope: None. **A.B. Barron:** None. **J.A.R. Marshall:** None.

Poster

422. Invertebrate Learning and Memory

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Program #/Poster #: 422.22/HHH58

Topic: H.01. Animal Cognition and Behavior

Title: Biased randomness: A connectivity mechanism for associative brain centers

Authors: *S. J. CARON, E. AMEMATSRO, K. ELLIS
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Abstract: Uncovering fundamental mechanisms of neuronal connectivity that enable associative brain centers to learn efficiently is an important goal of neuroscience. In the *Drosophila melanogaster* mushroom body, the constituent Kenyon cells receive input from olfactory projection neurons. Each projection neuron connects to one of the fifty glomeruli in the antennal lobe, the primary olfactory processing center. We and others have shown that these connections are random in that there are no sets of glomeruli converging preferentially onto a given Kenyon cell. However, we found that the glomeruli are not represented with equal frequency among Kenyon cell inputs. Certain glomeruli form many more connections than expected under a uniform distribution, whereas other glomeruli form far fewer connections than expected. We are testing the idea that this non-uniform distribution, which we termed 'biased randomness', serves an important biological function, namely to predispose the learning ability of the mushroom

body towards certain ethologically pertinent stimuli. To test this idea, we built two mathematical models of the mushroom body: one model was built using the biased distribution of input that we measured experimentally, while the other model uses a uniform distribution of input. Both models generate very similar representations for most of the tested odors. However, we found that each model generates strikingly different representations for a few ethologically relevant odors. Odors activating overrepresented glomeruli activate many more Kenyon cells than odors activating underrepresented glomeruli do. Consequently, although both models show overall similar learning performance, they perform differently in tasks involving these ethologically relevant odors. We are proposing that overrepresentation serves a biological function, namely to enable odors that must be learned in many different contexts, pheromones for instance, to be represented by a large number of Kenyon cells. In contrast, underrepresentation might be used as a strategy to prevent the mushroom body from representing — and possibly learning — odors with strong innate valence. We are currently testing this idea further by measuring the biases in connectivity in the mushroom body of other *Drosophila* species that have evolved in different ecological niches and therefore have different olfactory preferences. Altogether, our work supports the idea that 'biased randomness' is a wiring mechanism that predisposes associative brain centers to learn efficiently.

Disclosures: E. Amematsro: None. **K. Ellis:** None.

Poster

423. Hippocampal Circuits and Cognition

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Program #/Poster #: 423.01/HHH59

Topic: H.01. Animal Cognition and Behavior

Support: NSERC DG8318

Title: Hippocampal damage causes retrograde memory loss and delayed cue-place memory acquisition in a two-platform water task in rats

Authors: *J. Q. LEE[1], R. J. MCDONALD[2], R. J. SUTHERLAND[3]
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Abstract: Hippocampal (HPC) damage is known to cause retrograde amnesia (RA) for a broad range of long-term memory (LTM) tasks in rodents, including those dependent on cue- and place-memory guided behaviour. By contrast, HPC damage does not cause anterograde amnesia (AA) for cue memory, but causes AA for some aspects of spatial memory. In the present experiments, we examined cue and place aspects of LTM in parallel using a two-platform water task in rats with HPC damage made before or after training. We have found that HPC damage

before training causes a delay in expression of cue-place behaviour, followed by task performance that is comparable to control animals, including spatial aspects of memory performance. By contrast, HPC damage after learning causes robust RA for cue-place memory. Our findings support a view whereby the hippocampus is essential for an unexpectedly wide range of memory types if it is intact during a learning episode. Based on alternation of cue- and place-preferred strategies in over-trained control animals, we further hypothesize that cue and place information develop competitive associative strengths within the HPC system when both aspects of LTM are conditionally relevant to task performance.

Disclosures: J.Q. Lee: None. **R.J. McDonald:** None. **R.J. Sutherland:** None.

Poster

423. Hippocampal Circuits and Cognition

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Topic: H.01. Animal Cognition and Behavior

Support: JSPS KAKENHI 16H06543
JSPS KAKENHI 16H02840
JSPS KAKENHI 16K13115

Title: Development of the reconfigurable maze - Various shapes of maze in a single environment

Authors: *S. HOSHINO, K. IDE, S. TAKAHASHI
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Abstract: Several shapes of maze have been used for elucidating the neuronal mechanism on cognitive functions such as spatial navigation, working memory and decision-making. However, conventional maze has only a few experimental variables because the position of parts of the maze such as paths, walls, gates and feeders cannot be easily changed after construction. A novel maze system which is able to easily change the shape in a single environment, enables us to investigate the neural mechanisms more multi-directionally. Here, we realized such a maze system for small animals, called reconfigurable maze, in which the parts of the maze can be systematically reconfigured. In addition, to change scheduling of sensors and/or actuators in the maze in association with examining experimental variables, we developed a controller on an Arduino microcontroller and a user interface using Matlab programming language. To evaluate efficacy of reconfigurable maze, we produced several shapes of maze such as figure-eight maze and cross-to-square maze and recorded activity of place cells from the hippocampus CA1 of rats during some behavioral tasks. It is possible that the gap between paths in the maze hinders the animal's behavior. However, the rats could run through the maze at a speed comparable to that of the conventional mazes. Moreover, the size and shape of the place field are similar to those

previously reported in the conventional maze. Previous studies reported that while the animal briefly paused, place cells are sequentially reactivated as if they represent recently traveled trajectory; when a rat travels across the place field of a place cell, the firing timing shifts earlier in phase relative to the theta rhythm of the local field potential; the place field can be remapped in response to the morphing of square or circular walls. We thus checked whether the replay, phase precession and remapping of the place field can be observed in the maze. The results suggest that our reconfigurable maze system has compatibility with conventional maze. Our reconfigurable maze together with single cell recordings produces a complementary effect among the current maze tests and might provide a novel insight into the neural underpinnings of spatial navigation.

Disclosures: S. Hoshino: None. **K. Ide:** None. **S. Takahashi:** None.

Poster

423. Hippocampal Circuits and Cognition

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Topic: H.01. Animal Cognition and Behavior

Support: McNair Scholarship
Summer Undergraduate Research Experience Fellowship, USD
College of Arts and Sciences, USD

Title: Effects of partial hippocampal lesions in rats on the traveling salesman problem

Authors: *E. A. PETTY, G. COLLINS, K. LONG-IYER, J. PAUL, R. BLASER, J. B. HALES
Psychological Sci., Univ. of San Diego, San Diego, CA

Abstract: The Traveling Salesman Problem (TSP) is a spatial navigational task that differs from many other behavioral techniques because it allows the observation of behavior in a more naturalistic setting. The goal of the task is not to verify if an animal can do a certain behavior, but to record how the animal behaves in natural foraging conditions. This task may involve a variety of cognitive functions, such as spatial processing, memory, attention, route planning, and decision making. Given the established role of the hippocampus in both spatial processing and spatial memory, we examined how hippocampal damage affects rats' performance in the TSP. The rats were pretrained on the TSP, which involved learning to retrieve bait from targets in a variety of spatial configurations. Matched for performance, rats were then divided into two groups, receiving either a partial hippocampal lesion or a control sham surgery. After recovering from surgery, the rats were tested on eight new configurations. A variety of behavioral measures were recorded, including distance traveled, number of revisits, span, and latency. The results showed that the sham group outperformed the lesion group on most of these measures, with the

lesion group demonstrating more pronounced impairment on the more complex configurations. Based on histological tissue analysis of each group, we determined that the hippocampus appears to be involved in finding efficient routes, particularly in complex versions of the TSP.

Disclosures: G. Collins: None. **K. Long-Iyer:** None. **J. Paul:** None. **R. Blaser:** None. **J.B. Hales:** None.

Poster

423. Hippocampal Circuits and Cognition

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Program #/Poster #: 423.04/III1

Topic: H.01. Animal Cognition and Behavior

Support: NSERC

Title: The effects of lesions to the head direction cell circuit on behavioural demonstrations of direction learning in rats

Authors: *D. M. SKINNER, M. A. WASEF, V. C. J. HARVEY, C. M. THORPE
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Abstract: To navigate successfully an animal must have knowledge of its location and directional heading. These two components of navigation are well represented in a spatial network in the mammalian brain that contains place cells, grid cells, and head direction (HD) cells. It has been suggested that the HD signal originates sub-cortically in the reciprocal connections between the dorsal tegmental nucleus (DTN) and the lateral mammillary nucleus (LMN). Lesions to the LMN or DTN have been shown to disrupt HD cell firing in downstream structures, such as the anterior dorsal nucleus of the thalamus (ADN) and the postsubiculum. Lesions to the DTN have also been shown to produce severe impairments in directional heading on a foraging task and in directional learning in a water maze. In the present study, rats with bilateral electrolytic lesions of the LMN were compared to sham controls on a battery of spatial tasks that involve directional heading. LMN-lesioned rats were impaired, relative to sham controls, on water and dry-land versions of the direction problem, a foraging task, and a spatial discrimination on a radial arm maze. These results build on previous behavioural and cell-recording research and demonstrate the importance of the HD system to spatial learning. Current studies are examining the effects of discrete lesions to components of the HD system on response reversal learning. We have previously shown that a change in the orientation of the apparatus when the response contingency changes facilitates reversal learning to the same extent as a change in rooms. Whether this effect depends on the integrity of the HD circuit remains to be determined.

Disclosures: D.M. Skinner: None. **M.A. Wasef:** None. **V.C.J. Harvey:** None. **C.M. Thorpe:** None.

Poster

423. Hippocampal Circuits and Cognition

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 423.05/III2

Topic: H.01. Animal Cognition and Behavior

Support: NSF IIS RI Grant 1703340

Title: Modeling of multiscale spatial navigation in complex environments

Authors: *A. WEITZENFELD[1], P. SCLEIDOROVICH[1], B. HARLAND[2], J.-M. FELLOUS[3]
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Abstract: Studies suggest that spatial navigation is supported by the multiscale neural system of the hippocampus and associated structures. The dorsal hippocampus (DH) has a critical role in spatial learning and memory, but most of the supporting experiments were conducted using simple spatial tasks in small arenas. Such conditions may have not equired the integration of spatial representations at different scales along the dorso-ventral axis of the hippocampus, and hence may have not relied on ventral hippocampal (VH) computations. Our overall hypothesis is that the interactions of spatial maps at multiple scales along the dorso-ventral axis of the hippocampus allows for spatial navigation in large and complex environments. We are currently developing a computational model to simulate goal-directed navigation tasks in a large environment with different density of obstacles. The current work will present preliminary results related to this computational model and evaluation with autonomous robotic systems.

Disclosures: A. Weitzenfeld: None. **P. Scleidorovich:** None. **B. Harland:** None. **J. Fellous:** None.

Poster

423. Hippocampal Circuits and Cognition

Location: SDCC Halls B-H

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Program #/Poster #: 423.06/III3

Topic: H.01. Animal Cognition and Behavior

Support: NSF 1631465
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Alberta Innovates Health Solutions Polaris Award
DARPA FG20678 440880/24900

Title: Testing the hippocampus' role in a rapidly-acquired, novel spatial sequence task in rats

Authors: *S. KILIANSKI, R. E. HOKENSON, A. SAHAGIAN, B. L. MCNAUGHTON
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Abstract: It is thought that the hippocampus generates an "index code" that contains pointers to neocortical modules storing the content of a memory. In this model, the hippocampus coordinates reactivation of new memory traces distributed across the neocortex during sleep, and these reactivations support the gradual development of corticocortical connections that allow for eventual memory retrieval independent of the hippocampus. To test the hypothesis that the hippocampus coordinates neocortical reactivation, an experimental subject must encounter a new experience and thus generate a new index code that can be reactivated during sleep. To this end, we developed a 4-element spatial sequence task in which subjects must learn a novel sequence in a novel environment every day. We shaped water-restricted rats (n = 3) to continuously run a 4-element sequence of cued arms in an 8-arm radial maze for sweetened-water reward. Cued sequences were interleaved with noncued sequences, during which the blinking LED cue indicating the correct arm was on an 8 second delay so rats had to navigate to the correct arms from memory. All rats were trained on at least 36 new sequences, each in a new environment. To investigate the role of the hippocampus in this task, we inactivated it by infusing the $GABA_A$ agonist, muscimol, or saline before training on alternating days. All three rats learned the novel sequences in a single 45-minute training session under both muscimol and saline infusion conditions. In two rats, hippocampal inactivation retarded, but did not abolish, learning of the new sequence. While these results suggest that hippocampal inactivation might retard acquisition of the spatial sequence task, all 3 rats showed a significant improvement in performance across a single session irrespective of infusion condition. Given the rats' ability to rapidly acquire these sequences and the apparent role of the hippocampus in this process, this task is viable for use in future experiments probing the role of the hippocampus in neocortical reactivation of new memory traces. Ongoing experiments aim to investigate the role of the hippocampus in consolidation of memory for this task using a 2-day training/testing paradigm and inactivating the hippocampus immediately following initial training.

Disclosures: S. Kilianski: None. **R.E. Hokenson:** None. **A. Sahagian:** None. **B.L. McNaughton:** None.

Poster

423. Hippocampal Circuits and Cognition

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Program #/Poster #: 423.07/III4

Topic: H.01. Animal Cognition and Behavior

Support: THE ISRAEL SCIENCE FOUNDATION – FIRST Program (grant no. 281/15)
Frankel center at the Computer Science Department, Ben Gurion University
Helmsley Charitable Trust through the Agricultural, Biological and Cognitive Robotics Initiative of Ben-Gurion University of the Negev

Title: Representation of borders, velocity and speed in the goldfish brain

Authors: *E. VINEPINSKY, O. BEN-SHAHAR, O. DONCHIN, R. SEGEV
Ben Gurion Univ., Beer Sheva, Israel

Abstract: Navigation is one of the fundamental cognitive skills found in many animals across the animal kingdom. This ability is important for finding food, shelter, and mates in order to survive. However, almost nothing is known about the neural representation of space in the brain of animals outside the mammalian class. Goldfish, which is part of the largest animal class, the bony fish, also have the cognitive ability to navigate, using both allocentric and egocentric cues. In the goldfish brain, there is a specific region, the lateral pallium, which is associated with allocentric navigation and is a possible homolog of the mammalian hippocampal formation. Using a novel wireless recording system, we measured the activity of single cells in the lateral pallium while fish swam freely. We found three unique cell types: border cells, velocity cells and speed cells. Border cells are cells which are active when the fish is near the boundary of the environment. Velocity cells encode swimming direction and speed, while speed cells encode only the speed independent of direction. Those cells types resemble cell types which are found in the mammalian hippocampal formation and believed to be the building blocks which drive the navigation system. Our study sheds light on how spatial information is encoded in the fish brain and whether the mechanisms of the neural navigation system are preserved across evolution.

Disclosures: E. Vinepinsky: None. **O. Ben-Shahar:** None. **O. Donchin:** None. **R. Segev:** None.

Poster

423. Hippocampal Circuits and Cognition

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Program #/Poster #: 423.08/III5

Topic: H.01. Animal Cognition and Behavior

Support: Picower Fellowship
NIH grant EY007023
NIH grant NS090473

Title: A feedback circuit shaping spatial and reward expectation during visually guided locomotion

Authors: *E. M. ADAM, M. SUR
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Abstract: The ability to build up expectations, and hence predict and anticipate, is at the heart of cognition. This ability is enabled by assimilating prior experience, and, in light of it, completing acquired partial information, which is reincorporated as experience to build future expectations. We are interested in how this interaction is biologically implemented and how it is leveraged through feedback mechanisms to drive expectation and anticipation. We have developed a behavioral task where head-fixed mice run on a treadmill through a virtual linear track, then stop and wait at fixed visual landmarks to collect specified rewards. Mice learn to perform the task reliably, by moving quickly through the track and recognizing landmarks. By altering reward contingencies, we dissociate spatial information from reward information, thereby setting up rule-specific expectations. We hypothesize that the task biologically recruits an interplay of two pathways: one relaying spatial information, mediated through retrosplenial cortex (RSC), and one relaying reward information, mediated through prefrontal cortex (PFC). Retrograde tracing experiments reveal reciprocal connections between caudal anterior cingulate cortex (ACC), a subdivision of medial PFC, and both rostral and caudal RSC, as an anatomical basis for this interplay. Preliminary two-photon calcium imaging in caudal RSC and caudal ACC reveals neural population activity that is locked to locomotion activity during task performance. The low-dimensional dynamics of the population activity contain significant information reflecting the animal's speed. Using projection-specific in-vivo two-photon imaging, coupled with causal optogenetic manipulations, we are further investigating the information content and functional role of these feedforward and feedback pathways, with the goal of understanding their specific contributions to experience and expectation.

Disclosures: E.M. Adam: None. **M. Sur:** None.

Poster

423. Hippocampal Circuits and Cognition

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 423.09/III6

Topic: H.01. Animal Cognition and Behavior

Title: Super-active and ultra-sparse neurons: Hippocampal neurons with persistently high and low propensities for representing space across environments and time

Authors: *J. LEE, J. BRIGUGLIO, S. ROMANI, A. K. LEE
HHMI / Janelia Res. Campus, Ashburn, VA

Abstract: Hippocampal CA1 neurons exhibit clear location-specific (place field) activity. In a small environment, most CA1 pyramidal neurons are active at a single location or not active at all. In a large environment (48-meter-long track), Rich et al. (Science 345: 814-7, 2014) found that most CA1 pyramidal neurons have a few or no place fields, but a small population of CA1 neurons has many (e.g., >10) place fields, and that the distribution of place fields per cell across the population was well-described by a gamma-Poisson model. However, it was not known whether a neuron's place field propensity was specific to each environment and/or point in time. Therefore, we used 2-photon calcium imaging and a head fixed-virtual reality system to follow the spatially tuned activity of >500 simultaneously imaged CA1 cells over a month in mice that explored multiple, distinct 40-meter-long virtual environments. We found that the number of place fields of individual hippocampal CA1 neurons was stable across days within each environment. The number of place fields of each neuron was also stable across different environments while place field maps were remapping. These results imply that a neuron's place field propensity is a stable feature of the cell and likely reflects pre-existing differences between CA1 cells that have a large impact on how the hippocampus represents space.

Disclosures: J. Briguglio: None. **S. Romani:** None. **A.K. Lee:** None.

Poster

423. Hippocampal Circuits and Cognition

Location: SDCC Halls B-H

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Program #/Poster #: 423.10/III7

Topic: H.01. Animal Cognition and Behavior

Support: JSPS KAKENHI 18K07357

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JSPS KAKENHI 16H01490
MEXT-Supported Program for the Strategic Research Foundation at Private Universities

Title: Effects oflesionsoftheretrosplenialcortexontracingalearned routethat includes asmallchange inspatial structure

Authors: T. HAYASHI[1], *N. SATO[2]
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Abstract: It has been thought that the retrosplenial cortex plays an important role in spatial navigation and spatial learning. Here, we investigated the function of the retrosplenial cortex of rats in route learning. We used a lattice maze which consists of five vertical and five horizontal paths. We used twenty-one male rats as subjects. Of the rats, eleven had excitotoxic lesions of the retrosplenial cortex and nine had sham lesions. The rats were required to go to a goal box from a start box in the lattice maze. In the learning phase, there were 5 trials in a daily session. We trained the rats until they could reach the goal without any mistakes in the consecutive 8 trials (2 days). After the rats fulfilled the criterion, we conducted tests. In the test phase, there are 6 trials in a daily session. The first trial was the same procedure as the learning phase. In the remaining trials, we added one of seven novel bypassing routes to the learned route, and the rats were required to go to the goal box from the start box. We focused on the behavior when the rats faced the bypassing route. In the learning phase, there were no significant difference in the number of sessions to reach the criterion between the retrosplenial cortex lesion and sham groups. In the test phase, the rats with lesions of the retrosplenial cortex tended to make the greater number of deviations from the learned route than the control group. This result suggests the rats with lesions of the retrosplenial cortex cannot trace the learned route without mistake, and that the retrosplenial cortex may be related to tracing a learned route.

Disclosures: T. Hayashi: None. **N. Sato:** None.

Poster

423. Hippocampal Circuits and Cognition

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Topic: H.01. Animal Cognition and Behavior

Support: NINDS NS-053907

Title: Modeling egocentric bearing selectivity in the rat parahippocampal region

Authors: *P. A. LACHANCE, J. S. TAUBE
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Abstract: Head direction cells, grid cells, and other spatially responsive neurons (including conjunctive cells) have been shown to be intermingled with one another in the postsubiculum (PoS), parasubiculum (PaS), and medial entorhinal cortex (MEC) of the rat. Population analyses across these regions reveal a rough transition from direction to location encoding, with PoS cells largely encoding head direction and MEC cells primarily encoding location information. These functional differences are consistent with the anatomical connectivity of these areas, as PoS receives projections from the head direction circuit and MEC provides dense inputs to the hippocampus where place cells are found. However, mechanisms underlying the functional transition from direction to position encoding have not been fully characterized.
We developed a method for modeling aspects of this direction-location transformation by analyzing the encoding of egocentric place bearing (EPB) in parahippocampal cells. A subset of cells in the posterior parietal cortex fire in response to an animal's directional heading relative to a salient environmental cue (Wilber et al. 2014). It is therefore possible that cells could provide information about egocentric bearing relative to arbitrary places within an environment (i.e. egocentric place bearing), such that a navigating animal is constantly aware of how it is oriented with respect to its surroundings. The parahippocampal region, with its unique mixture of direction and location encoding, provides a potential substrate for this representation. Preliminary evidence suggests that EPB is indeed represented by parahippocampal neurons in a manner independent of allocentric head direction or location, with a general increase in EPB selectivity from PoS through PaS to MEC. Such area-specific differences could provide critical insights into fundamental mechanisms behind the transformation from direction to location encoding in the mammalian brain.

Disclosures: P.A. Lachance: None. **J.S. Taube:** None.

Poster

423. Hippocampal Circuits and Cognition

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Topic: H.01. Animal Cognition and Behavior

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PER-SU
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Title: How does the cerebellum modulate hippocampal coding?

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Abstract: The cerebellum is involved in many cognitive processes through its interaction with different association areas in the brain. In particular, it has an important role in shaping the hippocampal place code during self-motion based navigation thus evidencing the existence of cerebello-hippocampal interactions. However, little is known about the organization and functioning of the circuits supporting such interactions.
By using a retrograde tracing strategy the team has recently identified three polysynaptic pathways from the cerebellum to the hippocampus (HPC) involving lobule VI-caudal fastigial nuclei, Crus I-dentate nuclei and paraflocculus-vestibular nuclei. We have later explored the electrophysiological relevance of these pathways in behaving mice by measuring the levels of coherence between simultaneously recorded local field potentials from the HPC and the cerebellar cortex in the lobule VI, Crus I and lobule II/III as a control. We have consistently found higher levels of coherence in the theta range (6-12 Hz) between lobule VI and/or Crus I and the HPC compared with the control. In particular, Crus I-HPC coherence dynamically changed depending on the behavioral and sensorial context, being maximal during goal-directed behavior. However, in order to further investigate the role of the cerebellum in shaping the hippocampal spatial code we need a causal approach that allows us to address direct modulation of the hippocampal activity by specific manipulation of the cerebellum.
To optogenetically control the activity of discrete cerebellar circuits, we are using transgenic mice expressing channelrhodopsin specifically in the cerebellar Purkinje cells (L7-ChR2 mice), the only output of the cerebellar cortex. Using a virtual reality (VR) setup, we are now performing acute-like experiments in head-fixed mice with craniotomies exposing the cerebellar cortex and the cortex above HPC. We are using silicon probes to record the laminar profile of LFPs and units in the dorsal HPC while we stimulate precise spots on the cerebellar cortex with an optic fibre. Stimulation at specific hotspots generates heterogeneous hippocampal responses, either inhibitory or excitatory, at single cell level. We now aim at recording stable place cells in the VR and at testing the effect of cerebellar manipulation on their activity. Using this strategy we plan to explore to which extent and through which sensory information cerebellar manipulation can affect the hippocampal place code.

Disclosures: A. Torres Herraez: None. **L. Rondi-Reig:** None.

Poster

423. Hippocampal Circuits and Cognition

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Program #/Poster #: 423.13/III10

Topic: H.01. Animal Cognition and Behavior

Support: LMU

Title: Turning direction and image selectivity of hippocampal neurons during virtual reality navigation

Authors: *D. FETTERHOFF[1], C. LEIBOLD[1,2]
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Abstract: Virtual reality is useful to study spatial navigation because it enables environmental manipulations that would be unfeasible in real world setups. In our study, male Mongolian gerbils (Meriones unguiculatus) were exposed to two different virtual hallways that could be identified based on both turning direction and sequence of images on the walls of straight hallways. Specifically, maze A had two right turns and images of zebra skin, stars and targets, while maze B had two left turns and images of moons, pyramids and leaves. One objectives of this study was to identify activity of place cells during navigation of a maze with a well-known sequence of images compared to one which had images shuffled between two different image sequences. By shuffling between image sequences, identification of neuronal activity corresponding to spatial location versus image selectivity will be possible.
After learning how to navigate mazes with well-known sequences, a microdrive with eight individually movable tetrodes was implanted into hippocampal CA1 and CA3 regions and single units and local field potential recordings were made before, during and after virtual maze navigation. On testing days, gerbils were exposed initially to 20 laps with the well-known images, while the last 20 laps contained the initial image from one maze type but the final 2 images from the opposite maze type (i.e., mixed maze A contained zebra skin, pyramid and leaf; mixed maze B contained moon, stars and targets.)
Behavioral results showed that gerbils learned how to navigate virtual mazes. Analysis of electrophysiological data collected when images were shuffled between mazes showed that hippocampal principal cells fired specifically to either spatial location, turning direction or to specific images, while some cells only fired during the initial maze but not after shuffling. The results show that specific hippocampal cells respond to spatial location based on turning direction, to images or to a specific combination of the two.

Disclosures: D. Fetterhoff: None. **C. Leibold:** None.

Poster

423. Hippocampal Circuits and Cognition

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Topic: H.01. Animal Cognition and Behavior

Support: BBSRC Grant BB/P002455/1

Title: Lesions of the head direction cell system impair directional discriminations

Authors: *A. E. SMITH[1,2], O. A. CHEEK[1], E. L. C. SWEET[1], P. A. DUDCHENKO[2], E. R. WOOD[1]
[1]Univ. of Edinburgh, Edinburgh, United Kingdom; [2]Univ. of Stirling, Stirling, United Kingdom

Abstract: Previous work has shown that rats are better able to discriminate otherwise identical maze compartments when these face different directions, compared to when the compartments all face the same direction (Grieves et al., 2016). Additionally, hippocampal place cells show repetition of place fields between compartments that are parallel to one another, but not in the same compartments arranged at a 60^{o} angle to one another. This suggests that both place cell fields and spatial discrimination abilities are influenced by a directional input. Consistent with the former, lesions to the lateral mammillary nuclei (LMN, part of the head direction network) lead to place field repetition in the maze compartments facing different directions (Harland et al, 2017). In the current study we tested the hypothesis that a directional input is similarly necessary for rats to discriminate, behaviourally, maze compartments facing different directions. Adult, male Lister Hooded rats were randomly assigned to undergo either bilateral ibotenic acid lesions of the LMN (LMNx group, n=8) or a sham surgery (sham group, n=6). Once recovered from surgery, animals were mildly food deprived and trained on a four-way odour-location task in a maze with four radially arranged compartments. In this task, every compartment contained four sand wells, each scented with a different household spice. The same scented wells were replicated in each compartment, but a food reward (a buried cereal piece) was associated with a different sand well in every compartment. Thus, the rats' task was to discriminate between the otherwise identical maze compartments in order to dig in the correct sand well in each. The location and identity of rewarded odours was counterbalanced between groups, and experimenters were blind to the condition of the animals at the time of testing. Only 4 of 8 LMNx animals reached learning criterion for the four-way odour-location task, whereas all sham animals (6 of 6) reached this criterion. In addition, LMNx animals took significantly longer to reach criterion in an early stage of acquisition of the task which involved discrimination between two compartments, although group differences were not as evident in later stages of training. Analysis of probe trials indicated that neither group used the scent of the food reward to detect rewarded wells. These preliminary findings suggest that directional input may contribute to the ability to discriminate behaviourally between maze compartments facing different directions.

Disclosures: A.E. Smith: None. **O.A. Cheek:** None. **E.L.C. Sweet:** None. **P.A. Dudchenko:** None. **E.R. Wood:** None.

Poster

423. Hippocampal Circuits and Cognition

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Support: MEXT KAKENHI 15H04265
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CREST JPMJCR13W1

Title: Learning efficient search for reward by CA3 recurrent network model

Authors: *T. HAGA, T. FUKAI
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Abstract: Sequence learning is one of the essential functions of hippocampus. During run, sequential activities of place cells along the path is observed in every theta oscillation cycle (theta sequences), and place-cell sequences during run are replayed during immobility. These hippocampal firing sequences correlate with future paths of rats during exploration, and often designate paths to the predictable reward. In one-dimensional tracks, replay in the reversed order (reverse replay) is often observed when rats are consuming reward. Because reward modulates its occurrence frequency, reverse replay presumably contributes to goal-directed path learning in hippocampus. In contrast, in a two-dimensional open field, it has been reported that firing sequences triggered at the rewarded position are not biased to reverse replay of the recent path. The mechanism how hippocampus realize this goal-directed path learning, and the role of reverse replay in one-dimensional environments and unbiased firing sequences in two-dimensional environments are still unknown. In this work, we built the CA3 recurrent network model with the combination of Hebbian plasticity and short-term depression (or afterdepolarization). In our model, a firing sequence selectively potentiates synaptic transmissions in the reverse direction. Therefore, after a traversal on a one-dimensional track, this model generates reverse replay from goal to start, which realizes goal-directed learning by potentiation of forward synaptic transmission from start to goal. Notably, reverse replay in our model sometimes deviates from recent path and bifurcates into previously experienced paths, by which the network can optimize many possible goal-directed paths through one rewarded experience. Such “joint replay” has been also observed in experiments and our model suggests their importance in generalization of spatial memory. When we applied our model to a two-dimensional open field, firing sequences triggered at reward sites isotropically propagate over the space, and they create bias of sequence propagation to reward from any point in the space. This property of our model suggests that we can extend the role of one-dimensional reverse replay for goal-directed learning to unbiased

sequence propagation in two-dimensional space. We demonstrate that this model can quickly learn efficient search for reward in the task similar to Morris water maze. Our hippocampal model proposes the experimentally testable mechanism for goal-directed path learning in one-dimensional and two-dimensional environments.

Disclosures: T. Haga: None. **T. Fukai:** None.

Poster

423. Hippocampal Circuits and Cognition

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Topic: H.01. Animal Cognition and Behavior

Support: NIH MH079511
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UCLA Depression Grand Challenge
UCLA NSIDP

Title: Hippocampal sharp-wave ripples precede high-effort movements

Authors: *A. G. HOWE[1], G. J. BLAIR[2], H. T. BLAIR[2]
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Abstract: Sharp-wave ripples (SWR) are an electrophysiological local field potential (LFP) phenomenon found in the hippocampus during periods of waking stillness and sleep. SWRs have been proposed to participate in a variety of cognitive processes such as deliberative planning, memory consolidation, and credit assignment during reinforcement learning. Here, we investigated the role of SWRs in deliberative planning by recording LFP signals from area CA1 in freely behaving rats as they acquired and expressed a simple T-maze task with a fixed reward location. We identified episodes of movement initiation during which the rat was continuously still (speed < 5 cm/s) for at least 3 s, and then transitioned to a period of movement (speed > 10 cm/s) lasting for at least 3 s. We observed a bimodal distribution of speeds during movement episodes, allowing for behavioral discrimination between low-effort (10-25 cm/s) versus high-effort (35-55 cm/s) movement episodes. Preliminary analyses indicate that during the 3 s of stillness preceding movement onset, SWRs occurred at a significantly higher rate prior to high-effort versus low-effort movements. Furthermore, the probability of SWR events peaked approximately 1 s prior to high-effort movement initiation. As a complementary analysis, we categorized the movement traces during these episodes of transition from stillness to movement into those preceded by one or more SWR and those preceded by no SWR. Statistical comparison

of the average speed trace for each category revealed that the average speed trace for episodes preceded by one or more SWR is significantly higher than the average speed trace for episodes preceded by no SWR, which is consistent with the first analysis. Based on these results, we hypothesize that SWR rate increases prior to the execution of effortful actions, as would be expected if SWR events are involved in deliberative planning of goal-directed actions that are subsequently executed with vigorous effort.

Disclosures: A.G. Howe: None. **G.J. Blair:** None. **H.T. Blair:** None.

Poster

423. Hippocampal Circuits and Cognition

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Program #/Poster #: 423.17/III14

Topic: H.01. Animal Cognition and Behavior

Support: R01: MH090127
VA: I01BX003195

Title: The causal role of oxidative kynurenine metabolism in mediating cognitive impairment by remodeling actin cytoskeleton structure in a model of chronic inflammation

Authors: *M. MITHAIWALA, A. M. GARRISON, J. C. O'CONNOR
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Abstract: Cognitive impairment is a comorbidity associated with multiple neurological conditions accounting for reduced quality of life and significant socioeconomic burden. Currently, there is no FDA approved therapy for cognitive dysfunction and the pathogenic mechanisms remain poorly understood. Inflammation-induced disruption in the kynurenine pathway (KP) of tryptophan metabolism has been implicated in mediating behavioral dysfunction. In the hippocampus, a pronounced shift toward oxidative metabolism of kynurenine occurs during inflammatory conditions, and this shift appears to mediate hippocampal-dependent cognitive deficits caused by inflammation. We hypothesized that increased oxidative KP metabolism during inflammation leads to increased production of QA in the hippocampus that is responsible for cognitive impairment. In this study, we infected WT and 3-Hydroxyanthranillate-3,4-dioxygenase (3-HAAO; enzyme that produces QA) null mice with the Bacillus of Calmette-Guérin (BCG) to induce chronic inflammation. BCG challenged WT mice exhibit significant deficits in hippocampal-dependent spatial learning and a significant reduction in spine density CA-1 basal and dentate gyrus neurons. These impairments were absent in 3-HAAO null mice. Chronic direct administration of QA (10μM) to primary hippocampal neuronal cultures for 5 days significantly decreased the number of dendritic spines. Interestingly, we also observed an increase in the levels of the actin-severing protein-cofilin in hippocampal tissue lysate in WT-

BCG mice that was absent in 3-HAAO null mice. Together, these data indicate that inflammation-induced increases in hippocampal QA production contribute directly to cognitive impairments, likely via structural remodeling of hippocampal dendritic structure and alterations in synaptic plasticity.

Disclosures: M. Mithaiwala: None. **A.M. Garrison:** None. **J.C. O'Connor:** None.

Poster

423. Hippocampal Circuits and Cognition

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Topic: H.01. Animal Cognition and Behavior

Support: JSPS KAKENHI Grant-in-Aid for Young Scientists (B) (17K13272)
Brain Science Foundation Research Grant
Special Postdoctoral Researchers Program in RIKEN

Title: The hippocampal engram maps experience but not place

Authors: *K. Z. TANAKA[1], H. HE[1], A. TOMAR[2], K. NIISATO[1], A. J. Y. HUANG[1], T. J. MCHUGH[1]
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Abstract: Episodic memories are encoded by a sparse population of hippocampal neurons. In mice optogenetic manipulation of this memory "engram" established these neurons are necessary and sufficient for memory recall. However, nothing is known about their *in vivo* activity or precise role in memory. Here we find that during memory encoding only a fraction of the CA1 place cells are engram neurons, distinguished by firing repetitive bursts paced at the theta frequency, a pattern effective in strengthening synapses. During memory recall these neurons remained highly context specific, yet demonstrated preferential spatial remapping of their place fields. These data demonstrate a dissociation of precise spatial coding and contextual indexing by distinct hippocampal ensembles and suggest the hippocampal engram serves as an index of memory content.

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Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

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Topic: H.01. Animal Cognition and Behavior

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NIH Grant R01DC007703

Title: Interactions of taste and place coding in the hippocampus

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Abstract: The hippocampus plays a key role in spatial learning and memory, and hippocampal place cells respond to specific locations as animals explore their environment. Place cells have also been shown to respond to non-spatial stimuli such as odors, visual cues, textures and tones, forming a mental map based on environmental context and task demands. However, little is known about how place cells respond to tastes, which is surprising, given that one of the most important purposes of having a mental map is to find food. To characterize how the hippocampus responds to tastes, we recorded from neurons in the dorsal CA1 hippocampal region of freely behaving male Long-Evans rats (n=5) as they received different taste solutions delivered via intra-oral cannula either randomly or at specific locations on a linear track. We identified a subset of hippocampal neurons (~20% of recorded cells) that included both putative principal cells and interneurons that responded to as well as discriminated between tastes. Out of these taste-responsive cells, those classified as place cells discriminated between tastes exclusively when stimuli were delivered within their place field. Taste experience in specific locations on the linear track also led to an increased number of cells with place fields that represented locations of tastant delivery. Together, these results suggest that hippocampal taste responses allow animals to form associations between tastes and contexts, using past experience to locate food sources. These findings add to a growing body of literature indicating that place cells do not just respond to locations—rather, they form a mental map that encompasses both spatial and non-spatial aspects of an animal's environment. Future work will further assess the taste and place components of hippocampal responses, and examine the reactivation of taste-responsive cells during sharp-wave ripples.

Disclosures: L.E. Herzog: None. **L. Pascual:** None. **D.B. Katz:** None. **S.P. Jadhav:** None.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 424.02/III17

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01MH112661
Whitehall Foundation

Title: Odor-place associative memory in the hippocampal-prefrontal network

Authors: *C. A. SYMANSKI, S. K. GUHA, E. KULLBERG, S. P. JADHAV
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Abstract: Rodents use olfactory cues to learn and remember information about their environment. This requires forming associations between odors and locations, and then recalling these associations to guide future behavior. The physiological mechanisms by which these odor-place associations are formed, retrieved, and maintained during spatial behavior are thought to involve a brain-wide network that includes the hippocampus and the prefrontal cortex (PFC) (Tse 2007; Fujisawa 2011; Igarashi 2016; Rangel 2016). Recent evidence suggests that activity in the hippocampus and PFC, and especially the coordination between the two regions, is important for associative memory, memory formation and recall, and memory-guided decision making (Churchwell 2010; Spellman 2015; Shin & Jadhav 2016). We therefore investigated how coordination between these two regions, along with the olfactory bulb, supports the retrieval of learned odor associations to guide spatial decision making in an odor cue-guided T-maze task. In this task, rats (n = 6) recalled familiar associations between odors and reward locations on the maze arms (75-90% performance accuracy). Multi-site tetrode recordings were used to simultaneously monitor neural activity in the olfactory bulb, medial PFC, and dorsal hippocampal CA1 in freely-behaving rats as they performed this task. We measured local field potential (LFP) activity in all regions, as well as spiking activity of ensembles in CA1 (n = 494 neurons) and PFC (n = 181) to investigate the physiological basis of the recall and maintenance of odor-place associations. During the period of acute olfactory sampling and recall, beta oscillations (15-35 Hz) were prominent in olfactory bulb, CA1, and PFC; further, beta coherence was enhanced between the three regions. Inter-regional phase locking of hippocampal and PFC neurons to beta oscillations was also observed, suggesting beta-driven coordination of these regions as part of a functional network. Individual neurons in both CA1 and PFC also exhibited odor/choice-selective responses, implicating their involvement in recall of odor-place associations. These results point to an olfactory-hippocampal-prefrontal network underlying the recall of olfactory-place associations. In particular, beta oscillations may organize spiking patterns conveying task-related information in the hippocampal-prefrontal circuit. Future

investigations will address ensemble mechanisms in this network for recall and maintenance odor-place associations during the memory-guided decision-making task.

Disclosures: C.A. Symanski: None. **S.K. Guha:** None. **E. Kullberg:** None. **S.P. Jadhav:** None.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 424.03/III18

Topic: H.01. Animal Cognition and Behavior

Support: R01MH112661
Whitehall Foundation

Title: Hippocampal theta supports distinct prefrontal representations on a behavioral timescale

Authors: *M. C. ZIELINSKI[1], J. D. SHIN[1], S. P. JADHAV[2]
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Abstract: Rhythmic coordination of network activity between the rodent hippocampus (CA1) and medial prefrontal cortex (PFC) supports working memory guided decision-making. Theta (6-12 Hz) oscillations are one such prominent mode of rhythmic hippocampal activity, seen during active, goal driven behavior. Theta cycles pattern the firing of hippocampal place cells, as well as temporally organizing PFC activity; a majority of PFC cells fire at distinct hippocampal theta phases during spatial behavior (*Siapas et al., 2005; Jones & Wilson, 2005; Jadhav et al., 2016*). In addition to coding of spatial location, the firing of place cells during theta cycles are hypothesized to support cognitive processes such as planning and evaluation of paths in space and time, with theta-paced PFC responses likely facilitating these processes (*see Zielinski et al. 2017 for review*). In support of this, sequences of place cells that sweep away from the animal's current position during theta have been described (*Wikenheiser & Redish, 2015; Amemiya & Redish, 2018*), but the potential role of PFC in these patterns and the dynamics of these phenomena during behavior are still unknown. To address this relationship, we simultaneously recorded neural ensembles in PFC and CA1 during performance of a W-track spatial alternation task, known to require spatial working memory and dependent on interactions between these two areas (*Wang & Cai, 2006; Maharjan et al., in preparation*). We observed that population dynamics in PFC can encode the animal's current position on a theta cycle timescale, and that prefrontal representations aligned with concurrent representations in CA1. Incorporation of corresponding hippocampal theta phase improved PFC ensemble decoding of space. Furthermore, on approach to goal locations, we observed spatially distant, non-local

representations of place in CA1 on a theta cycle timescale, which aligned with non-local representations of place in PFC. These non-local representations often represented recent or future goal locations. These findings show that PFC coordinates with CA1 at a theta cycle timescale during active behavior, that the hippocampal theta phase locking of PFC activity is relevant to coordinated representations of space, and that concordant non-local representations between the two regions can serve as hallmarks of goal-oriented cognitive processes.

Disclosures: M.C. Zielinski: None. **J.D. Shin:** None. **S.P. Jadhav:** None.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

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Program #/Poster #: 424.04/III19

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R21-MH104318
NIH Grant R01-MH112661
Whitehall Foundation

Title: Investigating thalamic contributions to abnormal hippocampal oscillatory activity in a mouse model of schizophrenia

Authors: *R. NANU[1], C. LIN[2], D. B. KATZ[3], S. P. JADHAV[3], H. PI[3], J. LISMAN[3]
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Abstract: Schizophrenia (SZ) patients show an elevation in the power of delta frequency (1-5 Hz) EEG oscillations in the awake state (Venables et al., 2009; Clementz et al., 1994). This elevation is unique to the diseased state of SZ and isn't seen in first-degree relatives of patients. Furthermore, optogenetic studies in rodents suggest that waking hippocampal delta oscillations may be causal in producing some of the symptoms of SZ (Duan et al., 2015). Previous studies from our lab have found that thalamic T-type Ca^{2+} channels support this delta abnormality in an NMDAR antagonist model of SZ (Zhang et al., 2012); however, NMDAR antagonists do not fully capture the development and progression of the disease. A stronger model is provided by the Df(16)1/+ (Df1) mouse which mimics the largest known genetic risk of SZ (~33%), human 22q11 deletion syndrome. These mice have already been found to exhibit many of the behavioral phenotypes associated with animal models of the disease (Karayiorgou et al., 2010), but in order to further establish this experimental system to study underlying neural mechanisms, it is necessary to determine if these mice also show waking delta abnormalities. In a key a first step, here we test whether delta is elevated in the Df1 mice. To this end, we use in-vivo electrophysiology to record from hippocampal area CA1 in 3-9 month old Df1 mice and WT

littermate controls. We then use EMG data to classify behavioral states as moving, quiescent waking or sleeping, and the baseline spectral properties of the local field potentials are compared between groups and states. Our preliminary data suggests a large variation among Df1 animals with some animals exhibiting an elevation in low-frequency oscillatory power compared to controls. Ongoing studies seek to confirm these results and investigate possible phenotypes to identify Df1 animals with delta elevations. We aim to establish an experimental system that fully encapsulates SZ development and can be used to thoroughly study the mechanisms underlying SZ pathophysiology. Our long-term goal is to establish whether the mechanisms responsible for delta elevations in the NMDAR antagonist model, thalamic T-type Ca^{2+} channels, are still valid and can be targeted for possible treatment of SZ.

Disclosures: R. Nanu: None. **C. Lin:** None. **D.B. Katz:** None. **S.P. Jadhav:** None. **H. Pi:** None. **J. Lisman:** None.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Training Grant T90DA32435-7
NIH Grant R01MH112661
Whitehall Foundation

Title: Dual-phase-locking in the hippocampal-prefrontal network

Authors: *R. YOUNG[1], J. D. SHIN[1], S. P. JADHAV[2]
[1]Grad. Program in Neurosci., [2]Neuroscience, Psychology and Volen Ctr. for Complex Systems, Brandeis Univ., Waltham, MA

Abstract: Network interactions among neural ensembles in distributed circuits are necessary for cognition and behavior. It is still unclear how groups of neurons organize information transmission, but rhythmic patterns are thought to coordinate activity to form functional networks (*Palmigiano et al., 2017*). Brain rhythms reflect synchronized activity across large ensembles of neurons, believed key for coordination during many cognitive tasks. It has been shown that neurons in local networks will anchor their spikes within different local field rhythms, and that rhythms can synchronize across communicating structures (*Fries, 2015; Rangel et al., 2016)*. Multiple network activity patterns are prominent features in hippocampal and prefrontal areas during memory-guided navigation (*Shin & Jadhav, 2016*). Coordination of theta rhythms, and theta-gamma coupling, is observed in hippocampal-prefrontal circuits during spatial memory tasks, accompanied by local phase-locked spiking to theta and gamma rhythms

(*Tamura, et al., 2017; Shin & Jadhav, 2016*). Here, we report an interregional phase-locking pattern between prefrontal spikes and two hippocampal rhythms at once, beta and theta, during a spatial alternation memory task. A similar pattern has been observed before within the hippocampus (phase-locking of hippocampus to its own rhythms; *Lansink et al., 2016*), but here we examine a novel inter-regional phenomenon with prefrontal cells phase-locking simultaneously to hippocampal beta and theta rhythms. Importantly, this dual-phase-locking pattern undergoes a phase shift between distinct sections of the spatial memory task that have different cognitive demands, forming two inverse phase-locking modes we call "Type A" and "Type B". Both types appear in working memory and non-working memory phases of the task. However, only one of the two modes, Type B, is greatly attenuated and disordered during error trials that require working memory. Further, both phase-locking modes strengthen with degree of task performance. The precise manner in which spikes of the distant prefrontal cortex coordinate to this pair of hippocampal rhythms is striking and are indicative of different circuit-level operations, utilized differentially in behavior.

Disclosures: R. Young: None. **J.D. Shin:** None. **S.P. Jadhav:** None.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Training Grant R90DA033463
NIH Grant R01MH112661
Whitehall Foundation

Title: Learning-associated changes in awake replay content in the hippocampal-prefrontal network

Authors: *W. TANG[1], J. D. SHIN[1], S. P. JADHAV[2]
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Abstract: The hippocampus replays place-cell sequences reflecting behavioral experiences during sharp-wave ripple (SWR) events in awake and sleep states. Awake replay can recapitulate past trajectories as well as predict future paths, suggesting both retrospective and prospective roles in memory consolidation, retrieval and planning. Additionally, we have previously shown that these hippocampal replay events are coordinated with prefrontal cortical (PFC) activity for memory reactivation (*Jadhav et al., 2016*; *Tang et al., 2017*). However, it remains unclear whether replay serves specific functions in different learning states, and how PFC cells

participate in replay.
To address these questions, we continuously monitored the activity from same neuronal populations in both hippocampal area CA1 and PFC using high-density tetrode recordings throughout the duration of learning of a W-track spatial alternation task (n = 4 rats). Consistent with previous studies (see *Zielinski et al. 2017*, *Tang and Jadhav, 2018* for review), we observed that sequences of CA1 activity were reactivated both in the order as experienced (forward replay) and in the opposite order (reverse replay) during awake immobility. Interestingly, the order of replay sequences (forward or reverse) has a specific relationship to possible past and future trajectories during correct choices, but not during incorrect choices. Furthermore, the content of forward and reverse replay differentially changed as learning progressed: reverse replay was retrospective for past paths only during early learning; whereas forward sequences were predominantly related to prospective paths only during late learning. Additionally, PFC cells reactivated more coherently with CA1 cells during the replay of current relevant trajectories (i.e., the immediate past or future) than during that of irrelevant trajectories. This coordinated reactivation was stronger during initial learning compared with well-learnt periods, consistent with our previous study (*Tang et al., 2017*). Thus, our results show that the content of hippocampal replay systematically changes during learning. These suggest a shift in the role of replay from retrospective evaluation during initial learning to prospective planning of upcoming trajectories during later performance. Moreover, the coordinated hippocampal-PFC reactivation may play a crucial role in learning through selective replay of task-relevant content during memory-guided behaviors.
* WT and JDS have contributed equally to this work.

Disclosures: W. Tang: None. **J.D. Shin:** None. **S.P. Jadhav:** None.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: NIH R90DA033463
NIH R01MH112661
Whitehall Foundation

Title: Development of hippocampal-prefrontal representations in parallel with behavioral learning

Authors: *J. D. SHIN[1], W. TANG[1], S. P. JADHAV[2]
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Abstract: Coordinated activity between the hippocampus and prefrontal cortex (PFC) is critical for many aspects of cognition. The development of task-related neural representations within this network is thought to provide the neural substrate that underlies many memory-related processes (Frank et al., 2000; *Wood et al., 2000; Baeg et al., 2003)*. In any given environment, hippocampal place cells have specific spatial firing patterns that tile all locations, forming a cognitive map that provides a substrate for the formation of salient associations. Similarly, PFC cells also exhibit spatially modulated firing patterns, and we have recently shown that spatial correlations within the hippocampal-prefrontal circuit during the initial stages of learning may facilitate the emergence of representational similarities in these two regions (*Tang et al., 2017*). The development of stable representations in this network can support memory formation and retrieval. However, the specific nature of these task-relevant representations, the relative timescales at which they emerge in the hippocampus and PFC during learning, and how they support subsequent stable performance is still unclear.
To address these questions, we continuously recorded neural ensembles simultaneously in PFC and CA1 region of the hippocampus across behavioral and sleep sessions using high-density tetrode recordings in rats (n = 6) during learning of a novel spatial working memory task in a single day. This single-day learning paradigm allows us to track the same population of cells over the entire learning process, enabling characterization of changes in representations and dynamics in parallel with behavioral learning. We found that over the course of learning, populations in the hippocampus and PFC rapidly develop stable, task-modulated firing patterns. These stable representations dynamically represent behavior on a trajectory-by-trajectory basis during memory-guided performance. Furthermore, our results indicate that changes in neural dynamics in PFC occur at a similar behavioral timescale as the hippocampus. Spatially stable representations, as well as direction selectivity, emerged in parallel in both regions. Additionally, choice selective representations developed in the hippocampal-prefrontal network in parallel with learning and became predictive of past and future choices. The emergence of these stable, task-related representations may reflect the establishment of hippocampal-prefrontal associations that are important for learning in novel environments, and can therefore provide the neural basis to support memory guided behavior.
*JDS and WT are equal contributors

Disclosures: J.D. Shin: None. **W. Tang:** None. **S.P. Jadhav:** None.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: NIH DP5 Early Independence Award

NARSAD Brain and Behavior Foundation
McKnight Foundation
Ludwig Family Foundation

Title: Visualizing and modulating memories during voluntary aerobic exercise

Authors: *K. DORST[1], O. P. MCKISSICK[2], S. RAMIREZ[3]
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Abstract: Aerobic exercise improves physical health as well as various aspects of cognition. Moreover, the structural and functional integrity of the hippocampus—a key site for episodic memory-related processes—are markedly enhanced after chronic bouts of running. However, the underlying circuitry mediating interactions between an episodic (e.g. contextual) memory and voluntary aerobic exercise remains unclear.
Here, we selectively label cells within the dentate gyrus (DG) and basolateral amygdala (BLA) that are preferentially activated during the encoding of a positive, negative, or neutral memory. Our preliminary data suggest that both the DG and BLA become active during bouts of voluntary running, as indicated by c-fos expression. However, the ensemble within the DG that was previously active during the encoding of a positive, negative or neutral memory does not become preferentially reactivated during bouts of voluntary exercise. Conversely, BLA cells previously activate during the formation of negative, but not neutral or positive memories, become preferentially reactivated during bouts of voluntary exercise.This suggests that the DG may be encoding a different context when mice are voluntarily running, while BLA cells processing fear may become preferentially engaged during such behavior.
Lastly, our current experiments focus on closed-loop optogenetic reactivation of DG- or BLA-mediated negative or positive memories specifically during bouts of running to test for potential suppression or enhancement-like effects on subsequent memory expression.Together, our experiments reveal a putative neuronal and behavioral interaction between discrete memory-related processes and aerobic exercise.

Disclosures: K. Dorst: None. **O.P. McKissick:** None. **S. Ramirez:** None.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: NIH DP5 Early Independence Award
NARSAD Brain and Behavior Foundation

McKnight Foundation
Ludwig Family Foundation
Boston University

Title: Visualization and modulation of ensembles in the hippocampus and amygdala during fear reinstatement

Authors: *W. MAU, Y. ZAKI, A. HAMIDI, E. DOUCETTE, S. L. GRELLA, N. J. MURAWSKI, E. MERFELD, M. SHPOKAYTE, S. RAMIREZ
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Abstract: Post-traumatic stress disorder (PTSD) is a condition that precipitates from a highly aversive experience and is manifested by overgeneralized fear in innocuous situations. Interestingly, a striking proportion of patients who undergo exposure therapy - which can lead to the suppression, or "extinction," of the original fear memory - are highly vulnerable to relapse, especially when the conditioned stimulus is delivered outside a clinical context. Here, we interrogated the neural substrates supporting the acquisition of fear as well as the subsequent extinction of fear to gain a causal understanding of its underlying neural components. We used a combination of activity-dependent labeling of neuronal ensembles in multiple brain regions associated with fear-related behaviors (basolateral amygdala, BLA; dorsal dentate gyrus of hippocampus, dDG) and further manipulated these ensembles using optogenetics to probe the changes that a fear memory undergoes during extinction and during fear reinstatement.
We first tagged BLA or dDG cells during the acquisition of a contextual fear memory in mice. All subjects then underwent extinction learning, and then received a shock in an unconditioned context (serving to reinstate the original context-specific fear). We inhibited the tagged fear ensemble during reinstatement or during a fear recall test in the original conditioned context the day after. We found that inhibition of the fear ensemble in BLA or dDG during the recall test actively disrupted fear expression in the conditioned context. This suggests that the original fear ensemble in BLA and dDG contributes to a context-dependent fear response following shock-induced reinstatement, providing key evidence that the neural correlate of fear reinstatement may be a reemergence of the original fear memory trace. Next, we employed *in vivo* calcium imaging using miniaturized microscopes to visualize ensemble dynamics within and across fear conditioning, extinction, reinstatement, and recall sessions. Preliminary results from CA1 and BLA suggest that population activity can predict freezing bouts, opening up the possibility to investigate which cells are involved with fear expression at different time points in extinction and recall. We also observed sequences of cell activity related to the shock stimulus. Future work will examine how these sequences evolve over extinction and reinstatement.

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Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 424.10/III25

Topic: H.01. Animal Cognition and Behavior

Support: NIH DP5 Early Independence Award
NARSAD Brain and Behavior Foundation
McKnight Foundation
Ludwig Family Foundation

Title: Population and projection-specific segregation of fear and reward in the ventral hippocampus

Authors: *M. SHPOKAYTE[1], O. MCKISSICK[2], S. X. LIU[3], S. L. GRELLA[2], E. DOUCETTE[2], E. MERFELD[2], N. J. MURAWSKI[2], Y. ZAKI[2], A. B. FINKELSTEIN[4], S. RAMIREZ[2]
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Abstract: The ventral hippocampus(vHPC) codes both contextual information as well as rewarding and aversive experiences. Still, the behavioral, anatomical, and molecular vHPC activity associated with rewarding and aversive experiences has not yet been fully characterized. Here, we utilize an activity dependent, inducible, all-virus cfos-tTA system for tagging cells processing rewarding or aversive memories in vCA1 projecting onto the basolateral amygdala (BLA) of c57BL/6 mice. We optogenetically manipulate vCA1 to BLA terminals and provide evidence of the their capacity to "switch" from driving reward-related behaviors to aversive-related behaviors and vice versa. Furthermore, we use fluorescence-activated cell sorting (FACS) to pull down cells from the vCA1 responsible processing either fear or reward as well as those that have had their terminals "switched." Using Gene Set Enrichment Analysis (GSEA), a computational method that determines whether a defined sets of genes are statistically significant between two biological states, we find that vCA1 fear and reward cells upregulate genes associated with Alzheimer's Disease and neuroprotection, respectively. To further characterize this cellular population, we provide evidence that vCA1 recruits two segregated populations of cells in response to either a rewarding or aversive stimuli using a novel dual memory tagging strategy that combines our c-fos-tTA doxycycline-regulated viruses with a transgenic fos-Cre tamoxifen-regulated line. Our ongoing experiments focus on anatomically tracing projection-specific targets in vCA1 cells using retrograde, clearing, and physiological strategies. Understanding how the hippocampus regulates gene expression, processes and stores reward and

fear, and recruits valence specific information may have clinical value for the treatment of psychiatric disease-related states.

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Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

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Topic: H.01. Animal Cognition and Behavior

Support: NIH DP5 Early Independence Award
NARSAD Brain and Behavior Foundation
McKnight Foundation
Ludwig Family Foundation
Boston University

Title: Optogenetic induced extinction-like behaviors in ethanol withdrawn mice

Authors: *C. CINCOTTA[1], N. J. MURAWSKI[2], S. L. GRELLA[2], E. DOUCETTE[2], E. MERFELD[2], M. SHPOKAYTE[2], Y. ZAKI[2], A. HAMIDI[1], K. DORST[3], S. RAMIREZ[2]
[2]Psychological and Brain Sci., [3]Grad. Program in Neurosci., [1]Boston Univ., Boston, MA

Abstract: Withdrawal from chronic alcohol impacts the brain's stress and memory systems, which may underlie individual susceptibility to persistent drug seeking and stress-induced relapse. Preclinical studies demonstrate impaired fear memory processes in rodents withdrawn from alcohol, including abnormally heightened fear responses that are resilient to subsequent attenuation by extinction training. The underlying neural circuits mediating, and sufficient to intervene with, impaired extinction following alcohol withdrawal have remained elusive. First, we demonstrate that mice withdrawn from chronic ethanol show impaired fear extinction and heightened fear renewal relative to controls. Next, control mice that received a brief reactivation session prior to extinction showed enhanced extinction and lower reinstatement relative to non-reactivated controls; ethanol-withdrawn mice did not demonstrate this putative reconsolidation-extinction enhancement. We next labeled neural ensembles in the dentate gyrus (DG) with an inducible and activity-dependent virus cocktail which allows expression of channelrhodopsin-2 (ChR2) or a control protein (eYFP) in cells active during the formation of a contextual fear memory in mice withdrawn from chronic alcohol. Mice were placed into a distinct context and received chronic light stimulation directed at the DG over five consecutive days. Chronic reactivation of fear ensembles led to context-specific reductions in fear responses in control mice

expressing ChR2 (but not eYFP). This paradigm was successful in producing optogenetic induced extinction-like behavior effects in ethanol withdrawn mice. These results show how chronic reactivation of fear ensembles in the hippocampus may offer a means to facilitate extinction following withdrawal from chronic alcohol exposure. A mechanistic understanding of fear processes following drug withdrawal will aid in the development of therapies to attenuate stress-related cognitive dysfunction following drug withdrawal.

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Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

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Program #/Poster #: 424.12/III27

Topic: H.01. Animal Cognition and Behavior

Support: NIH DP5 Early Independent Award
NARSAD Brain and Behavior Foundation
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Title: Reactivating hippocampus-mediated memories to disrupt the reconsolidation of fear

Authors: *S. L. GRELLA, A. H. FORTIN, J. H. BLADON, N. J. MURAWSKI, Y. ZAKI, E. DOUCETTE, E. MERFELD, M. SHPOKAYTE, S. RAMIREZ
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Abstract: Background: Fear conditioning has been used to model memory and stress-related behaviors in rodents. While fear is often adaptive, dysregulation of fear circuits can lead to maladaptive states comprising mood and anxiety disorders. A promising prospect of attenuating the strength of fear memories is through the disruption of reconsolidation - a process by which activated memories are susceptible to modification. In previous studies, interventions such as protein synthesis inhibitors and beta-blockers have been used to target and disrupt conditioned fear during the reconsolidation process. Here, we propose a novel intervention based on the hypothesis that optogenetic reactivation of a previously formed, hippocampus-mediated memory during reconsolidation will disrupt the original fear memory, thereby reducing the behavioral expression of fear. **Method:** We combined the use of the activity-dependent inducible c-Fos-tTA system for neuronal tagging in c57BL/6 mice to tag dorsal dentate gyrus (dDG) cells active during a positive, neutral, or negative experience, and channelrhodopsin 2-mediated optogenetics. Mice were fear-conditioned and given a 20-minute recall session the following

day. During recall, we optically stimulated the dDG during the first or last 10 minutes of the session to reactivate the tagged dDG-mediated positive, neutral, or negative memories. Mice then received extinction training and were tested for stress-induced reinstatement on subsequent days or spontaneous recovery two weeks later. **Results:** Artificial reactivation of a positive memory during the last ten minutes of the recall session resulted in real-time decreases in freezing whereas reactivation of a neutral and negative memory did not produce similar decreases in freezing. Artificial reactivation of positive, neutral, and negative memories during the first ten minutes of the recall session resulted in faster rates of extinction learning and the attenuation of stress-induced reinstatement as well as lower levels of spontaneous recovery. Since these effects were observed when stimulation occurred during the first half of the recall session, it suggests that artificial memory reactivation may induce a priming effect or a general system perturbation serving to interfere with reconsolidation (and /or other) memory processes. We are currently investigating how widespread, non-specific hippocampal activation using a CAMKIIa promoter affects reconsolidation. **Significance:** This work highlights the therapeutic value of memory modulation as a viable treatment for the suppression of fear responses, implicating dDG cells as specific nodes of intervention.

Disclosures: S.L. Grella: None. **A.H. Fortin:** None. **J.H. Bladon:** None. **N.J. Murawski:** None. **Y. Zaki:** None. **E. Doucette:** None. **E. Merfeld:** None. **M. Shpokayte:** None. **S. Ramirez:** None.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 424.13/III28

Topic: H.01. Animal Cognition and Behavior

Support: NIH DP5 Early Independence Award
NARSAD Brain and Behavior Foundation
McKnight Foundation
Ludwig Family Foundation
Boston University

Title: Chronically reactivating positive and negative memories to modulate hedonic and social behaviors

Authors: *E. DOUCETTE, E. MERFELD, J. LOGAN, Y. ZAKI, S. L. GRELLA, N. J. MURAWSKI, M. SHPOKAYTE, A. HAMIDI, S. RAMIREZ, K. DORST, A. FINKELSTEIN
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Abstract: Chronic stress in mice induces a variety of anxiety- and depressive-like phenotypes at the neuronal, circuit, and behavioral levels. More specifically, social and hedonic-like states are dramatically impaired across many psychiatric disorders, though the underlying mechanisms sufficient to precipitate or alleviate such impairments remain largely unknown.
Here, we utilize an activity-dependent and inducible tagging strategy to modulate hippocampus (HPC) cells processing positive, neutral, or negative memories prior to chronic immobilization stress exposure. We hypothesized that chronic negative memory stimulation would impair social behavior and decrease hedonic-like activity, while positive memory stimulation would promote social behavior and increase hedonic-like activity.
We find that chronically stimulating hippocampal cells processing a valence-specific experience is sufficient to affect social but not anxiety-like behaviors. Furthermore, chronic reactivation of positive and negative memories modulates the behavioral response to chronic stress. We also find that chronic memory stimulation followed by chronic immobilization stress differentially affects body weight.
Our current experiments aim to delineate the underlying cellular activity during a variety of behavioral assays following chronic memory stimulation. In addition, our ongoing research focuses on interrogating the neural circuitry underlying affected behaviors, particularly examining functional connectivity between the hippocampus and the basolateral amygdala. Together our results connect chronic memory modulation with discrete maladaptive behavioral states and simultaneously provide a mechanistic basis for inducing a defined set of behavioral impairments.

Disclosures: E. Doucette: None. **E. Merfeld:** None. **J. Logan:** None. **Y. Zaki:** None. **S.L. Grella:** None. **N.J. Murawski:** None. **M. Shpokayte:** None. **A. Hamidi:** None. **S. Ramirez:** None. **K. Dorst:** None. **A. Finkelstein:** None.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: NIH DP5 Early Independence Award
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McKnight Foundation
Ludwig Family Foundation
Boston University

Title: Manipulating a social ensemble to modulate socially induced reinstatement of a contextual fear memory

Authors: *A. B. FINKELSTEIN[1], A. HAMIDI[2], Y. ZAKI[2], E. MERFELD[2], S. L. GRELLA[2], N. J. MURAWSKI[2], M. SHPOKAYTE[2], S. RAMIREZ[2]
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Abstract: Social interactions provide some of the most influential experiences in a social animal's life. The basolateral amygdala (BLA) and ventral hippocampus (vHPC) are two brain regions implicated in fear, anxiety, and social behaviors. Lesions to the BLA have been shown to decrease anxiety and increase social behaviors in novel environments. In social defeat paradigms, mice that underwent an aggressive attack exhibited increased BLA activity, and decreased vHPC activity. Finally, work by Ortiz and Tye has demonstrated that excitatory projections from the BLA to vHPC mediate changes in social behaviors. Collectively, these studies highlight the complementary and important role that the BLA and vHPC play in mediating anxiety and social behaviors. Here, we show that social interaction with a mouse that has received a foot shock induces non-generalized reinstatement of an extinguished fear memory in a portion of cagemates. Optogenetic excitation of basolateral amygdala (BLA), but not dentate gyrus (DG), cells that were previously active during the social encounter causes freezing in social reinstators but not non-reinstating cagemates and in a frequency-specific manner. However, activation of this ensemble is not necessary for reinstatement, as optogenetic silencing does not prevent reinstatement. Future experiments will test whether chemogenetic inhibition of the fear memory during the social encounter reduces reinstatement, and will explore overlap in cellular representation of the social and fear memories.

Disclosures: A.B. Finkelstein: None. **A. Hamidi:** None. **Y. Zaki:** None. **E. Merfeld:** None. **S.L. Grella:** None. **N.J. Murawski:** None. **M. Shpokayte:** None. **S. Ramirez:** None.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

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Program #/Poster #: 424.15/III30

Topic: H.01. Animal Cognition and Behavior

Support: NHMRC grant APP1078159

Title: Effect of combined adult vitamin D deficiency and social stress on spatial cognition in BALB/c mice

Authors: *M. AL AMIN, R. SULLIVAN, S. ALEXANDER, T. H. BURNE
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Abstract: Background
Vitamin D (VD) deficiency is prevalent in adults and is associated with cognitive impairment. However, it has not been established that VD deficiency is a causal factor leading to adverse health outcomes. It is possible that those VD deficient individuals are exposed to an additional insult leading to the progression of brain diseases. Therefore, the main aim of this study was to determine if AVD deficiency would exacerbate the effects of a secondary exposure, in this case social stress, in BALB/c mice.
Methods
Adult male BALB/c mice (10 weeks old) were fed a control or VD deficient diet for 10 weeks. To induce social stress, we further divided the animals into two groups, separated (SH) or socially defeated (SD). SH mice were housed 2 per cage separated with a perforated Plexiglass divider. SD mice were subjected to ten days of social defeat stress with a CD-1 mouse for 10 min/day. We then tested mice in a social preference test, and on the active place avoidance test to measure social preference and spatial learning and memory formation respectively. We performed Golgi-cox staining to measure the spine morphology of hippocampal CA1 (proximal branches) and Dentate gyrus (DG) neurons.
Results
We found that AVD-deficient mice were more susceptible to the effect of social stress in the social preference test; SD mice interacted more than the SH mice. SD mice had a longer latency to enter the shock zone in the APA test. We observed a lower mushroom spine density in AVD-deficient mice in the CA1 neurons. Moreover, the head extension and head extension to the centre was reduced in AVD-deficient mice. Interestingly, we found an increased in the number of thin spines in SD mice.
Conclusions
Our results support the hypothesis that VD deficiency may alter behavioral outcomes in mice susceptible to social stress. Spatial learning of SD mice could be correlated with increased formation of a thin spine in the CA1 neuron. However, reduced mushroom spine formation may affect spatial learning in AVD deficient mice. We suggest that pharmacological and genetic approaches be used to investigate the role VD deficiency and social stress in hippocampal-dependent spatial learning deficits in future studies.
Keywords: Vitamin D; stress; hippocampus; spatial learning, CA1, mushroom spine

Disclosures: M. Al amin: None. **R. Sullivan:** None. **S. Alexander:** None. **T.H. Burne:** None.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R15AG045812
AFPE Pre-doctoral fellowship for underrepresented minorities
Alzheimer's Association Grant NIRG-12-242187
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NINDS Grant R21NS101506

Title: Effects of LEC-specific P301L tau on object recognition memory

Authors: *S. SETTI, R. T. HESLIN, Y. DU, M. N. REED
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Abstract: Alzheimer's disease (AD) is biologically characterized by accumulation of beta-amyloid plaques, neurofibrillary tangles containing hyperphosphorylated tau protein, and pervasive neuronal damage ultimately leading to neuronal death. Because of the irreversible nature of the neuronal death, early detection of AD is important for any treatment aiming to halt or prevent neuronal damage. Currently, there are no existing cognitive screening tasks for AD optimized to assess function of one of the first brain regions affected in AD, the lateral entorhinal cortex (LEC). Tasks aimed to assess LEC function, as it is the first area to exhibit tau pathology, would aid in early detection of AD, thereby offering a better prognosis for affected individuals. In this study, we used a viral vector to introduce mutant P301L tau into the LEC of otherwise healthy male mice. Then, after 4 weeks, we assessed performance on an object recognition task previously identified to be sensitive to LEC lesions. Afterwards, we determined the presence of AD-associated pathology by using immunohistochemistry. Results indicate that tauP301L mice exhibit early stage-specific tau pathology and subtle learning and memory deficits when compared to control mice. Thus, our task is sensitive to AD pathology occurring within the LEC. Additionally, the literature suggests that neuronal activity alterations may mediate cognitive deficits in AD and promote the spread of tau pathology. Therefore, our next steps include examining whether using optogenetics to manipulate neuronal activity in the context of tau pathology can rescue cognitive deficits in this model.

Disclosures: S. Setti: None. **R.T. Heslin:** None. **Y. Du:** None. **M.N. Reed:** None.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 424.17/III32

Topic: H.01. Animal Cognition and Behavior

Title: Prenatal cannabinoid exposure results in learning and memory deficits in rodent adolescent offspring: Elucidation of the mechanism and identification of a therapeutic target

Authors: *P. D. PINKY, J. BLOEMER, S. E. SETTI, R. T. HESLIN, M. N. REED, V. D. SUPPIRAMANIAM
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Abstract: Cannabis use during pregnancy has increased by 62% from 2002 through 2014 and is now the most commonly used illicit drug during pregnancy with use ranging from 2-5% in most studies but as high as 15-28% among urban, low-income pregnant women. Human brain imaging studies show that functional network activity underlies the typical cognitive and behavioral processes reportedly altered by prenatal cannabinoid exposure (PCE), and that aberrant connectivity is linked to atypical functional development in other disorders. However, very little is known about the effects of prenatal cannabinoid exposure on early brain development in human infants, or on the formation of early functional networks that may underlie the cognitive and behavioral deficits reported in studies of exposed children. Here, we have investigated the impact of PCE in adolescent offspring in hippocampal dependent spatial learning and memory preforming a series of behavioral, electrophysiological and immunochemical studies. An osmotic pump filled with either N-Methyl Pyrulol (NMP) or the cannabinoid receptor full agonist WIN55,212-2 (2 mg/kg body weight/day) was implanted subcutaneously in Gestational Day-3 (GD-3) which delivered the drug at a constant rate until the delivery of the pups. Contextual Fear Conditioning (CFL) and Morris Water Maze (MWM) were performed to investigate the hippocampus based spatial memory which revealed significant deficits in the PCE animals. Electrophysiological experiment in Schaffer Collateral Pathway of Hippocampus also revealed significant impairment in NMDA mediated synaptic plasticity. In line with this, Immunochemical and Western blot data has shown increased Cannabinoid Receptor Type 1(CB1) expression followed by reduced Neural Cell Adhesion Molecule (NCAM) expression which postulates the observed behavioral deficits might be due to altered NMDA mediated glutamatergic neurotransmission in the hippocampus of these animals leading to impaired synaptic plasticity causing significant learning and memory deficits.

Disclosures: P.D. Pinky: None. **J. Bloemer:** None. **S.E. Setti:** None. **R.T. Heslin:** None. **M.N. Reed:** None. **V.D. Suppiramaniam:** None.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

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Topic: H.01. Animal Cognition and Behavior

Support: Packard Foundation
Lane Family
Friends and Alumni of Georgia Tech

Fulton County Elder Health Scholarship
Wright Family

Title: Non-invasive sensory stimulation targets deep brain structures in awake mice

Authors: *A. L. PAULSON[1], S. M. PRINCE[2], M. K. ATTOKAREN[3], L. ZHANG[3], A. C. SINGER[1]
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Abstract: Rhythmic brain activity is thought to be important for temporally precise coordination of neural activity to support a variety of brain functions. In the hippocampus, a deep brain region essential for spatial and episodic memory, rhythmic activity is thought to play a key role in learning and memory by modulating spiking across neurons and hippocampal subregions. Furthermore, in neurogenerative disease, such as Alzheimer's disease, there are deficits in rhythmic activity, particularly in the gamma band (30-50 Hz, also known as slow gamma). However, determining the causal role of rhythmic activity in hippocampal function or disease has been limited because of a lack of methods to drive such activity non-invasively in deep brain structures. Therefore, we developed a novel sensory stimulation technique to non-invasively drive rhythmic neural activity in deep brain regions. Prior work has shown that flickering light or sound stimulation drives rhythmic activity in primary sensory regions, visual cortex and auditory cortex, respectively, entraining neural activity to the frequency of the flickering stimulus. Surprisingly, we found that sensory flicker stimulation entrains activity beyond primary sensory areas. Using silicon probes, we recorded neural activity from awake, behaving mice as they were exposed to auditory or auditory-visual flicker stimulation at multiple frequencies between 20-100 Hz. We found that sensory flicker stimulation entrains single unit spiking activity not only in auditory cortex, but also in hippocampal area CA1 and prelimbic cortex, the mouse homolog of prefrontal cortex. Due to the non-invasive nature of this stimulus, driving rhythmic neural activity with sensory stimulation has a variety of applications, from understanding the role of rhythmic brain activity in cognitive functions, to developing therapeutics to treat neurodegenerative disease.

Disclosures: A.L. Paulson: None. **S.M. Prince:** None. **M.K. Attokaren:** None. **L. Zhang:** None. **A.C. Singer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognito Therapeutics.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 424.19/III34

Topic: H.01. Animal Cognition and Behavior

Support: Packard Foundation
Lane Family
Friends and Alumni of Georgia Tech
National Science Foundation Graduate Research Fellowship

Title: CA1 neural activity during spatial navigation in the 5XFAD mouse model of Alzheimer's disease

Authors: *S. M. PRINCE[1], A. L. PAULSON[2], L. ZHANG[3], M. K. ATTOKAREN[3], S. AMIGUES[3], J. H. TIPTON[3], A. C. SINGER[2]
[1]Emory Univ., Atlanta, GA; [2]Coulter Dept. of Biomed. Engin., Georgia Inst. of Technol. and Emory Univ., Atlanta, GA; [3]Georgia Inst. of Technol., Atlanta, GA

Abstract: Extensive research has revealed protein accumulation, synapse loss, and neurodegeneration in Alzheimer's disease, the most common form of dementia. However, it remains unclear how these changes lead to disruption of neuronal activity and cognitive impairment. Alterations in electrophysiological activity have been found in both humans with Alzheimer's disease (AD) and animal models of AD. Previously, we have shown decreases in gamma (30-100 Hz) power and gamma-modulated spiking activity in a well-established mouse model of Alzheimer's disease (5XFAD). These deficits were found specifically during hippocampal sharp-wave ripples, high frequency oscillations that are essential for spatial learning and memory. Not only is the hippocampus affected early in AD, but these neural deficits were also found before behavioral deficits emerged. These results, combined with proposed role of gamma in learning and memory, suggest that altered gamma activity contributes to memory deficits in Alzheimer's disease. Yet, the mechanism underlying these deficits in gamma activity during behavior remains unknown. In order to determine how neural circuits produce aberrant neural activity in a 5XFAD mouse model of Alzheimer's disease, we examined the contributions of different cell types to gamma activity during spatial navigation. We hypothesized that deficits in gamma oscillations occurred in 5XFAD mice due to changes in interneuron activity. To test our hypothesis, we designed a virtual-reality spatial navigation task that could be performed by both 5XFAD and wild-type (WT) animals. We then used silicon probes to record local field potential and single unit spiking activity from hippocampal subregion CA1, where gamma activity has been well characterized. All recordings were performed in awake, behaving, male 5XFAD mice and WT littermates during spatial navigation, and experimenters were blinded to genotype. Using spike rate, width, and autocorrelograms to classify putative cell types, we determined how spiking activity of putative pyramidal cells and interneurons differs between 5XFAD and WT mice. This work has broad applications in revealing how neural circuits for memory are disrupted in mouse models of Alzheimer's disease, and in bridging the gap between molecular pathology, neural activity, and cognitive deficits.

Disclosures: S.M. Prince: None. **A.L. Paulson:** None. **L. Zhang:** None. **M.K. Attokaren:** None. **S. Amigues:** None. **J.H. Tipton:** None. **A.C. Singer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognito Therapeutics.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 424.20/III35

Topic: H.01. Animal Cognition and Behavior

Support: NSERQ
CIHR

Title: OLM interneuron activity during goal-directed behaviors in a mouse model of Alzheimer's disease

Authors: *L. PENAZZI, J.-B. BOTT, C. LEGRAND, B. RIVARD, S. WILLIAMS
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Abstract: Oriens lacunosum-moleculare (OLM) cells represent a key class of GABAergic interneurons in controlling hippocampal network state and downstream behaviors. Their activitiy is associated to successful learning of new information. Recent reports demonstrate that OLM cells may be active in response to specific events such as aversive stimuli delivery (e.g. shock or air-puff) or to the locomotion state of the animal. However, it is still unknown whether functionally distinct OLM interneurons are selectively engaged during one of those events or both. In the pathology of Alzheimer's disease (AD), altered excitatory network activity is associated with cognitive deficits. Whether an impairment of OLM interneuron activity contributes to abnormal hippocampal function during learning and memory has not yet been determined.
We aim to characterize OLM activity patterns during distinct behavioral events underlying procedural and reference memory processes. To maximize the diversity of behaviors to investigate OLM activity, we performed calcium imaging in freely moving mice using the miniscope imaging technique. We used the Chrna2-cre mouse line to identify and control specifically OLM interneurons. To determine whether abnormal OLM cells contribute to the neuropathological feature of AD, we generated a novel transgenic Alzheimer mouse model Chrna2-J20 over-expressing a mutant form of human amyloid precursor protein with Swedish and Indiana mutations. OLM activity was analyzed during a diversity of behavioral events such as "avoidance" of aversive area, uptake of the sucrose-sweetened water, "reactivity" to aversive stimuli delivery, "locomotion" and "immobility".
This study using calcium imaging will help to reveal some of the key roles played by OLM interneurons in hippocampal function during goal-directed behaviors.

Disclosures: L. Penazzi: None. **J. Bott:** None. **C. Legrand:** None. **B. Rivard:** None. **S. Williams:** None.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 424.21/III36

Topic: H.01. Animal Cognition and Behavior

Title: Characterization of sex differences in hippocampus-dependent learning and memory

Authors: J. M. STRAUSS[1], K. D. STEVANOVIC[1], N. H. WU[2], *J. D. CUSHMAN[1]
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Abstract: Basic neuroscience research currently has a deficit in awareness of sex differences in neurobehavioral function; stifling translation to the human population. The current project is intended to characterize sex differences in hippocampus-dependent behavioral tasks and examine the underlying mechanisms. We assessed spatial, contextual and temporal learning using a battery of tests in two-month-old C57Bl6Tac mice: spontaneous alternation, novel context recognition (exploratory habituation), object recognition and tone and contextual fear conditioning. There were no sex differences in percent alternation, indicating similar spatial working memory, however females showed an overall increase in the number of arm entries. Males and females showed similar levels of exploratory behavior over two days of exposure to the same open field environment, including the typical reduction in exploratory behavior on the second day (referred to as exploratory habituation or novel context recognition). Novel object recognition was similar in males and females, with both showing a preference for investigating the novel object 24 hours after the familiarization phase. In the object location task males showed increased exploration of the displaced object after a 24 hour delay, whereas females did not. For tone fear conditioning we assessed hippocampus-independent delay, hippocampus-dependent trace and two non-associative controls: explicitly unpaired and sensitization (no tone present during training). Delay conditioning was similar; however, trace fear conditioning was enhanced in males. Females showed increased freezing in the sensitization group, responding fearfully to a novel tone after context-only fear conditioning. Overall these findings show task-specific sex differences indicative of a relative enhancement in hippocampus-dependent spatial and temporal learning in males. The enhanced non-associative sensitization learning has important implications as non-associative controls are rarely used, thus differences could be erroneously assumed to be due to altered associative learning when they are in fact due to non-associative changes. In addition, this finding could serve as a basis for modelling increased anxiety disorders in women. These findings are not only informative about general sex differences, to be studied in their own right; but they also set the baseline for interpreting data in transgenic models and advanced in vivo techniques.

Disclosures: J.M. Strauss: None. **K.D. Stevanovic:** None. **N.H. Wu:** None. **J.D. Cushman:** None.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

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Program #/Poster #: 424.22/III37

Topic: H.01. Animal Cognition and Behavior

Support: Cihr Grant
Fyssen fundation postdoctoral fellowship

Title: The role of medial septal glutamate neurons in CA1 principal cell activity during freely behaving navigation investigated with both calcium imaging and optogenetic silencing in mice

Authors: *J.-B. BOTT[1], L. PENAZZI[2], A. KY[1], S. WILLIAMS[3]
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Abstract: Recent evidence suggests that medial septum (MS) glutamatergic neurons through their projections to hippocampus may code for ongoing locomotion velocity. Since these results were obtained in head-fixed mice running on a treadmill, the role of MS glutamatergic cells in more cognitive-demanding behavior such as navigation remains underexplored.
Using miniscope calcium imaging, we characterized the spontaneous activity of identified glutamatergic neurons during navigation tasks in freely behaving mice. MS glutamatergic neurons form heterogeneous subpopulations of cells that are differentially active in relation to specific behaviors. MS glutamate neurons do not code for locomotion *per se* but rather for the spatial destination of both future and ongoing trajectories. The consequences of optogenetic silencing MS glutamatergic neurons before the onset of active navigation on both the precision of the navigation and CA1 principal cells activity was further explored using a combination of optogenetic and calcium imaging on the same freely-behaving mice.
Our results suggest that one subpopulations of medial septum glutamatergic neurons are indispensable for goal directed navigation.

Disclosures: J. Bott: None. **L. Penazzi:** None. **A. Ky:** None. **S. Williams:** None.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 424.23/III38

Topic: H.01. Animal Cognition and Behavior

Support: CIHR

Title: Investigating replay activity during Slow-wave and REM sleep in freely behaving mice

Authors: *J. CHOI[1], G. ETTER[3], S. WILLIAMS[2]
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Abstract: Several studies have pointed out the importance of sleep in memory functions. Periods of sleep can further be divided into rapid eye-movement (REM) periods characterized by prominent hippocampal theta (~8Hz) oscillations, and non-REM sleep that display slower hippocampal oscillations and occasional ultra-fast oscillations termed ripples. A large body of evidence indicates that ripple events observed during non-REM sleep are crucial for memory consolidation as they replay neuronal activations observed during wakefulness. Moreover, previous studies have pointed out increased ripple frequency after novel experience. More recent studies from our laboratory show however that altering hippocampal activity during REM sleep can induce a significant reduction in memory consolidation. We combined in vivo electrophysiology and calcium imaging using the miniscope technique to see whether wakefulness-related neuronal activity could be replayed during ripple-events but also during REM periods. In addition, we determined whether the temporal organisation of the cell assemblies during sequences is preserved between REM and wakefulness brain states. This would support previous studies that suggest that hippocampal temporal firing is governed by internal, intrinsic properties, rather than external stimuli only. Importantly, we observed that the proportion of the cells that were reactivated during REM sleep increases after the mouse is running the open field. This study sheds light on the mechanisms that underlie memory consolidation during different sleep stages.

Disclosures: J. Choi: None. **G. Etter:** None. **S. Williams:** None.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

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Program #/Poster #: 424.24/III39

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant ES102805

Title: Sex-specific effects of locus coeruleus norepinephrine loss on hippocampus-dependent learning

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Abstract: Locus coeruleus norepinephrine (LC-NE) signaling is involved in the modulation of diverse behaviors and physiological processes, including anxiety and learning. Yet, little is known about the long-term effects of embryonic loss of LC-NE on these behaviors, primarily due to the inability to selectively target the LC. Global synthesis of NE can be disrupted by mutating the dopamine β-hydroxylase (*Dbh*) gene, but *Dbh* null mice die *in utero*. While the embryonic lethality can be rescued by supplying pregnant dams with a synthetic precursor of NE, the prenatal treatment obscures any developmental phenotypes and does not permit dissection of brain-specific functions. To overcome these limitations, we generated a conditional knockout allele of *Dbh* (*Dbh cKO*). To specifically target the LC, we crossed *Dbh cKO* with $En1^{cre}$ (LC-NE mutants). LC-NE mutants survive to adulthood, allowing us to evaluate the consequences of embryonic disruption of LC-NE on adult behavior. We examined the performance of mutant and littermate control mice in a series of behavioral tasks. As previously reported for *Dbh* null mutants, LC-NE mutants showed normal levels of anxiety in the elevated plus maze and in the open field, as well as deficits in context-dependent, but not cue-dependent fear conditioning. Unlike the *Dbh* null mice, however, context-dependent learning was affected only in male LC-NE mutants, suggesting that early disruption of LC-NE affects hippocampus-dependent memory in a sex-specific manner. Moreover, while the deficits in contextual learning were no longer observed in *Dbh* null mice two weeks after training, those exhibited by LC-NE males persisted. This finding suggests that the contextual fear deficit in LC-NE males is not specific to short term retrieval. Mutant males also showed deficits in pre-exposure-dependent contextual fear conditioning, which has previously been shown to depend on LC input to hippocampal area CA3. In addition, LC-NE mutants exhibited sex-dependent changes in other aspects of hippocampal-dependent learning, including novel context and object recognition tasks.

Together, these findings suggest that embryonic disruption of LC-NE has selective, sex-specific effects on hippocampus-dependent memory and confirm the utility of our *Dbh cKO* allele for investigating the long-term consequences of early disruption of NE signaling.

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Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 424.25/III40

Topic: H.01. Animal Cognition and Behavior

Support: CIHR
NSERC
FRQS

Title: Essential role of acetylcholine in memory consolidation during REM-sleep

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Abstract: Spatial memory has been recently shown to be consolidated during rapid eye movement (REM) sleep since disrupting normal theta activity specifically during this state through the optogenetic silencing of medial septum (MS) GABAergic neurons impaired spatial and contextual fear memory (Boyce et al., 2016). In this study, we have examined the effects of MS cholinergic neuron inhibition on hippocampal activity during REM sleep following fear conditioning learning.
Viral delivery of the inhibitory opsin ArchT (n=7) or control eYFP (n=7) were done targeting the MS of ChAT-Cre mice. An optic fiber was implanted in the MS and tungsten electrodes were placed in the CA1, CA3 and subiculum of the hippocampus to record local field potentials. Three weeks later, mice received contextual fear conditioning (30s of cue tone followed by 50uA of electric foot shock) and were then returned to their home cage. Optogenetic silencing was performed specifically during REM sleep for a 4hr period following conditioning. On the following day, contextual and cue memory were tested by measuring the freezing duration. We found that silencing MS cholinergic neurons specifically during REM sleep modulated theta-gamma cross-frequency coupling. Following fear conditioning, phase-amplitude coupling between theta (5-12 Hz) and gamma band (30-60 Hz) significantly increased in the CA1 of eYFP control group during REM sleep (118±10%, Modulation Index compared before and after

learning). However, such an increase was suppressed in the ArchT mice group where cholinergic neurons were silenced (85±8%). This reduction in the modulation index was specific to the CA1 area and was not observed in CA3 or subiculum.
Our study demonstrates that MS cholinergic neurons play an essential role for hippocampal-dependent contextual memory consolidation during REM sleep. In addition, the present results propose that the modulation of cross-frequency coupling is an important mechanism underlying how acetylcholine promotes memory consolidation.

Disclosures: S. Williams: None. **J. Kang:** None. **J. Bott:** None. **G. Etter:** None. **F. manseau:** None.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

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Topic: H.01. Animal Cognition and Behavior

Support: NSERC/IRSC

Title: Role of medial septum parvalbumin neurons in supporting hippocampal predictive coding in normal and Alzheimer's disease conditions

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Abstract: The exact role of the hippocampus in spatial learning and memory is still not fully understood. Earlier work and the discovery of place cells has comforted the existence of a cognitive map that relies on distance and direction codes that arise from entorhinal cortex grid cells which represent one of the main input to hippocampal principal neurons. Alternative theories support the role of the hippocampus in predictive coding, both online, during exploration through phase precession (firing of place cells ahead of their place fields) and offline, during sharp-wave ripples (preplay of trajectories to be explored). More recent models inspired by reinforcement learning frameworks propose that the hippocampus codes for representations that maximize future reward by performing continuous predictions (Stachenfeld et al., 2017). Importantly, hippocampal sequences can only be reliably observed in environment involving a memory task (Pastalkova et al., 2008) and medial septum pharmacological inactivations are associated with perturbed place cells offline, but not online processing (Wang et al., 2014). Importantly, place fields persist (Koenig et al., 2011) and can emerge in new environments (Brandon et al., 2014) under pharmacological medial septum inactivations that are associated with reduced hippocampal theta oscillations and grid fields abolition. However, since

these experiments were performed in an environment that was devoid of a memory task, it is difficult to conclude on the impact of decreased theta on hippocampal predictive coding. We propose that hippocampal theta rhythms that are supported specifically by medial septum parvalbumin interneurons activity contribute to offline predictions of the future behaviourally relevant locations to be explored. We combined chronic recordings of large CA1 hippocampal cell assemblies (>1000 neurons per subject) in mice performing in a spatial working memory task that involved enriched multimodal sensory cues. Because of the neurochemical complexity of the medial septum, we combined our calcium imaging recordings with targeted optogenetic modulation of medial septum parvalbumin cells to transiently modulate or abolish theta rhythms. In parallel and as a negative control, we performed calcium imaging in an APP mouse model in similar tasks to verify the relationship between CA1 predictive coding and spatial working memory performances.

Disclosures: G. Etter: None. **B. Rivard:** None. **S. Williams:** None.

Poster

424. Animal Cognition and Behavior: Learning and Memory: Hippocampal Circuits IV

Location: SDCC Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: Grants-in-Aid for Science Research on Innovative Areas "Brain Information Dynamics" (18H05114)
Kaken-hi (17H05939; 17H05551)

Title: Encoding of elapsed time in the order of minutes by hippocampal pyramidal neurons

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Abstract: The hippocampus is involved in the formation of episodic memories including spatial and temporal contexts. While it has been well established that hippocampal pyramidal neurons encodes spatial information, little has been tested about how hippocampal neurons represent temporal contexts. Recently, several studies have demonstrated an interplay across brain regions to process seconds-range time intervals with tens of seconds. However, neural correlates of time periods in the order of minutes by hippocampal pyramidal neurons have not been extensively examined. Here, we developed a novel behavioral task in which rats were placed in a test chamber (25 cm x 25 cm) with a feeding port at a specific location. The animals were rewarded if they poked a reward port to collect single 45-mg food pellets that were presented for 2-3 s every five-minute waiting time period. Trained animals showed a gradual increase in their frequency of nose-poke behavior in individual five-minute waiting time periods. Chronic in vivo

electrophysiological recordings using tetrode assemblies from the dorsal hippocampal CA1 area revealed that about half of hippocampal neurons showed a gradual increase or decrease in their firing rates, so-called ramping activity, during the waiting time periods. The firing rates of these neurons were specifically correlated with elapsed times without being affected by poke behavior, animal's positions, and animal's head direction. Our results suggest that hippocampal neurons periodically and repeatedly show ramping activity to cover a duration on a time scale of minutes.

Disclosures: Y. Shikano: None. **T. Sasaki:** None. **Y. Ikegaya:** None.

Poster

425. Learning and Memory: Molecular Mechanisms

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Program #/Poster #: 425.01/III43

Topic: H.01. Animal Cognition and Behavior

Support: MH078064

Title: Transcriptome analysis of state-dependent memory transition from recent to remote

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Abstract: Overwhelming stressful experiences can lead to mental illnesses known as dissociative disorders. They are thought to arise when normally integrated functions of consciousness, such as memory, perception, and identity awareness become disrupted. Dissociative symptoms are often debilitating, and persist for a long time after the initial stress has ended. While many dissociative symptoms are intrinsic to human sufferers, and therefore cannot be studied in rodents, this is not the case with dissociative amnesia, which can be modeled in rodents using state-dependent learning. Fear-inducing memories can be state-dependent, meaning that the retrieval of a memory is most efficient when occurring under the same state of consciousness as when the memory was encoded. We have previously demonstrated that encoding of state-depended memories required a distinct molecular mechanism from encoding under normal conditions. State-dependent memories are long-lasting and they retain their state-dependence, suggesting that distinct mechanisms initiated at encoding are maintained during consolidation and transition from recent to remote. Here we analyzed the global changes in gene expression using high throughput RNA sequencing to identify individual genes and cellular pathways contributing to the transition or fear-inducing memories from recent to remote under normal and state-dependent conditions. For these analyses, mice were fear conditioned under normal or state-dependent conditions, and samples collected 1 day (recent) or 30 days (remote) later. Our results show that consolidation of memories and their transition from

recent to remote under normal and state-dependent conditions occurs through the same cellular pathways, yet involving mostly distinct sets of genes belonging to these pathways. These results suggest that changes of cognitive states require alterations of the activity of distinct sets of genes within conserved pathways.

Disclosures: V. Jovasevic: None. **F. Sananbenesi:** None. **A. Fisher:** None. **J. Radulovic:** None.

Poster

425. Learning and Memory: Molecular Mechanisms

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Topic: H.01. Animal Cognition and Behavior

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Carver chair in Neuroscience to TA

Title: Role of nuclear receptor Nr4A in hippocampus dependent memory formation: A single-neuronal nuclear RNA sequencing (sn-nuc rna seq) approach

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Abstract: New experiences are initially encoded as labile short-term memories and then converted into stable long-term memory by gene transcription-dependent processes. Gene expression after learning involves a transient wave of transcription that is critical for memory consolidation. Following early transcriptional activity, learning induces persistent long-lasting transcriptional changes that are reported to be involved in the storage of long-term memory. Increased transcript levels of 13 nuclear receptors, including all 3 members of the Nr4A subfamily, have been identified after learning. In this study, we show that transgenic mice expressing a dominant-negative form of Nr4A (Nr4ADN) in forebrain excitatory neurons have impaired long-term spatial memory. Following a spatial object recognition task, we removed dorsal hippocampi from Nr4ADN mice and wildtype controls and performed single-neuronal nuclear RNA sequencing (Sn-Nuc RNA seq). This employs a Drop-Seq based method to allow nuclei from single neurons to be processed individually. We found significant downregulation of genes related to long-term spatial memory and synaptic plasticity in CA1 and CA3 excitatory

neurons. Differentially expressed genes were validated at the single-cell level using the *in situ* hybridization technique RNAscope. Although no cellular ligands have been identified for the Nr4A sub-family of transcription factors, we and others have observed that synthetic molecules like C-DIM drugs bind to Nr4A and modulate its transactivation function. In additional studies, we found that C-DIM12, a putative NR4A2 ligand enhances spatial memory and Nr4A regulated genes in adult mice. These results further underscore the importance of Nr4A during hippocampus dependent memory storage.

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Poster

425. Learning and Memory: Molecular Mechanisms

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Topic: H.01. Animal Cognition and Behavior

Support: Science Foundation Ireland
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Title: Characterising the function and transcriptional regulation of a cluster of genes core to hippocampal memory consolidation

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Abstract: Memory formation and recall are fundamental processes that involves information processing and consolidation that control cognitive function. Understanding the molecular background underlying normal cognition would provide insights to identify impairments underpinning range of neurodegenerative and neuropsychiatric disorders. During the learning event, a cascade of molecular signals are initiated recruiting genes that translate to proteins to mediate morphological alterations of synapses in the hippocampus, a central region for memory consolidation. Previous studies suggest that the early phase (0-6h) of memory consolidation involve several waves of gene expression regulation and subsequent protein expression change that drives synaptic restructuring involving formation of new synaptic connections. This growth phase seems to be followed by selective retention and pruning of synapses to restore the circuit to the baseline level of connectivity. Much of the molecular underpinnings of these events remain poorly elucidated.
Recently, we have revisited a temporal microarray study we conducted looking at increasing time points following either water maze or passive avoidance learning. We found 609 and 700 genes to be transcriptionally regulated across 24h post-learning following spatial learning and

passive avoidance conditioning, respectively. Comparing those gene lists, we were able to find an overlapping cohort of 135 genes regulated following both tasks. This cohort could identify a core memory consolidation-specific program that is recruited regardless of the nature of the task. We have begun to characterise the function and transcriptional regulation of these genes. Using primary hippocampal cultures, we were able to characterise morphological changes exerted by two members of the cluster, midkine and klotho, which seem to mediate distinct effects on the growth of neuronal processes. Furthermore, we have been able to show that the plasticity-associated neurotransmitter glutamate can control the expression of klotho but not midkine suggesting the need for convergent signals during actual memory formation to regulate the cognition cluster as a coordinated whole. Finally, we have used genomatix promotor analysis software to identify several potential transcriptional regulators of the cognition-associated genes and have confirmed the classic, memory-associated transcription factor CREB to be a regulator of several genes in the cognition-associated cluster. These studies begin to dissect the function and transcriptional regulation of a core cognition-associated gene cluster regulated during memory consolidation.

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Poster

425. Learning and Memory: Molecular Mechanisms

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Title: Defects in Arc turnover impair cognitive flexibility

Authors: *M. A. GHANE[1], M. J. WALL[3], D. R. COLLINS[4], S. L. CHERY[1], Z. D. ALLEN[1], E. D. PASTUZYN[5], A. J. GEORGE[1], V. D. NIKOLOVA[6], S. S. MOY[7], B. D. PHILPOT[8], J. D. SHEPHERD[5], J. MULLER[9], M. D. EHLERS[10], A. M. MABB[2], S. A. CORREA[11]
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Abstract: Arc/Arg3.1, an immediate early gene and molecular coordinator of synaptic plasticity, is transcriptionally and translationally regulated by network-wide activity. The temporal expression of Arc protein is largely mediated through the ubiquitin-proteasome pathway, yet the importance of such turnover is currently unknown. We created an Arc knock-in mouse (ArcKR) wherein the predominant Arc ubiquitination sites were mutated. Arc mRNA and protein expression are altered in ArcKR mice, leading to reduced mGluR-LTD threshold and enhanced mGluR-LTD amplitude. ArcKR mice had intact spatial learning but showed specific deficits in selecting an optimal strategy during reversal learning. Alterations in reversal learning suggest a global deficit in cognitive flexibility and is likely a result of underlying changes in neural coding mediated through proper turnover of Arc.

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Poster

425. Learning and Memory: Molecular Mechanisms

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Title: Reduced dendritic mRNA localization and AMPAR surface expression by RNG105/caprin1 deficiency

Authors: *R. OHASHI[1,2], Y. SHINODA[3,4], S. SHIGENOBU[2,5], Y. KIMORI[2,6,7], T. FURUICHI[4], N. SHIINA[1,2,8]
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Genomics Facility, Nat'l Inst. for Basci Biol., Okazaki, Japan; [6]Dep. of Imaging Sci., Nat'l Inst. for Nat. Sci., Okazaki, Japan; [7]Fukui Univ. of Technol., Fukui, Japan; [8]Exploratory Res. Cent. on Life Living Systems, Okazaki, Japan

Abstract: Protein synthesis in neurons is essential for long-term memory formation, in which dendritic mRNA transport and local translation are believed to play roles. However, molecular mechanisms involved are not fully understood. In this study, we focused on RNG105/caprin1, a major RNA-binding protein in RNA granules that are responsible for mRNA transport and local translation, because RNG105-deficient mice display impaired long-term memory formation.
We comprehensively identified mRNAs whose dendritic localization was dependent on RNG105. In this identification, the hippocampal CA1 region, in which somas of pyramidal neurons are aligned in the stratum pyramidale (SP) and dendrites elongate in the stratum radiatum (SR), was used. We microdissected and isolated SP and SR from 12-week-old mice, extracted mRNAs from the isolated layers, and subjected them to RNA-seq. We identified 1,122 SR-enriched mRNAs (dendritic [D-] mRNAs) and 2,106 SP-enriched mRNAs (somatic [S-] mRNAs) in control mice (*Rng105*$^{f/f}$). Comparison between control and RNG105 conditional knockout (cKO) mice (*Camk2a-Cre; Rng105*$^{f/f}$) revealed that a considerable subset of D-mRNAs was reduced in the SR of RNG105 cKO mice. GO enrichment analysis of the RNG105-dependent D-mRNAs found that a category of "Regulation of Arf (ADP-ribosylation factor) protein signal transduction", which included Arf regulators GEFs and GAPs, had a high-fold enrichment score. RNG105-dependent dendritic localization of Arf GEF and GAP mRNAs was confirmed by imaging of mRNAs fused to the MS2-binding sequence with MS2-GFP in primary cultured cerebral neurons from wild-type and RNG105 KO mice. These results indicated that RNG105 was a key regulator of dendritic localization of mRNAs for the Arf regulators.
Arf is known to be involved in AMPAR recycling in dendrites. In addition, fEPSP amplitude of hippocampal CA1 neurons was markedly reduced in RNG105 cKO mice. Given these results, we analyzed AMPAR (GluR1) surface expression in primary cultured neurons with immunostaining. The number of surface GluR1 puncta in dendrites was significantly reduced in RNG105 KO neurons compared with wild-type neurons. Taken together, these results suggested that RNG105-dependent dendritic localization of mRNAs for regulators of AMPAR distribution, such as Arf regulators, is an underlying mechanism for synaptic potentiation and long-term memory formation.

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Poster

425. Learning and Memory: Molecular Mechanisms

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Title: RNG105/caprin1, an RNA granule protein, regulates structural spine plasticity and is required for long-term memory formation

Authors: *K. NAKAYAMA[1,2,3], M. ABE[4], M. YAMAZAKI[4], A. FUJIKAWA[5], M. NODA[5,3], A. FUTATSUGI[6], K. MIKOSHIBA[7], K. SAKIMURA[4], N. SHIINA[1,2,3]
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Abstract: Local translation in neuronal dendrites plays an essential role in synaptic plasticity. Local translation is mediated by RNA granules, large RNP complexes containing mRNAs, ribosomes and RNA-binding proteins. However, the relevance of RNA-binding protein components of RNA granules to long-term memory in vertebrate animals remains elusive. In this study, we show roles of RNG105/caprin1, a major RNA binding protein in RNA granules, in structural spine plasticity and long-term memory formation. By applying the glutamate-uncaging technique to control neurons (*Rng105*$^{flox/flox}$) and RNG105 conditional knockout (cKO) neurons (*CMV-Cre*; *Rng105*$^{flox/flox}$), we found that RNG105 was required for the late phase of structural spine plasticity, but not for the initial phase. Furthermore, in the hippocampal neurons of RNG105 cKO mice (*CaMK2a-Cre*; *Rng105*$^{flox/flox}$), the proportion of the number of large spines to total spines was significantly reduced as compared with neurons of control mice (*Rng105*$^{flox/flox}$). We then examined whether RNG105 is involved in learning and memory. In Morris water maze, spatial memory was markedly impaired in RNG105 cKO mice. Furthermore, in contextual fear conditioning tests, 5-minute memory was normal, but one-day or longer memory was affected in RNG105 cKO mice. These results demonstrated that RNG105 regulates the structural plasticity of spines and is required for long-term memory formation.

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Poster

425. Learning and Memory: Molecular Mechanisms

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Title: Enhancer RNAs are necessary and sufficient for gene transcription and neuronal function

Authors: *N. CARULLO[1], A. J. SALISBURY[3], R. C. SIMON[2], K. D. BUNNER[2], J. S. REVANNA[4], K. E. SAVELL[2], F. SULTAN[2], J. J. DAY[2]
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Abstract: Enhancer elements in the DNA regulate gene expression programs important for cell fate and function in the developing and adult brain. The majority of DNA sequence variants linked to neuropsychiatric diseases and intellectual disabilities fall in non-coding DNA regions, and numerous variants have been linked to altered enhancer function. Moreover, enhancer activity drives transcription changes in response to a variety of signals. A significant fraction of regulatory DNA regions, like enhancers, are subject to bidirectional, RNA polymerase II-dependent transcription that results in non-coding enhancer RNAs. However, the potential role of eRNAs in neuronal function and activity-dependent responses remains unclear. Here, we used primary cortical neuronal cultures to investigate enhancers and eRNAs in the rat genome. We first identified actively transcribed enhancers using RNA sequencing from non-polyadenylated RNA fractions. This revealed thousands of active enhancers, with enrichment near genes involved in neurodevelopment, synaptic communication, and neuropsychiatric disease. We validated enhancer function at selected candidate enhancer-promoter pairs using a CRISPR-dCas9 system in which dCas9 is fused to a strong transcriptional activator (VPR) to allow for targeted activation of specific genomic loci. CRISPR-VPR mediated activation of enhancers promoted eRNA synthesis and produced corresponding increases in mRNA in all candidate pairs, whereas activation of promoters increased target mRNA but not eRNA. To explore the potential role of eRNAs in more detail, we focused on specific eRNAs arising from enhancers surrounding *Fos* (or *c-Fos*), an immediate early gene that codes for a transcription factor implicated in neuronal plasticity and cognitive processes. Transcription of *Fos* eRNAs is dynamically modulated by various forms of neuronal activity, requires RNA polymerase II, and

precedes activity-dependent *Fos* mRNA induction. Single-molecule FISH revealed nuclear localization of eRNAs, activity-dependent increases in eRNA arising from the most distal *Fos* enhancer and *Fos* mRNA, as well as a positive correlation between eRNA and mRNA within the same cells. Anti-sense based *Fos* eRNA knockdown decreased *Fos* mRNA expression, whereas mRNA knockdown did not affect eRNA levels. In contrast, CRISPR-targeted delivery of eRNA to a *Fos* enhancer elevated mRNA induction following neuronal depolarization. Finally, we show that knockdown of a single *Fos* eRNA is sufficient to alter neuronal activity patterns in vitro using a multi electrode array system. Overall, these findings indicate that eRNAs modulate gene expression and neuronal physiology.

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Poster

425. Learning and Memory: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 425.08/III50

Topic: H.01. Animal Cognition and Behavior

Support: Sh.Rustaveli Science Foundation, Grant 217592

Title: Micro-RNA130b-3p and its targets in the recognition memory of imprinting in domestic chicks

Authors: *R. O. SOLOMONIA[1], G. MARGVELANI[1], M. MEPARISHVILI[1], T. KIGURADZE[2], B. J. MCCABE[3]
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Abstract: Visual imprinting is a learning process whereby young animals come to recognize a visual stimulus by being exposed to it (training) and subsequently prefer it to other objects. Much evidence has implicated a restricted forebrain region, the intermediate medial mesopallium (IMM), in memory for visual imprinting in the domestic chick. Learning-related, time-dependent molecular changes occur in the IMM after imprinting, indicating molecular regulation during memory formation. We have inquired whether certain micro-RNAs (miRNAs), which typically suppress protein translation, are involved in such regulation. Twenty-four hours after training, miRNA spectra in the left IMM were compared between chicks termed good learners, characterised by high preference score (a measure of memory strength), and chicks with low preference scores (poor learners); a μParaflo™ microarray assay was used**.** Using criteria of effect size and expression level, we chose gga-miR-130b-3p for further study and inquired

whether amount of miRNA was correlated with preference score. In left and right IMM, amount of gga-miR-130b-3p decreased significantly with preference score, but only when learning was observed. No effects were detected in the posterior pole of the nidopallium (PPN), a brain region not involved in imprinting. In both left and right IMM, residual variance from the regression with preference score was significantly lower than the variance of untrained chicks. Since the variances of trained and untrained chicks did not differ significantly from each other, the correlations were attributable not to training but to a predisposition, i.e. propensity to learn, independent of training.
We studied two gga-miR-130b-3p targets, cytoplasmic polyadenylation element binding proteins 1 (CPEB1) and 3 (CPEB3), in two subcellular fractions (P2 membrane-mitochondrial and cytoplasmic) of IMM and PPN. Only in the left IMM was a learning-related change observed: a positive correlation between amount of membrane CPEB3 and preference score, with protein amount increasing only when learning occurred. Residual variance from the regression with preference score was not significantly different from the variance of untrained chicks. Taken together, the results suggest that the increase in membrane CPEB3 with preference score was a results of training and specific to learning.
Our results indicate that animals predisposed to learn well possess reduced miR-130b-3p activity in the IMM. One of this miRNA's targets, membrane CPEB3, is up-regulated by training, reflecting the learning that occurs. This effect is lateralised, being restricted to the left IMM.

Disclosures: R.O. Solomonia: None. **G. Margvelani:** None. **M. Meparishvili:** None. **T. Kiguradze:** None. **B.J. McCabe:** None.

Poster

425. Learning and Memory: Molecular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 425.09/III51

Topic: H.01. Animal Cognition and Behavior

Support: CIHR PJT-153311
CRC 950-231066
FRSQ 35457

Title: Cell-specific knockdown of TSC1 increases mTORC1 signaling and facilitates late LTP induction in somatostatin interneurons, and promotes long-term hippocampal memory

Authors: *J. ARTINIAN, E. HONORE, A. W. JORDAN, A. KHLAIFIA, I. LAPLANTE, J.-C. LACAILLE
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Abstract: Long-term synaptic plasticity is a prime candidate as cellular substrate for learning and memory which remains largely unexplored in inhibitory interneurons. In the hippocampal CA1 region, excitatory synapses onto somatostatin interneurons (SOM-INs) show cell type-specific metabotropic glutamate receptor 1 (mGluR1)-mediated long-term potentiation (LTP) that regulates hippocampal network plasticity, persist 24h and requires translation via Mechanistic Target Of Rapamycin Complex 1 (mTORC1). We previously showed that impaired mTORC1 signaling in SOM-INs prevents learning-induced LTP in SOM-INs, reduces CA1 network metaplasticity and decreases contextual fear and spatial memories, indicating that mTORC1-mediated LTP in SOM-INs is necessary for intact hippocampal memory (Artinian et al., SfN 2017 abstract 428.21).
In this study we investigated if mTORC1 activity in SOM-INs is sufficient to modulate translation-dependent interneuron LTP and hippocampal memory, by using a knockdown of Tuberous Sclerosis Complex 1 (TSC1), an upstream repressor of mTORC1, selectively in SOM-INs (SOM-TSC1$^{+/-}$ mice).
We first determined that basal ribosomal S6 protein phosphorylation, a downstream effector of mTORC1, was increased in SOM-INs of SOM-TSC1$^{+/-}$ mice and that repeated treatment with mGluR1a agonist failed to further increase S6 phosphorylation in SOM-TSC1$^{+/-}$ mice. Whole-cell recordings revealed normal intrinsic properties of SOM-INs. A single treatment with mGluR1 agonist was sufficient to induce late-LTP (increased minimally-evoked excitatory transmission) in SOM-INs of SOM-TSC1$^{+/-}$ mice, whereas repeated treatment was necessary in WT mice, indicating that TSC1 knock-down upregulates mTORC1 activity and facilitates late-LTP induction in SOM-INs. At the behavioral level, SOM-TSC1$^{+/-}$ mice showed slightly decreased locomotion and normal anxiety in the open-field test. They showed improved long-term spatial reference memory in the Barnes maze, as well as stronger contextual fear memory and context discrimination deficits. Short-term contextual fear and long-term cued-fear memories were intact. These results indicate that increasing mTORC1 function in SOM-INs is sufficient to promote hippocampal memory consolidation.
Our findings show that cell-specific knockdown of TSC1 in SOM-INs is sufficient to increase mTORC1 signaling and facilitate mGluR1-mediated late LTP induction in SOM-INs, and to promote long-term hippocampal memory. Thus mTORC1 activity in SOM-INs appears necessary and sufficient for learning-induced persistent LTP in SOM-INs, up-regulation of CA1 network plasticity, and hippocampal memory precision.

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Poster

425. Learning and Memory: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 425.10/III52

Topic: H.01. Animal Cognition and Behavior

Support: Emmy Noether grant (OL437/1-1)
Travel grant from Graduate Academy University of Heidelberg

Title: Mimicking age-associated Gadd45γ decline results in memory impairments in young mice

Authors: *D. VILHENA CATARINO BRITO, J. KUPKE, K. GÜLMEZ KARACA, B. ZEUCH, A. MM OLIVEIRA
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Abstract: With an increasingly aged population age-associated cognitive decline is a major health and socio-economic burden. Understanding the mechanisms underlying progressive cognitive loss is required to develop future therapies. The underlying causes of age-associated cognitive decline are largely unknown. We found that Gadd45γ expression is decreased during basal conditions and spatial learning in the hippocampus of old male mice, compared to young adult mice. This decrease is selective, as other family members are expressed at comparable levels in young adult and aged mice.
We hypothesized that if Gadd45γ would be involved in age-associated cognitive decline, then abolishing its expression in the hippocampus of young mice should promote memory deficits. Indeed, we found that knocking down Gadd45γ in the hippocampus of young adult male mice, generates age-like memory deficits in long-term and short-term memories. Abolishing the expression of other family members did not produce the same phenotype. Hence, our findings show a selective role of Gadd45γ in memory formation, which may underlie memory deficits observed during ageing. Next, we identified signaling pathways regulated by Gadd45γ that may mediate its activity during synaptic plasticity. Specifically, decreasing the expression of Gadd45γ disrupted MAPK signaling and downstream targets in dissociated hippocampal neurons. Importantly these alterations were not due to altered stimulus-dependent intracellular calcium dynamics.
This data suggests that age-associated decrease in Gadd45γ expression may be associated with age-dependent cognitive deficits.

Disclosures: D. Vilhena Catarino Brito: None. **J. Kupke:** None. **K. Gülmez Karaca:** None. **B. Zeuch:** None. **A. MM Oliveira:** None.

Poster

425. Learning and Memory: Molecular Mechanisms

Location: SDCC Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: Emmy Noether grant (OL437/1-1)
SFB1134 (C01)
Travel Grant from Graduate Academy, University of Heidelberg
Travel Grant from HBIGS, Heidelberg

Title: Learning triggered Dnmt3a2 overexpression within hippocampal neuronal ensembles is sufficient to enhance memory

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Abstract: It is currently thought that memories are stored within neuronal ensembles in the brain. Although it has been shown that the recall of memories depends on the reactivation of neurons active at the time of learning, the molecular and cellular mechanisms within the neuronal ensembles that underlie their formation and stabilization are not known. Epigenetic mechanisms play a critical role in memory storage, therefore appear like good candidates in the regulation of these processes. However, until now, neuronal ensemble-specific manipulations aimed at the elucidation of the molecular mechanisms underlying their formation and stabilization have not been performed. Here, we hypothesized that Dnmt3a2, a *de novo* DNA methyltransferase critically involved in cognition, improves the formation and stabilization of the neuronal ensembles engaged in hippocampus-dependent memory, and its overexpression within neuronal ensembles is sufficient to strengthen memory. To address this, we used a synthetic, neuronal activity dependent promoter, E-SARE, to manipulate the levels of Dnmt3a2 specifically within the neuronal ensembles formed in the DG of the hippocampus of adult male mice upon contextual fear learning. We found that overexpressing Dnmt3a2 in the DG neuronal ensembles after a hippocampus-dependent fear learning was sufficient to enhance long-term fear memory. Furthermore, we showed that this cognitive enhancement required an ensemble-specific Dnmt3a2 upregulation, as overexpressing Dnmt3a2 in a similarly sized but random subset of neurons in the DG did not improve long-term fear memory. Currently, we are investigating the mechanisms underlying the cognitive enhancement triggered by Dnmt3a2 overexpression within the ensembles associated with spatial memory. Overall, in this study, we performed for the first time a neuronal ensemble-specific manipulation of a DNA methyltransferase and show that this is sufficient to impact memory storage. This study brings a novel perspective on how epigenetic factors may contribute to cognitive function and opens up a new venue to explore memory storage mechanisms at a neuronal ensemble level.

Disclosures: K. Gülmez Karaca: None. **J. Kupke:** None. **B. Zeuch:** None. **A.M. Oliveira:** None.

Poster

425. Learning and Memory: Molecular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 425.12/III54

Topic: H.01. Animal Cognition and Behavior

Support: KAKENHI JP 22500301
KAKENHI JP 16K08511

Title: Involvement of calmodulin kinase IIalpha in hippocampus- vs. amygdala-dependent memory revealed by kinase-dead knock-in mouse

Authors: *Y. YAMAGATA[1,3], Y. YANAGAWA[4], K. IMOTO[2]
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Abstract: Ca^{2+}/calmodulin-dependent protein kinase IIα (CaMKIIα) has been thought to be a key mediator of activity-dependent neuronal modifications and play a key role in the molecular mechanisms of learning and memory. So far, several types of CaMKIIα knock-in and knock-out mice have confirmed its essential role in hippocampal synaptic plasticity and behavioral learning. However, it is still not clear how CaMKIIα is involved in different types of memory. To better understand its involvement in amygdala-dependent memory as compared to hippocampus-dependent memory, here we performed biochemical analyses and behavioral memory tests using the kinase-dead CaMKIIα (K42R)-knock-in mouse (Yamagata et al., J Neurosci, 2009). In the Morris water maze tasks, homozygous mutants performed well in the visible platform trials, while they failed to form spatial memory in the hippocampus-dependent hidden platform trials. In fear conditioning, these mice were impaired but showed a certain level of amygdala-dependent cued fear memory that lasted as long as four weeks, while they showed virtually no hippocampus-dependent context discrimination memory. Neither stronger stimulation nor repetitive stimulation compensated for their memory deficits. The differential outcome of hippocampus- and amygdala-dependent memory in the mutant mouse was not due to differential expression of CaMKIIα between the hippocampus and the amygdala, because biochemical analyses revealed that both kinase activity and protein levels of CaMKII were indistinguishable between the two brain regions. These results indicate that kinase activity of CaMKIIα is indispensable for hippocampus-dependent memory, but not necessarily for amygdala-dependent memory. There may be a secondary, CaMKIIα activity-independent pathway, in addition to the CaMKIIα activity-dependent pathway, in the acquisition of amygdala-dependent memory.

Disclosures: Y. Yamagata: None. **Y. Yanagawa:** None. **K. Imoto:** None.

Poster

425. Learning and Memory: Molecular Mechanisms

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Topic: H.01. Animal Cognition and Behavior

Support: National Natural Science Foundation of China 31430032
National Natural Science Foundation of China 81329003

Title: ErbB4 signaling in the infralimbic cortex regulates fear extinction

Authors: *Y. CHEN[1], L. BI[2], S. ZHANG[2], T. GAO[2]
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Abstract: Many psychiatric diseases such as post-traumatic stress disorder (PTSD) are characterized by abnormal processing of emotional stimuli particularly fear. The medial prefrontal cortex (mPFC) is critically involved in fear extinction, which is defined as the learned reduction of fear. However, the molecular mechanisms underlying this process are largely unknown. ErbB4 receptors, the only tyrosine kinase that can both bind to NRG1 and become a functionally active homodimer, are abundant in parvalbumin (PV)-expressing interneurons in the PFC. In this study, we aimed to determine how NRG1/ErbB4 signaling in the mPFC modulates fear extinction and found that fear extinction increased NRG1 expression in the mPFC. Fear extinction was impaired following neutralization of endogenous NRG1 and specific inhibition or genetic ablation of ErbB4 in the infralimbic (IL) cortex but not in the prelimbic cortex. Furthermore, ErbB4 deletion specifically in PV neurons impaired fear extinction. Notably, overexpression of ErbB4 in the IL cortex is sufficient to reverse impaired fear extinction in PV-Cre;ErbB4− / −mice. Together, these findings identify a previously unknown signaling pathway in the IL cortex that regulates fear extinction. Our study may shed new light on the pathophysiology of this disorder and help to improve its treatments.

Disclosures: Y. Chen: None. **L. Bi:** None. **S. Zhang:** None. **T. Gao:** None.

Poster

425. Learning and Memory: Molecular Mechanisms

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Program #/Poster #: 425.14/III56

Topic: H.01. Animal Cognition and Behavior

Support: Korea NRF 2013M3C7A1056102
NIH NS087112
NIH NS034007
NIH NS047384
US Public Health Service MH18501

Title: Inositol polyphosphate multikinase mediates extinction of fear memory

Authors: J. PARK[1], F. LONGO[2], *S. KIM[1], S. PARK[1], S. LEE[1], M. BAE[3], J.-H. HAN[1], E. SANTINI[4], E. KLANN[2], S. H. SNYDER[5]
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Abstract: Inositol polyphosphate multikinase (IPMK), the key enzyme for the biosynthesis of higher inositol polyphosphates and phosphatidylinositol 3,4,5-trisphosphate, also acts as a versatile signaling player in regulating tissue growth and metabolism. To elucidate neurobehavioral functions of IPMK, we generated mice in which IPMK was deleted from the excitatory neurons of the postnatal forebrain. These mice showed no deficits in either novel object recognition or spatial memory. IPMK conditional knockout mice formed cued fear memory normally but displayed enhanced fear extinction. Signaling analyses revealed dysregulated expression of neural genes accompanied by selective activation of the mTOR regulatory enzyme p85 S6 kinase 1 (S6K1) in the amygdala following fear extinction. The IPMK mutants also manifested facilitated hippocampal long-term potentiation. These findings establish a novel signaling action of IPMK that mediates fear extinction.

Disclosures: J. Park: None. **F. Longo:** None. **S. Kim:** None. **S. Park:** None. **S. Lee:** None. **M. Bae:** None. **J. Han:** None. **E. Santini:** None. **E. Klann:** None. **S.H. Snyder:** None.

Poster

425. Learning and Memory: Molecular Mechanisms

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Program #/Poster #: 425.15/III57

Topic: H.01. Animal Cognition and Behavior

Support: JSPS Grant 16K19007

Title: Plasmalogens enhance spatial memory by increasing synaptic plasticity

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Abstract: It was previously known that the plasmalogens (Pls) are reduced in the Alzheimer's disease (AD) brains. Pls are the type of ether phospholipid characterized by the presence of a vinyl ether linkage at the sn-1 position. To understand how these lipids are reduced in the brain, we previously reported that aging, inflammation and stress signals downregulate the gene encoding a Pls-synthesizing enzyme, *Gnpat* (Glyceronephosphate O-Acyltransferase). We also reported that the reduction of Pls in the brain increased the glial activation. However, the role of Pls in the synaptic plasticity and memory function was mostly elusive. The clinical study showed that the oral intake of sPls (Pls extracted from the scallop) improved the cognition among mild AD patients. In the microarray study, we noticed that various synaptic function related genes were upregulated when the neuronal cells were stimulated by the sPls. To examine the role of Pls in the memory and cognition, we reduced the Pls in the murine brain hippocampus by shRNA against *Gnpat* (shGNPAT) and observed a significant reduction of spatial memory ($p<0.01$, n=7). This evidence suggest that Pls have an important role in the hippocampal dependent memory and it may be due to the increased expression of memory related gene expression. This was further supported by our recent findings that Pls drinking for three months in adult B6 mice improved memory by enhancing the BDNF-TrkB signaling which was associated with an increased expression of synaptic related gene expression. The Pls treatments increased the dendritic spines in the cultured neuronal cells. The golgi cox staining also showed that the Pls drinking increased the dendritic spines in the murine hippocampal neurons which is believed to be associated with the increased synaptic potential. We still do not know how the Pls can increase the gene expression. However, our previous study showed that Pls can activate ERK and Akt signaling, which may play a role behind the gene regulation. The future study will be conducted to investigate the detail mechanism of Pls-mediated memory related gene expression. Our research outcome may help us to understand the sPls-mediated cognition improvement among the mild AD patients.

Disclosures: M. Hossain: None. **T. Fujino:** None.

Poster

425. Learning and Memory: Molecular Mechanisms

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Program #/Poster #: 425.16/III58

Topic: H.01. Animal Cognition and Behavior

Support: NIMH

Title: M1 muscarinic receptor-dependent protein signaling underlying visual recognition in monkey

Authors: *B. A. CORGIAT[1], C. MUELLER[2], R. C. SAUNDERS[4], J. L. OLDS[3], L. LIOTTA[2], M. MISHKIN[5], J. N. TURCHI[6]
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Abstract: Visual recognition memory is critically dependent upon the perirhinal cortex (PRh). More specifically, visual memory formation requires cholinergic activation of PRh M1 muscarinic receptors and is also characterized by enhanced multiunit activity in the upper middle and deep PRh layers. However, the M1 muscarinic-dependent intracellular signaling pathways underlying the critical synaptic changes induced during visual memory formation remain unknown. Here, we used a proteomic approach to assess M1-dependent protein signaling in specific PRh layers and in other implicated brain regions after visual recognition memory training.
Experimentally naïve monkeys (n=4) first learned the delayed non-matching-to-sample (DNMS) rule in a Wisconsin General Testing Apparatus using 1200+ junk objects as stimuli. Once criterion was met, they received forebrain commissurotomies plus unilateral optic tract transections. Once recovered, animals were retrained on DNMS and the stimulus list-lengths (LL) and trials completed per day were gradually increased. The mnemonic difficulty of the DNMS task was tailored to each animal and variable LLs (range: 3-10) were used so each animal performed at criterion (above 75% correct in 78-80 trials). After a testing session, monkeys were quickly sedated, euthanized, and specific brain structures were excised and snap-frozen in bilateral pairs. Samples from two behaviorally naïve control subjects receiving the same surgery were collected in the same manner 7 months post-op.
Layers III and V/VI of ventral part of anterior area TE (TEav) and PRh, layers II/III and V/VI of entorhinal (ERh), and the pyramidal cell layer of hippocampal subregions CA1, subiculum, and the dentate gyrus were microdissected and then lysed. Along with the microdissected lysates, whole region lysates created from TEav, PRh, ERh, hippocampus, tail of the caudate nucleus, primary visual cortex, posterior parietal cortex, and prefrontal regions (areas 11/13, area 45, area 9, lateral area10) were printed onto reverse phase protein microarrays (RPPAs). We stained RPPAs with antibodies against M1-dependent signaling proteins and compared the phosphorylation states in the hemisphere which received more extensive visual mnemonic training to the contralateral control hemisphere.

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Poster

425. Learning and Memory: Molecular Mechanisms

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Program #/Poster #: 425.17/III59

Topic: H.01. Animal Cognition and Behavior

Support: AFOSR

Title: Proteomic analysis determines molecular pathway differences between tDCS current intensity groups in rats

Authors: *J. WAGNER[1], S. H. JUNG[4], C. N. HATCHER-SOLIS[5], Q. V. QUALLEY[7], M. P. JACKSON[8], R. J. MOORE[2], N. A. BECHMANN[3], J. A. MARTIN[6], R. JANKORD[5]
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Abstract: Previous studies have demonstrated the positive effects of transcranial direct current stimulation (tDCS) on cognitive performance; however, no study has been conducted to determine the molecular processes involved in the modulation of behavior, specifically protein expression in the hippocampal synapse. tDCS at anodal current intensities of 250µA and 500µA was applied to Sprague Dawley rats for 30 minutes prior to both training and testing of a passive avoidance test (PAT); hippocampal tissue was harvested and synaptic proteins were profiled. Multiple bioinformatics methods were employed to analyze the protein regulation difference between the two current intensity groups. Interestingly, pathway analysis between 250µA and 500µA showed differences; highlighted processes of interest are: cadherin signaling pathway, glutamate receptor pathways, G-protein mediated pathways, wnt signaling pathway, oxidative stress response, and some neuronal diseases. DAVID Annotation clustering analysis identified multiple clusters, including mitochondrial membrane (enrichment score: 2.3), prenylation (1.6), immunoglobulin domain (1.24), etc. The Ingenuity Pathway Analysis (IPA) network analysis created 12 networks, and the 6 networks with a networks score greater than 20 are mainly associated with cell death, metabolisms, molecular transport, protein trafficking, and free radicals. Further analysis regarding canonical pathways of IPA networks analysis found similarly related functions such as: mitochondrial dysfunction, some neuronal diseases, protein trafficking, cell death and survival, free radial scavenging, and cell morphology. Based on the IPA Upstream Analysis of causal networks and IPA results of brain-associated diseases and functions we are able to show that the anodal 500uA of tDCS are related to negative molecular and functional changes in hippocampal synapse: e.g., decreases in growth of neurites, neuronal density and cell viability and increases in inflammatory signaling, apoptosis of neurons, and cell death. The upstream analysis of IPA causal networks identified multiple upstream candidate regulators,

including lrrc4, fbxw8, prkcz, bdnf, il3, thpo, mir-214, etc. In conclusion the data has successfully shown significant findings utilizing a shot gun proteomic experiment, focusing on hippocampal synapse, which has further elucidated mechanisms involved in the behavioral effects of tDCS in rats.

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Poster

425. Learning and Memory: Molecular Mechanisms

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Program #/Poster #: 425.18/III60

Topic: H.01. Animal Cognition and Behavior

Support: NIH-NINDS Child Neurologist Career Development Award (CNCDP-K12)
UC Irvine School of Medicine Department of Pediatrics

Title: A unique mouse model of early-life exercise enables lasting hippocampal synaptic plasticity and memory

Authors: T. YU[1], E. A. KRAMÁR[3,4], S. PARIEVSKY[2], T. L. VU[2], M. A. WOOD[3,4], *A. S. IVY[1,4]
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Abstract: Exercise in adulthood is a powerful regulator of cognitive function. Molecular mechanisms underlying exercise's effects on hippocampus-dependent learning and memory have been extensively characterized in rodent models. Less known is whether exercise taking place during early-life periods of hippocampal development can regulate its function later in life. It is well established that exogenous experiences occurring during early-life developmental periods can have long-lasting effects on memory and neuronal function. We address the hypothesis that aerobic exercise occurring during specific periods of postnatal hippocampal maturation can have enduring consequences on hippocampus-dependent memory and synaptic plasticity. In addition, we explore molecular and epigenetic mechanisms that may give rise to the persistent changes in neuronal function engaged by early-life exercise. Wild-type C57Bl/6J mice were placed in cages with either locked or unlocked running wheels on day of weaning (postnatal day (P) 21) for three exercise durations: a 3-week juvenile-adolescent period (P 21-41); a 1-week juvenile period (P21-27), and a 1-week period of later adolescence (P35-41). Subthreshold and threshold training durations for long-term memory formation were identified in the Object Location Memory

(OLM) task in a separate group of age-matched sedentary mice. Between P43-P50, mice from running-wheel exposed groups were then tested in the OLM task or were used to examine long-term potentiation (LTP), a form of synaptic plasticity, in hippocampal CA1 Schaffer collateral pathway. Both the 3-week exercise group and the 1 week juvenile-exercise group formed long-term memory for novel object location when exposed to a typically subthreshold training duration for memory acquisition. LTP was increased in adolescent mice that underwent early life exercise during the juvenile-adolescent period. Notably, input/output curves generated in the Schaffer collaterals showed greater fEPSP responses in early-life exercised groups compared to sedentary controls. Neuronal gene expression and histone modifications after OLM training of exercised mice will be measured by RT-qPCR and ChIP-qPCR, respectively, for candidate genes identified by transcriptome sequencing. These preliminary results suggest that early-life exercise can improve spatial memory and synaptic plasticity and identifies a juvenile, critical period of hippocampal plasticity during which an exercise experience can have a lasting effect on memory function.

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Poster

425. Learning and Memory: Molecular Mechanisms

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Topic: H.01. Animal Cognition and Behavior

Support: NSFC 81471123
NSFC81671071

Title: Molecular profiles in the brain are involved in fear memory induced by physical and psychological stress

Authors: *J. H. WANG[1], W. LU[2]
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Abstract: Fear memory induced by physical and/or psychological stress influences human's life, which may lead to anxiety or depression. The removal of fear memory is important to reduce the occurrence of psychological disorders. Molecular mechanisms underlying fear memory remain to be elucidated. We have used the intruder/resident model to investigate the alternation of molecular profiles in the brain areas related to sensation, emotion and cognition from mice that have experienced physical and/or psychological stress, in which mRNA and microRNA profiles have been analyzed by high throughput sequencing. The strength and maintenance of fear memory induced by physical and psychological stress are higher and longer than those induced

by psychological stress alone. Numerous mRNAs and microRNAs are differentially expressed among the prefrontal cortex, nucleus accumbens, amygdala, visual cortex and somatosensory cortex in the mice that experience physical/psychological stress versus psychological stress. Genes of encoding molecules are engaged in the following signaling pathways, such as dopaminergic synapse, cholinergic synapse, serotonergic synapse, GABAergic synapse, glutamatergic synapse, calcium signaling, Wnt signaling, MAPK signaling, PI3K-Akt signaling, axon guidance, cell adhesion molecules and so on. Through these analyses, we are able to figure our molecular profiles related to fear memory induced by physical and psychological stress in brain region-specific manner, which should be helpful to attenuate and remove fear memory.

Disclosures: J.H. Wang: None. **W. Lu:** None.

Poster

425. Learning and Memory: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 425.20/III62

Topic: H.01. Animal Cognition and Behavior

Support: Canadian Diabetes Association
St. Boniface Hospital Foundation
Manitoba Health Research

Title: Secreted amyloid precursor protein alpha overexpressing neural stem cells increase cognition in healthy mice

Authors: *G. W. GLAZNER[1], B. AULSTON[1], G. L. ODERO[2]
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Abstract: Secreted amyloid precursor protein alpha (sAPPα) is a neurotrophic factor that plays a pivotal role in learning and memory acquisition. Numerous studies demonstrate that sAPPα administration in the brain can enhance cognition in multiple species of animals and decreased sAPPα levels are hypothesized to contribute to cognitive impairment associated with Alzheimer's disease. Here, we tested the hypothesis that sAPPα overexpressing neural stem cells (sAPPα-NSCs) could improve cognition in healthy in mice. sAPPα-NSCs and wild-type NSCs (Wt-NSCs) were engrafted into the hippocampi of 7-month old SAMR1 mice and cognition evaluated 6 weeks later using the Morris water maze (MWM). Both types of NSCs survived implantation and differentiated primarily into astrocytes. Strikingly, sAPPα-NSC injected mice performed better in both the acquisition trials and in the probe trial compared to Wt-NSC injected mice and artificial cerebrospinal fluid (ACSF) treated controls. These datum demonstrate that NSCs can be utilized to improve cognition via clinically available methods and warrant additional studies examining the therapeutic potential of sAPPα-NSCs

Disclosures: G.W. Glazner: None. **B. Aulston:** None. **G.L. Odero:** None.

Poster

425. Learning and Memory: Molecular Mechanisms

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Topic: H.01. Animal Cognition and Behavior

Support: NSFC Grant 31500968
NSFC Grant 81601121

Title: Neuropeptide s ameliorates pathological damages and spatial memory impairment in the app/ps1 mouse model of Alzheimer's disease

Authors: *P. ZHAO, X. QIAN, N. SUN, C. WEI, Y. NIE, G. CHAI
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Abstract: Alzheimer's disease (AD) is a common neurodegenerative disorder with the characteristics of a progressive deterioration of memory and cognition. However, there is no effective cure for this devastating disease at present. Neuropeptide S (NPS) is an endogenous peptide in central nervous system which has comprehensive functions. Central administration of NPS produces anxiolytic-like effects, promotes arousal, inhibits food intake, and plays an important role in learning and memory processes. The mechanisms by which NPS modifies cognitive processes and the potential therapeutic roles of NPS in AD have not been determined. In the present study, we examined the effects of NPS on the pathological changes and spatial memory impairment of eight month-old amyloid precursor protein/presenilin 1 (APP/PS1) transgenic AD mice. The results showed that NPS intracerebroventricular (i.c.v) injection (1nmol) for a week ameliorated spatial memory deficits and promoted dendrite ramification and spine generation in hippocampal CA1 neurons, which was accompanied by the upregulation of postsynaptic density protein 95, synapsin1 and synaptophysin. We also found that i.c.v injection of NPS decreased cerebral amyloid plaques through the up-regulation of γ-secretase activity and by decreasing the phosphorylation of APP at Thr668. Furthermore, application of NPS reversed the deficits in hippocampal late-phase long-term potentiation. These findings suggested NPS attenuated cognitive deficits by reducing pathological features in APP/PS1 mice and NPS might be a potential therapeutic agent for Alzheimer's disease. (1387)

Disclosures: X. Qian: None. **N. Sun:** None. **C. Wei:** None. **Y. Nie:** None. **G. Chai:** None.

Poster

425. Learning and Memory: Molecular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 425.22/III64

Topic: H.01. Animal Cognition and Behavior

Support: DP130103687
DE150101478

Title: The role of the basolateral amygdala complex in consolidation of second-order conditioned fear

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Abstract: *Background.* This study pursued our recent findings that consolidation of a second-order fear memory requires DNA methylation, but not de novo protein synthesis, in the basolateral amygdala complex (BLA). It specifically used an animal model (fear conditioning in rats) to examine whether the BLA gene and protein networks that have been implicated in the consolidation of first-order conditioned fear (e.g., established through tone-shock pairings) are also involved in the consolidation of second-order conditioned fear (established when tone-shock pairings in stage 1 of training are followed by light-tone pairings in stage 2). The genes of interest were analysed using RT-PCR: they were selected for their relevance to kinase signalling pathways (e.g., PKA, PKC, CaMKII, CaMKIV, MAPK1, MAP2K7) and nuclear processes (e.g., CREBBP, EGR-1 and DNMT3A) that have been differentially implicated in consolidation of first- and second-order conditioned fear. The protein networks were analysed using TMT-10plex LC-MS/MS.
Results. Relative to controls that received tone-shock pairings in stage 1 and context alone exposures in stage 2, rats that underwent second-order conditioning (i.e., that received tone-shock pairings in stage 1 and light-tone pairings in stage 2) exhibited a unique profile of gene and protein changes in the BLA. There were three major findings. First, all genes included in our analyses were upregulated in the control group that was re-exposed to the conditioned context. Second, after second-order conditioning, the majority of these genes were NOT upregulated; and a network of proteins was explicitly downregulated. Third, this dowregulated network of proteins included Mapk1, Map2k7, Mras and Rasa1, which we have previously shown to play a limited role in consolidation of second-order fear.
Conclusions. Taken together, these results provide molecular support for our recent findings that consolidation of second-order fear does not require ERK/MAPK signalling or de novo protein

synthesis in the BLA. They are discussed with respect to the established roles for CaMK signalling and DNA methylation in consolidation of second-order fear and other types of memory.

Disclosures: N.M. Holmes: None. **K. Clemens:** None. **A. Shvetcov:** None. **M. Mirzaei:** None. **F. Westbrook:** None.

Poster

425. Learning and Memory: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 425.23/III65

Topic: H.01. Animal Cognition and Behavior

Title: Molecular dissection of active forgetting in *Drosophila*

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Abstract: A highly flexible nervous system requires not only the generation of memories but also forgetting. The pioneering studies have identified two small G proteins, Rac1 and Cdc42, which separately regulate active forgetting of labile anesthesia-sensitive memory (ASM) and consolidated anesthesia-resistant memory (ARM) in *Drosophila*. It remains unclear how the two proteins actively regulate forgetting of different memory components. Here, the current study reveals downstream molecular signaling pathways underlying Rac1- and Cdc42-dependent active forgetting mechanisms respectively: WAVE acts as downstream of Rac1 in ASM forgetting, while WASP functions as the downstream of Cdc42 in ARM forgetting. These two proteins contribute to both natural memory decay and interference-induced forgetting. Knocking down them in mushroom body neurons leads to slower memory decay and significant resistance of interference-induced forgetting. Conversely, overexpressing them accelerates memory decay. Surprisingly, Arp2/3 complex, which is widely reported to be a shared downstream effector, is only required in ARM forgetting, but not in ASM forgetting. Furthermore, we also provide an explanation of active forgetting of labile memory at synaptic level. Thus, learning itself activates two pathways to actively erase the formed memory: Rac1/WAVE pathway for labile memory lasting for hours and Cdc42/WASP/Arp2/3 pathway for consolidated memory lasting for days. Such two pathways may manage forgetting through different actin remodeling mechanisms which determine presynaptic structure changes.

Disclosures: Y. Gao: None. **Y. Peng:** None. **X. Zhang:** None. **Y. Zhong:** None.

Poster

425. Learning and Memory: Molecular Mechanisms

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Program #/Poster #: 425.24/III66

Topic: H.01. Animal Cognition and Behavior

Support: KAKENHI
JSPS fellowship
Brain-MINDS
CREST-AMED
Takeda Science Foundation

Title: Specification of a remote memory cell ensemble during cortical tagging

Authors: *R. KIM, Y. KONDO, T. KAWASHIMA, M. INOUE, Y. ISHII, K. INOKUCHI, K. SAKAI, M. NONAKA, M. SAKAMOTO, H. OKUNO, H. BITO
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Abstract: Formation of long-term memories (LTM) for episodes and events require two distinct mechanisms of consolidation. Initially, recent memories are rapidly acquired in the hippocampus through synaptic plasticity, and then strengthened via a cellular consolidation process through new gene expression and protein synthesis. Subsequently, however, via a time-dependent mechanism referred to as systems consolidation, these LTM are slowly consolidated into the neocortical network through dynamic interaction between the hippocampus and the neocortex. Although molecular and genetic mechanisms for cellular consolidation have been intensively investigated, mechanistic insights on systems-level memory consolidation still remain elusive. In this study, we introduce a novel transgenic mouse line that can permanently label and manipulate a temporally-defined active neuronal ensemble. Using this, we investigated the cellular and molecular basis of the memory dynamics that underlies time-dependent systems-level memory consolidation. We found that a cortical "proto-engram" is generated during encoding, in parallel to the formation of a hippocampal engram. Memory engram transfer from the hippocampus to the neocortex was in fact accounted for by a functional awakening of this silent cortical "proto-engram", which was specified during cortical tagging. How can a proto-engram remain resistant to recall during a recent memory phase, but subsequently slowly mature into an "engram" that is activatable during remote memory retrieval? To address this question, we have investigated several activity-dependent pathways implicated in memory consolidation mechanisms. Altogether, our results indicate that a cellular consolidation-like process critically governs key steps of systems consolidation.

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Poster

425. Learning and Memory: Molecular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 425.25/III67

Topic: H.01. Animal Cognition and Behavior

Title: Proteomic modification in hippocampal synapse following noninvasive brain stimulation

Authors: *S. H. JUNG[1,2], A. QUALLEY[2], J. WAGNER[2], R. MOORE[2], N. BECHMANN[2], M. JACKSON[2], J. MARTIN[2], C. HATCHER[2], R. JANKORD[2]
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Abstract: Transcranial direct current stimulation (tDCS) is known to enhance cognitive performance and neuroplasticity, but its underlying mechanisms in the brain are still unknown. The purpose of this study was to investigate effects of tDCS on protein regulation in the hippocampal synapse. Two different current intensities (anodal 250uA and 500uA) of tDCS were applied to Sprague Dawley rats (male, 9-10 weeks old) for 30 min for 2 days, just before training and testing of passive avoidance test (PAT). Hippocampal synaptic proteins were profiled by the Waters Acquity UPLC M-class LC and normalized proteomic data were analyzed by multiple bioinformatics methods. Compared to the sham group, anodal 250uA tDCS upregulated 26 proteins, and 17 protein expression were down-regulated. The expression levels of only 20 proteins were significantly modified by anodal 500uA of tDCS when compared to the sham group. Multiple signalings were identified by GO enrichment analysis. From both tDCS intensities, membrane (GO:0016020) and synapse (GO:0045202) were significantly detected. Multiple terms associated with mitochondrial function were identified only from the anodal 500uA. Pathway analysis identified both shared and unique pathways between 250uA and 500uA. Inflammatory pathway (P00031) detected from both current intensities. Annotation clustering analysis identified some unique annotations, including PDZ domains (Enrichment score: 2.3 & 1.2, respectively). Multiple IPA networks were created for anodal 250uA and 500uA. The networks for both intensities are associated with Protein Synthesis and Cellular Development, Growth and Proliferation. Neurological Disease is the top disease detected from the comparison of 500uA and sham. Only one module created by WGCNA was significantly correlated with PAT latency, and 3 proteins (*gdi1*, *ran* & *ndufs6*) resulted in the most weighted connections in the module. Expression levels of these proteins were significantly associated with PAT latency. Best subset regression method to compare all possible regression models for both

PAT latency and current intensity compared more than 800 models and identified 2 and 6 models for the variables of current intensity and PAT latency, respectively. *Fabp7* were detected from all 8 regression models, and significantly inverse correlation between the expression of *fabp7* and PAT latency were detected while a negative trend was observed with the increased intensity of tDCS. In conclusion, our data provide molecular evidence for effects of tDCS on the hippocampal synapse and suggest candidate synaptic molecules in the hippocampus that are modulated by anodal tDCS.

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Poster

426. Human Cognition and Behavior: Working Memory

Location: SDCC Halls B-H

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Program #/Poster #: 426.01/III68

Topic: H.02. Human Cognition and Behavior

Support: CONACYT 238826
UNAM DGAPA PAPIIT IG300618

Title: Mediators of spatial and verbal working memory decay across the adult life span

Authors: *S. CANSINO[1,2,3], F. TORRES-TREJO[1], C. ESTRADA-MANILLA[1], J. G. MARTÍNEZ-GALINDO[1], E. HERNÁNDEZ-RAMOS[1], M. AYALA-HERNÁNDEZ[1], T. GÓMEZ-FERNÁNDEZ[1], M. D. RAMÍREZ-GONZÁLEZ[2], S. RUIZ-VELASCO[3]
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Abstract: Working memory abilities significantly decrease with advancing age; hence, the search for factors that may increase or mitigate this decline is critical. Several factors have been identified that influence working memory beside age, such as education, socioeconomic status, nutrient intake and health status, among many others. However, the influence of these variables has mostly been analyzed separately and rarely together with other factors in the same sample. The aim of the present study was to identify factors that jointly act as mediators of working memory decay across the adult life span. We examined a total of 120 variables that included sex; education; income; nutrient intake; alcohol and drug consumption; medication intake; health status; glucose, cholesterol and triglyceride levels; heart rate; physical, cultural and mental activities; and self-perception of memory ability. A lifespan sample of 1652 healthy adults (831 women) between the ages of 21 and 80 participated in the study. The n-back task was used to

evaluate working memory in the spatial and verbal domains. Structural equation modeling analyses were conducted to search for potential mediators that intervened between age and working memory. Age was treated as an exogenous variable and all the rest of the variables were included as mediators. All models were found to have a power of 1 when the alpha level was set to .05, the null RMSEA to .05 and the alternative RMSEA to .08. The study is exploratory because to be able to estimate the effects of numerous factors on memory it is necessary to employ observational methods. Only 14 and 10 variables reliably mediated spatial and verbal working memory, respectively. Some factors positively mediate the effects of age on working memory decay in both domains (education, Mini Mental State Examination, believes about their own memory capacity, alcohol intake, computer use and social activities), while others negatively mediate these effects (anxiety related to memory performance; palmitic acid consumption). Bootstrapped test of mediation revealed that bias-corrected confidence intervals were significant for each of the individual indirect effects, and for the total indirect effects in both models. We identified the factors that are sufficiently powerful to influence working memory decline when they jointly interact, as in everyday living.

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Poster

426. Human Cognition and Behavior: Working Memory

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 426.02/JJJ1

Topic: H.02. Human Cognition and Behavior

Support: MH112206

Title: Frontoparietal theta tACS enhances verbal working memory in healthy humans with high working memory capacity

Authors: *Z. HU[1], A. J. WOODS[1], C. RANA[1], I. B. SAMUEL[1], S. MEYYAPPAN[1], R. GAO[2], M. DING[1]
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Abstract: Theta oscillations are hypothesized to play an important role in working memory by mediating the interaction between frontal and parietal structures. To date, evidence supporting the hypothesis has mainly come from correlative studies; the causal relation between theta oscillations and working memory remains to be better established. In this study, we stimulated the frontoparietal network at individual theta frequencies via two 4x1 electrode arrays centered at F3 and P3, respectively, in three schemes: in-phase, anti-phase, and sham. Twenty healthy

subjects performed a Sternberg verbal working memory task with three levels of memory load (load 2, 4 and 6), imposing three levels of cognitive demand, during stimulation and after stimulation. High-density EEG (128 channel) were recorded throughout the experiment. Individual differences in working memory abilities were assessed by working memory capacity (WMC) derived from the OSPAN task. Analyzing behavioral and EEG data from the after-stimulation session, we report two results. First, in-phase stimulation improved task performance only under the high memory load (load 6) condition as compared to sham stimulation in subjects with high WMC. Second, in-phase stimulation enhanced frontoparietal theta synchronization relative to sham stimulation during working memory retention under the high load condition in subjects with high WMC. Taken together, the present findings suggest that under high cognitive demand, in-phase theta tACS of the frontoparietal network using two high-definition stimulation arrays enhanced neuronal communications between prefrontal and parietal structures in healthy subjects with high WMC, and the enhanced frontoparietal communications may underlie improved task performance.

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Poster

426. Human Cognition and Behavior: Working Memory

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Program #/Poster #: 426.03/JJJ2

Topic: H.02. Human Cognition and Behavior

Support: JSPS KAKENHI Grant 17H06065
JSPS KAKENHI Grant 16H07483

Title: Elderly with high aerobic fitness maintain working memory via right ventrolateral prefrontal activation: A functional near infrared spectroscopy neuroimaging study

Authors: *K. HYODO[1], T. JINDO[1], H. SOYA[2], T. NAGAMATSU[1]
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Abstract: BACKGROUND: Prefrontal cortex (PFC) - mediated working memory (WM) declines with aging. On the other hand, several studies have reported that higher aerobic fitness is associated with greater WM performance in the elderly. However, the underlying neural mechanisms of the relationship are still not well understood. With aging, the brain activity pattern during a cognitive task becomes less lateralized in the PFC, and this change is thought to compensate for the neural dysfunction specific to task demand. Thus, the compensatory brain mechanism might be involved in the association between aerobic fitness and WM in elderly

people. In this study, we aimed to clarify the neural mechanisms underlying the relationship between the aerobic fitness and WM performance in elderly by using functional near infrared spectroscopy (fNIRS) neuroimaging method. **METHODOLOGY:** Twenty two healthy elderly women (mean age 68.8 ± 2.4 years) participated in a graded exercise test using a recumbent ergometer, and computer-based verbal WM task on separate days. During the exercise test, respiratory gas was measured and ventilatory threshold (VT) was analyzed as an indicator of submaximal aerobic fitness. As the WM task, verbal N-back task with letter stimuli was used in this study. The task consisted of three conditions: 0-back (no memory load), 1-back (low memory load), and 2-back (high memory load). Correct reaction time (RT) and correction rate (CR) in each condition were measured. During the task, we monitored prefrontal activation with multichannel fNIRS. fNIRS probes were set to cover lateral PFC activation foci, and neighboring channels were combined for the dorsolateral PFC (DLPFC), ventrolateral PFC (VLPFC), and frontopolar area (FPA) in each hemisphere using virtual registration. **RESULTS:** Compared to the 0-back and 1-back condition, RT was slowed and CR was declined in 2-back condition. The fNIRS data showed bilateral PFC activation during only 2-back condition. Partial correlation analyses revealed that there were significant relationships among higher VT, shorter RT in 2-back condition, and greater right-VLPFC activation during 2-back condition, with controlling age as covariates. **CONCLUSION:** Our results suggest that aerobic fitness is associated with high-loaded verbal WM performance via right-VLPFC activation in the elderly. Because verbal WM task recruits the left PFC predominantly, it is possible that the elderly with high aerobic fitness could maintain working memory by increasing compensatory brain activation.

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Poster

426. Human Cognition and Behavior: Working Memory

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Program #/Poster #: 426.04/JJJ3

Topic: H.02. Human Cognition and Behavior

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

Title: Visual working memory processes for objects' material properties in the human prefrontal cortex

Authors: *M. FUJIMICHI[1,2], H. TSUDA[1], H. YAMAMOTO[1], J. SAIKI[1]
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Abstract: We previously investigated neural bases of visual working memory for objects' material properties (VWMM) in the human visual cortex, and found VWMM-related activities in the higher-order ventral visual pathway and intraparietal sulcus. It remains unclear how the brain

regions other than the visual cortex are related to VWMM. We hypothesized that VWMM is accomplished by the interplay between the visual cortex and the prefrontal cortex (PFC) that has been shown as a critical site for VWM. To test this, we conducted an fMRI experiment where participants repeatedly performed delayed material discrimination tasks in a 3T scanner. Two kinds of material discriminations were performed, one for glossiness and another for roughness. In each trial, participants first judged which of the two tasks to be performed by detecting which dimension material property of two sequentially presented samples were varied (material discrimination phase). For example, samples varied in glossiness, participants judged this task to be glossiness memory task. After the presentation of the samples, a numerical cue indicating which sample to memorize was presented. After an 11s delay (delay phase), a probe object was presented and participants indicated whether the probe object had higher glossiness (lower roughness) than the memorized one. A multi-voxel pattern analysis (MVPA) was applied to predict which task participants performed from the fMRI activities in the anatomically defined prefrontal regions, dorsolateral PFC (DLPFC), ventrolateral PFC (VLPFC), and frontal pole (FP). The results of MVPA in each phase were different in the three regions. FP was near chance accuracy in both phases. In DLPFC, accuracy was not significantly higher than chance level in the material discrimination phase. The accuracy significantly exceeded chance in the mid-delay phase and fell to chance in the late-delay phase. VLPFC showed above chance accuracy in the material discrimination phase, in contrast, only marginally significant accuracy throughout the delay phase. In comparison with the visual cortex, a similar pattern of MVPA results was obtained in VLPFC and the higher-order ventral visual cortex. Furthermore, in the mid-delay phase, the accuracies in DLPFC and in intraparietal cortex varied in a similar fashion. These results suggest that visual working memory for objects' material properties is supported by the interplay between the visual cortex and lateral PFC.

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Poster

426. Human Cognition and Behavior: Working Memory

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Program #/Poster #: 426.05/JJJ4

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant MH095984-04

Title: Are unattended memory items under cognitive control? Electrophysiological evidence

Authors: *J. M. FULVIO[1], B. R. POSTLE[2]
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Abstract: In our variant of the dual-serial retrocuing (DSR) paradigm, two sample items are presented simultaneously at the beginning of each trial, and recognition is tested twice in succession, each time after a delay-period retrocue has indicated (with 100% validity) which of the two items will be tested (thereby giving the probed item the temporary status of "attended memory item"; AMI). Importantly, the intitially-uncued item cannot be forgotten because it may be tested by the second memory probe, and so it temporarily takes the status of "unattended memory item" (UMI). Previous work has shown that multivariate pattern analysis (MVPA) evidence for an active representation of the UMI often drops to baseline, raising the possibility that the UMI may not be held in working memory, but instead may be transferred to long-term memory (LTM) and then retrieved from LTM on trials when it is cued by the second retrocue. Initial evidence that the UMI is held in working memory, not LTM, came from the demonstration that it can be "reactivated" by a single pulse of transcranial magnetic stimulation (spTMS): 1) spTMS transiently reinstated the MVPA decodability of the UMI from the concurrently measured electroencephalogram (EEG); and 2) spTMS increased false-alarm responses when the UMI was presented as the recognition memory probe (i.e., as a lure; Rose et al., 2016). Here, we tested predictions made by the model that an active representation of the UMI's spatial context is held in a dynamically reconfigurable parietal salience map: Behavioral and EEG reactivation of an uncued item with spTMS targeting IPS2 would only occur on DSR trials when the uncued item was a UMI, but not on single-probe trials, when the uncued item could be dropped from working memory ("dropped memory item"; DMI). (Critically, the temporal lag between sample presentation and retrocue was identical for DSR and single-probe trials.) Results supported these predictions, showing UMI reactivation effects - in behavior and in the EEG - only during the first delay of DSR trials and not on single-probe trials. The specificity of reactivation effects for UMIs, but not for DMIs, confirms that information about the UMI is held in working memory. The presence, and strength, of elevated effective connectivity between a frontoparietal salience map (including IPS2) and the posterior networks that can represent a recently presented stimulus may govern whether or not that stimulus remains "in working memory."

Disclosures: J.M. Fulvio: None. **B.R. Postle:** None.

Poster

426. Human Cognition and Behavior: Working Memory

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Program #/Poster #: 426.06/JJJ5

Topic: H.02. Human Cognition and Behavior

Support: CONACYT Fellowship 857898
CONACYT CB 255462

Title: Longitudinal brain connectivity changes in response to a working memory task

Authors: *A. BRZEZINSKI RITTNER, S. ALCAUTER, F. A. BARRIOS
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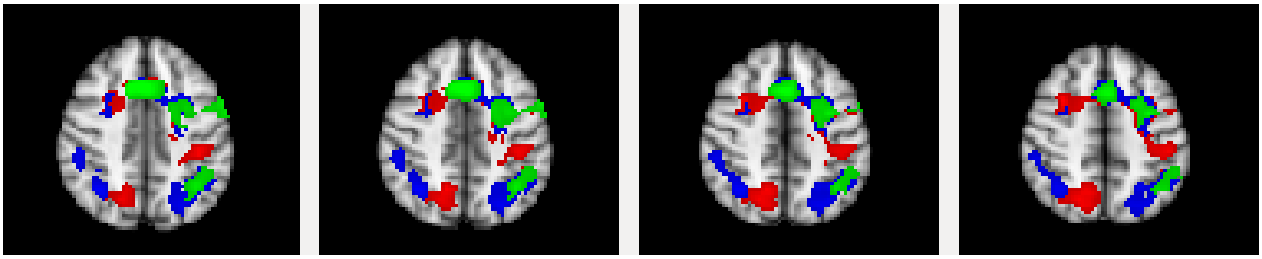
Abstract: Working memory (WM) consistently activates specific brain regions (Owen, et al, 2005). Some of these structures, such as the dorsolateral prefrontal cortex (DLPFC), part of the cingulate cortex and certain parietal regions are included in the Central Executive Network (CEN), (Bressler and Menon, 2010). It has been shown that practice in WM tasks can induce functional changes in neural activation patterns (Jolles, et al, 2010), presumably due to learning effects.

We tested if the brain activation and functional brain connectivity change when someone performs the same cognitive task along different time periods. Eight subjects (mean age 24.4 years) performed a N-back task (2-back) with visual stimuli during three fMRI sessions. The sessions where distributed with the second one taking place one week after the first, and three weeks before the last one. We found a decrease in BOLD signal extension comparing the average activation maps from each session. Then we performed a psycho-physiological interactions (PPI) analysis to study the functional connectivity; based on the BOLD signal average obtained from the third session, we selected different regions of interest (ROI) and created spherical masks to use them as seeds for the PPI analysis.

We found that, compared to the first session, the functional connectivity between the areas associated with the CEN and PPI connected structures grew stronger on the second session; for the third session, there was a decrease connectivity in certain of these areas. These PPI connectivity regions were mainly localized in frontal regions and the cingulate cortex. Overall, both in activation and connectivity, there was a left lateralization, since there was smaller activation in the right hemisphere. We think these findings are caused to a learning process over time considering that at the beginning, subjects started at a certain effort level to perform the task, and as they repeated it, they invested less resources to solve.



PPI maps for a seed ROI placed in the cerebellum. Maps from the first (red), the second (blue) and the third sessions (green) are overlapped.

Disclosures: A. Brzezinski Rittner: None. **S. Alcauter:** None. **F.A. Barrios:** None.

Poster

426. Human Cognition and Behavior: Working Memory

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 426.07/JJJ6

Topic: H.02. Human Cognition and Behavior

Support: MH112206

Title: Decoding working memory load from high density EEG

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Abstract: Working memory (WM) is an essential cognitive function. Manipulating working memory load and observing corresponding brain responses is a commonly applied technique for uncovering the neural mechanisms of WM. In this study we applied multivariate pattern analysis to high-density EEG data to examine the formation and development of WM representations in the brain. 128-channel EEG were recorded from twenty healthy subjects performing a Sternberg verbal working memory task with three levels of memory load (2, 4 and 6). On each trial, a memory array (cue), containing either 2, 4 or 6 letters to be remembered, was presented for 2000 ms (encoding period); to equalize the intensity of visual stimulation across memory load the non-letter positions in the array were filled with meaningless symbols. A time period (retention period) 3000 ms followed cue offset, and at the end of the time period, a probe letter was displayed for 1000 ms (retrieval period). Subjects responded via a button press to indicate whether this letter was contained in the cue array. Following EEG preprocessing, multivariate pattern classifiers were trained to decode load 2 condition versus load 6 condition during encoding, retention, and retrieval. There were four key findings. First, during encoding, decoding accuracy began to increase after cue onset, reached the highest level at ~500 ms, and decreased back to chance level at ~1500 ms. Second, during retention, following cue offset, decoding began to increase again, reached the highest level at ~500 ms after cue offset, and declined back to chance level at ~1100 ms after cue offset. Third, during retrieval, after probe onset, decoding accuracy once again began to increase, reached the highest level at ~500 ms after probe onset, and declined back to chance level at ~1500 ms after probe onset. Fourth, for the periods of encoding and retention when decoding accuracy was at chance level, we replaced voltage values by functional connectivity values as feature input into the classifiers, and found that the decoding accuracy became significantly above chance level. These results suggest that (1) the formation and development of the neural representations of WM is a highly dynamic process and (2) multivariate patterns of electrical activity and multivariate patterns of functional connectivity reflect different aspects of WM representations.

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Poster

426. Human Cognition and Behavior: Working Memory

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 426.08/JJJ7

Topic: H.02. Human Cognition and Behavior

Title: Working memory and falls risk in older adults

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Abstract: RATIONALE: The aging population is rapidly increasing, where currently the population of older adults (ages 60+) outnumbers the population of children. Falls risk is major concern for older adults, as falls can impair instrumental activities of daily living. No studies have investigated the differences between fallers, non-fallers, and individuals who are at moderate risk for falls. Thus, understanding the factors that attribute to falls risk is vital. Cognition function, specifically global cognition and executive function have shown to be impaired in fallers. However, working memory has not been examined as a falls risk factor, and the effects of impaired working memory across fallers, non-fallers, and moderate risk for falls. PURPOSE: To examine if there is a differences behaviourally and electrophysiologically in working memory between non-fallers, fallers, and moderate risk for falls in older community-dwelling adults. METHODS: Older adults (n=44, female=27) aged 60-80 years (m=68.6, SD=4.7) completed two sessions. The first session incorporated a battery of cognitive tests, Tinetti's Mobility Test, and general questionnaires that obtained demographic information and falls history. Participants were classified as a faller, non-faller, or a moderate risk for falls, based on Tinetti's Mobility Test (TiMT) scores, and their falls history. The second session had participants engaged in 3 versions (0, 1, 2) of the n-back test. Electroencephalograms (EEG) and behavioural performance were recorded. RESULTS: A one-way ANOVA with a post-hoc Tukey analysis was used to detect differences between groups. No significant differences between age, sex, or education were seen between groups. However, fallers group had a significantly higher physical activities score in comparison to the non-fallers group and moderate risk group. The moderate risk for falls group performed significantly ($p<0.05$) poorer in comparison to both the fallers and non-fallers with reaction time and error ($n^2=0.133$). The P3 component at the Fz electrode site displayed a significant difference in peak latency between non-target and target by classification. As well, peak latencies in the N2 and P3 component were significantly different in the 1, and 2-back test at the Fz. CONCLUSIONS: The moderate risk for falls group per is associated with poorer behavioural and electrophysiological performance on higher order

working memory tasks, specifically with cognitive processing and conflict resolution. The implications of this study suggests that those who are at risk for falling should be investigated and further research should consider the link between working memory and falls risk.

Disclosures: M. Wong: None. **L.S. Nagamatsu:** None.

Poster

426. Human Cognition and Behavior: Working Memory

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 426.09/JJJ8

Topic: H.02. Human Cognition and Behavior

Support: University of Guanajuato

Title: Quantitative electroencephalographic features of early postmenopausal women with low and high performance in a working memory test

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Abstract: Working memory is an executive function which is key for effective cognitive performance. Several studies have found that this memory displays deficits in early postmenopausal women, although the results are inconclusive. An electroencephalographic (EEG) approach during the application of a working memory test may aid in understanding these cognitive deficits. The aim was to explore EEG features of postmenopausal women through the execution of a test demanding working memory. EEG activity was recorded through the execution of the Wisconsin Card Sorting Test in the frontal, central, parietal and occipital leads in twenty-five early postmenopausal healthy women. The women were divided into two groups according to their execution in the test. A low execution group (n=11) and a high execution group (n=14) were established for comparisons. The criterion was based on the number of trials needed to reach the first category of the test. A large number of trials and 2 categories performed were indicative of low performance. Delta, theta, alpha1, alpha2, beta1, beta2 absolute power, the number of completed categories, correct responses (trials), perseverative errors and test errors were analyzed between two groups. When compared to the high execution group, the low execution group displayed lower number of completed categories ($p = 0.0001$), a higher number of trials to complete the category) ($p = 0.003$), a higher number of perseverative errors ($p = 0.02$) and greater number of errors committed ($p = 0.0001$) in the test, with a large size effect. The quantitative EEG analysis did not reveal significant differences between the groups, although the high execution group showed a tendency to have higher delta, alpha2, beta1 and beta2 absolute power. These findings indicate that the EEG activity was similar between the two groups, which

suggests that the women of low execution group used the brain resources to perform the test in a manner analogous to the high-performing group.

Disclosures: M. Solis-Ortiz: None. **E. Gonzalez-Pérez:** None.

Poster

426. Human Cognition and Behavior: Working Memory

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Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 426.10/JJJ9

Topic: H.02. Human Cognition and Behavior

Support: National Institute of Mental Health MH059299

Title: Network profiles of the dorsal anterior cingulate cortex in obsessive compulsive disorder during motor control and working memory

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Abstract: Background
Obsessive-Compulsive Disorder (OCD) is a mental disorder characterized by recurring rituals or reoccurring thoughts (Coluccia, 2017). Interest on its biological correlates has focused on network profiles of the dorsal Anterior Cingulate Cortex (dACC; Rosenberg et al., 2004). This interest is motivated by the dACC's hypothesized role as a principle control region (Paus 2001), dysfunction in which may be relevant for OCD. Previous studies have described dysfunctional network profiles of the dACC during working memory (Diwadkar et al., 2015) and motor control (Friedman et al., 2017). Here we compared the *relative* impairments in these profiles across these two task-active states.
Methods
fMRI data (3T Siemens Verio) was collected from 28 OCD (10 male) and 27 Health Control (HC) (10 male) subjects (Right-handed; $12 \leq Age \leq 22$). Groups were matched for age, handedness, and gender. Subjects underwent fMRI while performing working memory (WM, 2-back) and motor tasks. During WM, subjects maintained information on stimuli in a sequence presented for brief periods of time (presentation time: 500 ms; ISI: 2500 ms; 10 letters per run). During the motor task, subjects tapped their right forefinger in response to a flashing white probe (.5 or 1 hz, 30 s Epochs). Data were modeled using standard methods in SPM12 (Wellcome Trust Center). Psychophysiological Interactions (PPI) were employed with a seed in the dACC to examine the region's modulatory effects. 2nd level analysis utilized a two-factor design with Group (OCD/HC) and Task (Memory/Motor) as non-independent factors ($p<.005$ for all

analyses).
Results
A main effect of Group was observed in the precuneus, cuneus, occipital cortex, and left postcentral gyrus, with OCD characterized by greater modulation. The motor task induced greater dACC modulation in OCD in multiple regions including the occipital lobe, cuneus, insula, and precentral gyrus. Effects during the memory task were more circumscribed, observed in the inferior frontal gyrus and middle occipital cortex.
Discussion
Dysfunctional network profiles of the dACC are exaggerated in OCD across tasks. This effect is more salient during motor function, implying a relationship between task characteristic and expression in network dysfunction. Previous studies have characterized the dACC as being ideally positioned to translate intentions to actions, placing a heavy emphasis on the link between cognition and motor responses (Paus, 2001). Our findings support the primacy of the dACC in motor over memorial processes (Diwadkar et al., 2017) and indicate that this primacy is revealed in the pathology of brain network function in OCD.

Disclosures: T.D. Meram: None. **A.Z. Chowdury:** None. **P. Easter:** None. **T.J. Attisha:** None. **E. Kallabat:** None. **G. Hanna:** None. **P. Arnold:** None. **D.R. Rosenberg:** None. **V.A. Diwadkar:** None.

Poster

426. Human Cognition and Behavior: Working Memory

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Program #/Poster #: 426.11/JJJ10

Topic: H.02. Human Cognition and Behavior

Support: DGAPA-UNAM to AERC IN217918
DGAPA-UNAM to OPG IN215218
DGAPA-UNAM to MMD IA205218

Title: Differences in alpha band along maintenance and manipulation of information in working memory

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Abstract: Working memory (WM) is defined as the ability to maintain (Mt) and manipulate (Mp) information to guide subsequent behavior. The Mt refers to the ability to keep in mind information even if is not present. The Mp of information is the modification or processing of the

information that is being maintained. Delay-Match to Sample Task (DMST) allows the evaluation of different phases of the information processing in WM: encoding, delay period, and retrieval. The aim of this study was to evaluate the event-related synchronization (ERS) or desynchronization (ERD; by Hilbert transform) of alpha-band, along Mt and Mp processes. Two conditions of DMST were used: the Mt condition required keeping in mind two features (color and shape) of irregular figures; meanwhile, the Mp condition required the mental rotation (180º plane rotation) of these irregular figures. A higher percentage of correct responses and shorter reaction times was found for Mt *vs.* Mp ($p<0.001$). For encoding, a higher ERS was observed for Mt *vs.* Mp from 0-300ms, regardless region; also a higher ERS was observed at Occipital *vs.* Frontal and Central sites ($p<0.05$). Also, from 300-1000ms an ERD higher for Mp *vs.* Mt ($p<0.05$) was found. For delay period, in occipital site a ERS was larger for Mt than for Mp at 500-1000 ms. Also, an effect for region was found, occipital and parietal sites had larger ERS than frontal-central sites from 0 to 2500ms; and occipital had larger ERS than parietal *f*rom 0-1500ms occipital ($p<0.05$). Finally, in retrieval phase it was observed a higher ERD for Mt than for Mp at parietal and occipital regions ($p<0.05$); also, a higher ERD in parietal and occipital regions compared to frontal and central regions ($p<0.05$), regardless of the condition. Our results confirmed behaviorally and electrophysiologically that Mp is more difficult than Mt, associated with a higher desynchronization during encoding and the retention delay, predominantly in posterior regions, which could be related with differences in suppression of relevant features of the stimuli. Meanwhile, higher desynchronization for Mt than for Mp during retrieval could be associated with a more active searching process for comparing the actual stimulus which required a transformation.

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Poster

426. Human Cognition and Behavior: Working Memory

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Program #/Poster #: 426.12/JJJ11

Topic: H.02. Human Cognition and Behavior

Support: NSFC Grant 31671077
BMSCT Grant z171100000117007

Title: Neural circuit maintains simultaneously or sequentially presented multiple items in working memory

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Abstract: There are two streams of computational model in theoretical study on working memory. One claims that WM information is represented as persistent firing of neurons and the other argues that WM information is represented as brief bursts of spiking and stored in dynamic synapses. The working memory with simultaneously presented multiple items has been implemented by persistent firing model and the working memory given sequential multiple items has been implemented using brief bursts of spiking models. However, the brief burst model can not hold simultaneously presented information and the persistent firing model also has not been shown to maintain sequential presented information. Here we constructed a spiking neural network to maintain the simultaneously or sequentially presented multiple items in working memory. We use the prefer direction to label pyramidal cell with three compartments and placed them on a ring according to its prefer direction. We also include three different types of interneuron into the model. We adopt a structured connectivity between neurons. NMDA and AMPA receptors mediate the excitatory synaptic currents and GABA mediates inhibitory synaptic currents. Each item will evoke one localized activity bump which is a persistent gamma oscillation nested by beta oscillation during delay period. The population activity shows a persistent firing pattern but single neuron activity show a gamma oscillation nested by beta oscillation which is similar to the brief burst model. Importantly, our model can hold simultaneously presented information as well as sequentially presented information.

Disclosures: D. Wang: None. **Y. Zhang:** None. **J. Zhou:** None.

Poster

426. Human Cognition and Behavior: Working Memory

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Program #/Poster #: 426.13/JJJ12

Topic: H.02. Human Cognition and Behavior

Support: 2016R1D1A1B03930292
NRF- 2017M3C7A1029658
IBS-R001-D1

Title: Distinct neural coding schemes of anterior and posterior brain regions in forming cluster representations in visual working memory

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Abstract: We investigated neural codes reflecting how sensory representations are transformed into visual working memory. We capitalized on two recent advances. In visual cognition,

evidence indicates that sensory signal is abstracted to form a cluster representation around the mean of multiple items (Brady & Alvarez, 2011). Methodologically, the temporal generalization (TG) enables us to distinguish dynamic from stable neural coding (King & Dehaene, 2014): If a decoder built at each time generalizes only to the same time, it implies that different neural populations dynamically code a representation. However, if it generalizes to a long period, it means that same neural populations stably form a representation. Twenty people (9 females, mean age = 24.6) were included in the analysis after oculomotor artifacts rejection. They remembered 20 oriented bars and performed an old/new judgment for a probe bar after a 1.6-s delay while EEG was recorded. We built an orientation decoder with an inverted encoding model from the homogeneous block where all orientations were the same. Since all orientations are identical to their mean, the decoder should reflect both sensory and cluster representations. We then generalized the decoder to the heterogeneous block where four orientations (e.g., −30°, −10°, 10°, and 30° from the mean) were repeated five times. Since the decoder's orientation was identical to their mean orientation but different from the individual orientations, successful generalization should reflect only cluster representations. All analyses were separately performed for anterior and posterior electrodes to investigate neural coding schemes of each region. Subjects formed a cluster representation, as indicated by the largest, old responses at the mean orientation in the heterogeneous block. When TG analysis was performed within the homogenous block, the anterior decoder stably generalized over 200-800 ms, while the posterior decoder generalized to the time when the decoder was built, suggesting a stable coding in anterior regions and a dynamic coding in posterior regions. When TG analysis was performed over the heterogeneous block, only the anterior decoder successfully generalized (300 - 700 ms), implying the stable coding for cluster representations in that regions. Further, a behavioral measure of a cluster representation correlated with the decoding sensitivity across subjects only in the anterior regions. These results suggest that different posterior neural populations dynamically transform the sensory signal into cluster representations in visual working memory, while same anterior neural populations maintain the representations over time.

Disclosures: M. Kang: None. **B. Oh:** None. **Y. Kim:** None.

Poster

426. Human Cognition and Behavior: Working Memory

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Topic: H.02. Human Cognition and Behavior

Support: 106-2221-E-030-004- from the Ministry of Science and Technology, Taiwan

Title: Event-related phase-amplitude coupling for assessing the EEG correlates of auditory working memory load

Authors: T. HUANG[1], H.-M. DING[1], *Y.-L. TSENG[2]
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Abstract: Phase-amplitude coupling, also known as cross-frequency coupling, is a well-established concept to evaluate the neuronal encoding in brain regions and has been shown to be able to track learning and memory mechanisms. Previous study has presented that neural coding structure of memory retrieval is formed by the oscillations of gamma and theta brain signals. Although many studies have addressed the neural mechanisms underlying working memory, most of these studies have focused on the visual modality. In the past few years, neurologists suggested that different chords and tones can influence or induce the brain activations from the frontal, parietal, and temporal regions. In this study, we used auditory stimulation and focused on the relationship between activation of brain oscillations and working memory load using electroencephalography (EEG). The 32-channel EEG signals of 11 participants were measured while they were performing an auditory WM task. For the auditory n-back task, there were three experimental conditions, including two n-back task conditions of stimuli memorization with different memory load and a condition of passive listening to the stimuli. The independent components from frontal and parietal regions were further analyzed by event-related phase-amplitude coupling (ERPAC). ERPAC is used to calculate the degree of coupling between phase and amplitude in a single brain region. Different from other traditional methods with only spatial resolution, ERPAC can assess an index at each sampling point, and thus achieve better temporal resolution. The results suggested that there is significant theta-beta and theta-gamma ERPAC observed in the parietal region during auditory working memory. Furthermore, pronounced ERPAC has been found later (800 ms after the stimulus onset) during high working memory load than the lower conditions (400 - 800 ms after the stimulus onset). In conclusion, the findings demonstrate the existence of phase-amplitude coupling in the parietal regions during an auditory working memory task, and support the viewpoint of theta-gamma coding schemes that the amplitudes of high-frequency oscillations are modulated by the phase of low-frequency oscillations during the memory and learning processes.

Disclosures: T. Huang: None. **H. Ding:** None. **Y. Tseng:** None.

Poster

426. Human Cognition and Behavior: Working Memory

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Program #/Poster #: 426.15/JJJ14

Topic: H.02. Human Cognition and Behavior

Support: NIMH R01 MH104588

Title: Sensory acquisition functions of the cerebellum in verbal working memory: An fMRI investigation

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Abstract: **Objectives:** Several fMRI studies have shown that the superior cerebellum exhibits load-dependent activations during encoding of letters in a Sternberg verbal working memory (VWM) task. It has been hypothesized that the cerebellum regulates the acquisition of sensory data across all modalities (Bower, 1997), and thus, (1) VWM load activations may reflect high- vs low-load differences in sensory acquisition demands, and (2) increased difficulty in sensory data acquisition should elicit greater activation in the cerebellum. We tested sensory acquisition in VWM by presenting visually degraded and non-degraded stimuli with high and low memory loads, identifying load-dependent regions of interest in the cerebellum, and then testing if these regions showed greater activation for degraded stimuli. **Methods:** 15 healthy subjects (10 F, 5 M; mean age 31.6 years; age range 22-54 years), received 192 Sternberg VWM trials during fMRI scanning. Each trial was either high- (4 letters) or low-load (2 letters), and letters were either visually-degraded or non-degraded. Eye movements were recorded, and fMRI analyses in SPM8 focused on the encoding phase of the task. **Results:** Consistent with previous studies, load-dependent cerebellar activation was observed in hemispheric right and left VI, as well as in vermis Crus II. Using small volume correction, the degraded vs non-degraded contrast revealed significant activation in the vermis. However, significant activation was not found in the hemispheres. Eye movements for degraded vs non-degraded stimuli were not significantly different. Whole brain (corrected) analysis for the degraded vs non-degraded contrast revealed greater activation for degraded stimuli in left fusiform and right inferior occipital regions, indicating that the degradation manipulation successfully increased sensory acquisition demand. **Conclusion:** We found partial support for the sensory acquisition hypothesis in a load-dependent region of the vermis, which showed significantly greater activation for degraded relative to non-degraded stimuli. Because eye movements did not differ for these stimulus types, this activation appears to be related to perceptual rather than eye movement demands. In contrast to the vermis, hemispheric regions of the cerebellum that also exhibited load-dependent activation did not show increased activation for degraded stimuli. To explain these findings, an overall function of association-based prediction may underlie general cerebellar function, with perceptual prediction of stimuli from partial representations occurring in the vermis and articulatory prediction occurring in the hemispheres.

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Poster

426. Human Cognition and Behavior: Working Memory

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant GR035189

Title: Whole-brain functional connectivity predicts working memory performance in novel healthy and memory-impaired individuals

Authors: *E. AVERY[1], K. YOO[1], M. D. ROSENBERG[1], S. GAO[2], A. GREENE[3], D. L. NA[7], D. SCHEINOST[4], R. CONSTABLE[5,3,4], M. M. CHUN[1,3,6]
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Abstract: From reading a book to remembering items at the grocery store, our capacity to temporarily store and manipulate information is critical to our interactions with the world. While previous fMRI work has characterized neural correlates of working memory (WM; Magnuson et al., 2015; McNab & Klingberg, 2007) and predicted WM precision (Galeano et al., 2017), brain-based measures have yet to be used to predict WM performance. Using a data-driven technique known as connectome-based predictive modeling (CPM; Finn et al., 2015; Rosenberg et al., 2016; Shen et al., 2017), we here built models to predict individual WM performance from whole-brain functional connectivity (FC) patterns. FC patterns between each pair of 268 nodes of the brain (Shen et al., 2013) in 502 subjects (mean age 28±3.6) of the Human Connectome Project were used to construct CPMs predicting individual WM performance (2-back accuracy). We built two CPMs, one using WM (n-back) fMRI task data and one using resting state data. In leave-one-out cross validation, CPMs built from n-back or rest data successfully predicted previously unseen individuals' 2-back acc. (correlation between predicted/observed scores: task: $r=0.36$, $p=7.3*10^{-16}$; rest: $r=0.20$, $p=1.1*10^{-5}$). Model predictions also correlated with measures of fluid intelligence (gF; Penn's Progressive Matrices: $r=0.30$, $p=7.7\times10^{-12}$) and, with less strength, sustained attention (Short Penn Continuous Performance: $r=0.13$, $p=9.3*10^{-3}$). Analogously built CPMs of gF can predict WM score ($r=0.42$, $p=1.7\times10^{-20}$), as can CPMs of sustained attention, again with less strength ($r=0.22$, $p=1.4\times10^{-6}$). This reflects the documented, strong relationship between WM and gF (Colom et al., 2002, 2004) and weaker relationship between WM and sustained attention (Barrett et al., 2004). Anatomical feature analysis likewise revealed significant overlap (30.8% of connections) between WM/gF models, particularly in utilization of prefrontal and parietal regions, while overlap between WM/sustained attention models was observed in just 5.7% of connections. Indicating that our models characterize WM abilities in general rather than 2-back acc. in particular, the same CPMs of WM generalized to predict memory deterioration (Ahn et al., 2010; $r=0.32$, $p=5.4\times10^{-5}$) in an independent dataset of 157 older adults (mean age 68.7±9.6; 48 healthy, 54 amnestic mild cognitive impairment, 55 Alzheimer's disease) recruited from the Samsung Medical Center (Seoul, Korea). The present results indicate for the first time that whole-brain FC is a broadly applicable indicator of WM capability across the lifespan, correlating as expected with constructs such as gF and sustained attention.

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Poster

426. Human Cognition and Behavior: Working Memory

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Program #/Poster #: 426.17/JJJ16

Topic: H.02. Human Cognition and Behavior

Support: MH095984-04 to B.R.P.

Title: Tracking stimulus representation across a 2-back visual working memory task

Authors: *Q. WAN, Y. CAI, J. SAMAHA, B. R. POSTLE
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Abstract: In the dual serial retrocueing (DSR) task, after two items are encoded into working memory, a retrocue indicates which of the two will be the first to be tested (thereby designating it the "attended memory item" (AMI). Because there is a *p* of .5 that the initially uncued item will be tested later in the trial, it temporarily takes the status of "unattended memory item" (UMI). Although previous studies using multivariate pattern analysis (MVPA) often find that evidence for an active representation of the UMI drops to baseline levels (e.g., Rose et al, 2016), more recent studies employing multivariate inverted (or "forward") encoding modeling (IEM) suggest that the UMI may be held in an active state, but in a representational format that is different from the AMI (Yu & Postle, 2018). One question that remains unclear is whether the differential coding of AMI vs. UMI reflects a general property of varying attentional state, or, rather, is idiosyncratic to the DSR task. Here, we used a task in which memory items are also held in differing states of attentional priority, but one that lacks overt cuing, and for which the *p* of a UMI-to-AMI transition is 1 - the 2-back task. If subjects know with certainty that a memory item will be needed later during the trial, is it nonetheless recoded into a different format than the AMI, or is it held in the same state (as would be the case for, e.g., a conventional load-of-2 delayed-recognition (DR) task)? Stimuli were drawn randomly, with replacement, from 6 black-and-white gratings of 6 orientations. IEMs were successfully trained on EEG voltages from a 1-item DR task, with k-fold cross-validation, although the failure of cross temporal generalization indicated that the representational format at encoding differed from that during the delay. IEM reconstruction of stimuli during the 2-back task also revealed a dynamic representational trajectory: 1) After stimulus offset, when an item became a UMI, it could be reconstructed with the delay-period IEM, but not the encoding IEM; 2) after the offset of the subsequent item in the 2-back sequence, however, the opposite was true. These results suggest that a general principle of neural coding during working memory may be that information is recoded into a format that is different from visual perception while it is being retained for later use, then recoded back into a

perceptual format when needed to guide behavior (e.g., with a recognition decision or a recall response).

Disclosures: Q. Wan: None. **Y. Cai:** None. **J. Samaha:** None. **B.R. Postle:** None.

Poster

426. Human Cognition and Behavior: Working Memory

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 426.18/JJJ17

Topic: H.02. Human Cognition and Behavior

Support: Hearst Foundation Fellowship
5 T32 GM007517-36
5 R01 EY015260

Title: Interactions between working memory and perceptual or predictive task demands on an adaptive oculomotor delay response task

Authors: *K. SCHAPIRO[1], J. I. GOLD[2]
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Abstract: An ongoing question in neuroscience is how expectations inform decisions. Bayesian approaches have been used successfully to understand how prior expectations are used to improve accuracy and shape even low-level perceptual decisions. However, the majority of this work has focused on the effects of stable priors formed in static environments, whereas many decisions are made in more volatile conditions that require dynamic updating of the prior. In principle, this dynamic process relies upon the ability to represent and manipulate relevant information in working memory (WM). Information held in WM is subject to storage limitations and degradation over time. Thus, different task demands, such as whether to make a perceptual (retrospective) or predictive (prospective) inference, may differentially dictate what information is maintained and how it is manipulated to best perform within the constraints of WM. The goal of this study is to understand how different temporal delays affect decisions involving a dynamic prior under both perceptive and predictive task demands.
We used a novel adaptive Oculomotor Delay Response (ODR) task with noisy and volatile structure in the sequence of visual target locations to examine the effects of response delay and task demands on adaptive decision-making. This work builds on extensive work using the ODR task without this explicit sequential structure to understand visual-, memory-, and motor-related processing in the brain. Our task required subject to report, after each sample, either the perceived location of the previous sample or the predicted location of next sample after a varied delay period. Preliminary results show that the perceptual report was dominated by the most recent sample rather than the prior. Conversely, the effect of the most recent sample on the

prediction report decreased as the number of samples in the stable environment increased. These results suggest that the degree to which new information is represented and incorporated into an updated decision is dependent upon both environmental statistics and on task demands. Additionally, increased reporting delay in both decision conditions resulted in decreased average accuracy. This result suggests that at least part of the perceptual and predictive adaptive inference processes are executed in a WM framework that is subject to degradation over time. Possible differential effects of delay on perceptual and predictive adaptive inferences and the representation and modulation of these processes in the brain are the subjects of ongoing research.

Disclosures: K. Schapiro: None. **J.I. Gold:** None.

Poster

426. Human Cognition and Behavior: Working Memory

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 426.19/JJJ18

Topic: H.02. Human Cognition and Behavior

Support: CERC Grant #0000025914

Title: Training-induced changes to brain functional connectivity during cognitive tasks and rest

Authors: *J. EREZ[1,2], C. MACE[2,1], E. S. NICHOLS[2,1], A. M. OWEN[2,1], B. B. STOJANOSKI[2,1]
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Abstract: The primary goal of many ‘brain training’ games is to improve working memory (WM), thought to be a core component of higher cognitive function. The central tenet is quite appealing; by increasing one’s working memory capacity, other higher cognitive functions, such as planning and reasoning, might benefit as well. Yet, support for this idea remains inconsistent; although prolonged practice on one cognitive task yields significant improvements on that particular task, there is little evidence that this improvement transfers to unpracticed tasks that tax other cognitive domains. It is also not well understood whether these different aspects of higher cognitive function share common neural resources, and what degree of plasticity they have. Finding the neural mechanism of this limitation is a key question in cognitive neuroscience. In this study, 10 healthy young adults completed a series of ‘brain training’ sessions using tasks from a validated cognitive assessment tool (http://www.cambridgebrainsciences.com). Participants were assigned to one of two groups: the first group trained on a visuospatial working memory task (“Token Search”), while the second group trained on a reasoning task (“Double Trouble”). Each participant completed approximately 12-15 hours of training across 20 days, and was scanned 5 times over the training period (before,

after, and 3 times between training sessions, following every 2.5 hours of practice). fMRI scans were done as participants performed the tasks, as well as during a short period of rest before and after each scanning session (improvements in WM following training have been shown to be associated with increased neural connectivity at rest). This paradigm allowed us to (1) establish the neural network recruitment of each task before training, (2) investigate plasticity based changes to neural networks due to training, and (3) track whether network connectivity at rest changes as a function of cognitive training. Results revealed that 'brain training' on the two cognitive tasks produced domain-specific functional connectivity profiles (involving the frontoparietal network), but also produced domain-general effects consistent across both tasks (e.g. improved behavioral performance was associated with higher functional connectivity within the default mode network). In addition, prolonged cognitive training affected how these networks were represented in the resting state; that is, the neural profile during training became more divergent. These results provide key evidence as to the nature of the brain networks that are responsible for cognitive training.

Disclosures: J. Erez: None. **C. Mace:** None. **E.S. Nichols:** None. **A.M. Owen:** None. **B.B. Stojanoski:** None.

Poster

426. Human Cognition and Behavior: Working Memory

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 426.20/JJJ19

Topic: H.02. Human Cognition and Behavior

Support: NSFC Grant: 31671077

Title: The capacity and maintenance mechanisms of vibrotactile working memory

Authors: *C. WANG[1], X. ZHANG[2,3,4], D. WANG[1]
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Abstract: The limit capacity is a hallmark of working memory (WM). Previous studies demonstrated that 3 to 4 visual spatial items can be stored in WM, and 7 plus/minus 2 digits can also be hold in WM. Little is known about the WM capacity of tactile stimulus. Considering that tactile is an important sense and its neural coding is different from visual spatial stimulus and digit, we carried out three delayed match-to-sample experiments to investigate the capacity and maintenance mechanisms of vibrotactile WM. In Exp 1, vibrotactile stimuli were delivered to the fingertips of all five fingers of the left hand. In each trial, the sample and probe were delivered to

the same finger. Results showed that accuracies of five fingers were not significantly different, which suggested that regions of corresponding somatosensory cortex of each finger contribute equally to vibrotactile working memory. In Exp 2, two, three, four or five different vibratory frequencies were simultaneously delivered to two, three, four or five fingers of participants' left hand and randomly chose one of these fingers to test whether the participant memorized the delivered stimulus. Accuracy in the two frequencies condition was 63%, and performance in the three, four, and five frequencies conditions were near chance. The results suggested that there was interference when multiple frequencies were delivered to different fingertips simultaneously, and the sensory cortex can not process multiple vibratory stimuli simultaneously during vibrotactile working memory.Exp3 was designed to examine the capacity of vibrotactile working memory when subjects memorized sequentially presented vibratory frequencies. Vibrotactile stimuli were delivered to the left index finger. Participants were presented with two, three, four, or five 1000ms different vibratory frequencies, separated by an unfilled 1000ms period. Results showed that performance for all four conditions were above chance, and mean accuracies were 0.80, 0.75, 0.71, and 0.71 respectively. The mean capacity of vibrotactile working memory was 2.11. These results indicated that vibrotactile working memory can store multiple frequencies. Overall, our results suggest that the somatosensory cortex is equally recruited for each finger of the left hand in vibrotactile working memory tasks. However, the somatosensory cortex can not process multiple vibratory stimuli simultaneously. And the vibrotactile working memory can store about 2 vibratory frequencies.

Disclosures: C. Wang: None. **X. Zhang:** None. **D. Wang:** None.

Poster

426. Human Cognition and Behavior: Working Memory

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 426.21/JJJ20

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01 DC004290
Wellcome Trust Grant WT091681MA

Title: Active tracking of sound textures: A human intracranial study

Authors: *A. J. BILLIG[1], P. E. GANDER[2], W. SEDLEY[3], M. CHAIT[1], H. KAWASAKI[2], C. K. KOVACH[2], M. A. HOWARD, III[2], T. D. GRIFFITHS[3]
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Abstract: A network consisting of auditory cortex, hippocampus, and inferior frontal gyrus has been proposed to support auditory working memory (Kumar et al., J Neurosci 36:4492-4505, 2016). However, human intracranial recordings are yet to reveal hippocampal high gamma activity, a neural spiking correlate, during memory for tones. Recordings from the rat hippocampal complex indicate that cells that support navigation can also form discrete firing fields for particular sound frequencies (Aronov et al., Nature 543:719-722, 2017). Critically, this is the case only when the animal adjusts a sound to match the frequency of a remembered target, but not during passive listening. For humans performing such a task, recruitment of navigation circuits may stem from a cognitive association between height in pitch and in physical space (Rusconi et al., Cognition 99:113-129, 2006). Here, we study the neural underpinnings of memory for and tracking of sound textures that vary continuously in a single dimension but have no such spatial association. Stimuli were concatenated chords, each containing between 4 and 100 simultaneous 200-ms tones randomly distributed in frequency over a 4-octave range. Fixing the number of simultaneous tones ("density") while varying their frequencies from chord to chord gave rise to textures that were more "beep"-like at lower densities and more "noise"-like at higher densities. In each active trial, human subjects (patients implanted with electrodes to localize epileptic activity) listened to a 2-s sound of fixed target density, which they were to remember over a subsequent 2-s retention period. They then heard a 15-s texture, the density of which they adjusted using button presses to match the density of the target. Preliminary analyses of sound-induced neural activity revealed robust (high) gamma responses in Heschl's gyrus and superior temporal gyrus, with concurrent low frequency (4-30 Hz) power decreases over a range of frontal, temporal, and parietal sites. (High) gamma responses were also observed in right hippocampus and right inferior frontal gyrus; these were greater during active adjustment than in a passive listening condition with similar auditory stimulation. Activity at one hippocampal site was also present during target presentation and the subsequent silent retention period. Ongoing work includes conducting motor and attention control experiments, and using multivariate analyses to study the link between behaviour and the strength of target representations across the putative auditory working memory brain network.

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Poster

426. Human Cognition and Behavior: Working Memory

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Program #/Poster #: 426.22/JJJ21

Topic: H.02. Human Cognition and Behavior

Support: NWO (VIDI grant 452-13-016)
James S. McDonnell Foundation 220020373

ERC Starting Grant 678286
China Scholarship Council (CSC)

Title: Remapping of working memory across eye movements in early visual cortex

Authors: *T. HE[1], A. R. VANDENBROUCKE[2], M. EKMAN[1], F. P. DE LANGE[1]
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Abstract: Saccadic remapping, the mechanism that visually responsive neurons increase their activity to stimuli that will be brought into their receptive fields by an upcoming eye movement, may play a critical role in visual stability. Previous studies have demonstrated remapping of stimulus representations during perception. However, our brain can also process stimuli that are not directly available to our eyes. A working memory buffer allows us to access visual information even after it vanished from our visual field, and further guides behavior over a period of seconds. It remains unclear if saccadic remapping also occurs for stimuli that are held in working memory. In the current study, we investigated whether visual working memory representations are remapped. To this end, we directly compared the contents of visual working memory in early visual cortex for saccade and no-saccade conditions. Participants memorized one of two orientation gratings presented in the left or right visual field, and on some trials, subsequently made a saccade. Then, after a retention period participants reported whether a probe stimulus was tilted clockwise or count-clockwise with respect to the grating held in working memory. We used multivariate pattern analysis of fMRI data to probe item-related working memory representations of the remembered grating orientation from contralateral and ipsilateral early visual areas (V1 - V3). Preliminary results suggest that contralateral visual areas contain an item-specific memory trace that corresponds to the retinotopic location of the remembered item during working memory period. Importantly, after participants made a saccade to the opposite visual field, working memory representations appeared to shift from contralateral regions to ipsilateral regions. These results indicated that working memory representations may indeed be remapped after eye movements, suggesting that the storage of working memory, like perception, is dynamically updated with eye movements.

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Poster

426. Human Cognition and Behavior: Working Memory

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Program #/Poster #: 426.23/JJJ22

Topic: H.02. Human Cognition and Behavior

Support: Belgian Fund for Scientific Research -- Flanders (G.0883.14)
NIH R01MH111742

Title: P300 morphology during variations of N-Back task

Authors: ***V. PERGHER**[1], M. SHALCHY[2], A. PAHOR[2], S. JAEGGI[3], M. VAN HULLE[1], A. SEITZ[2]
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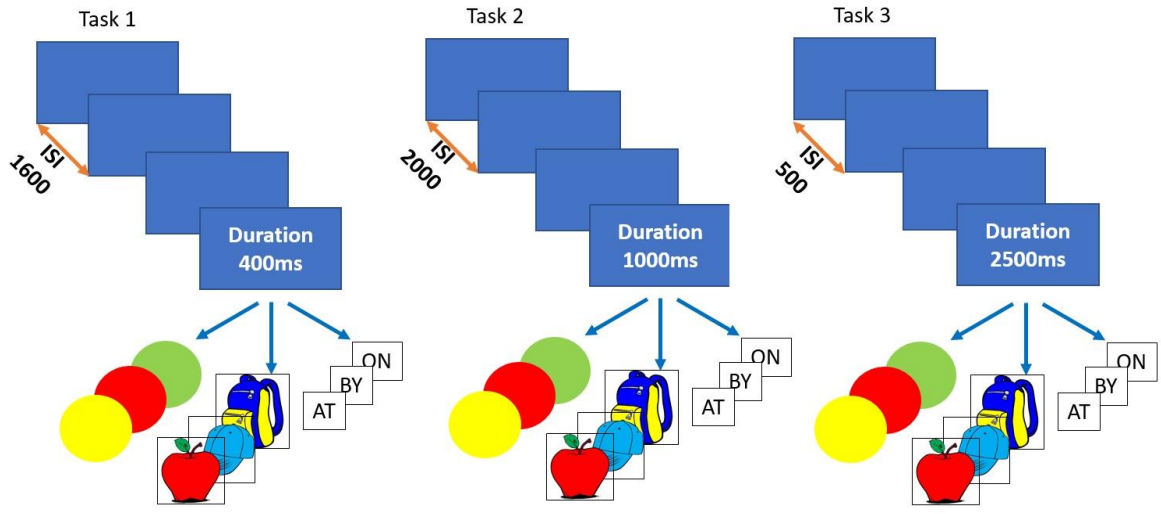
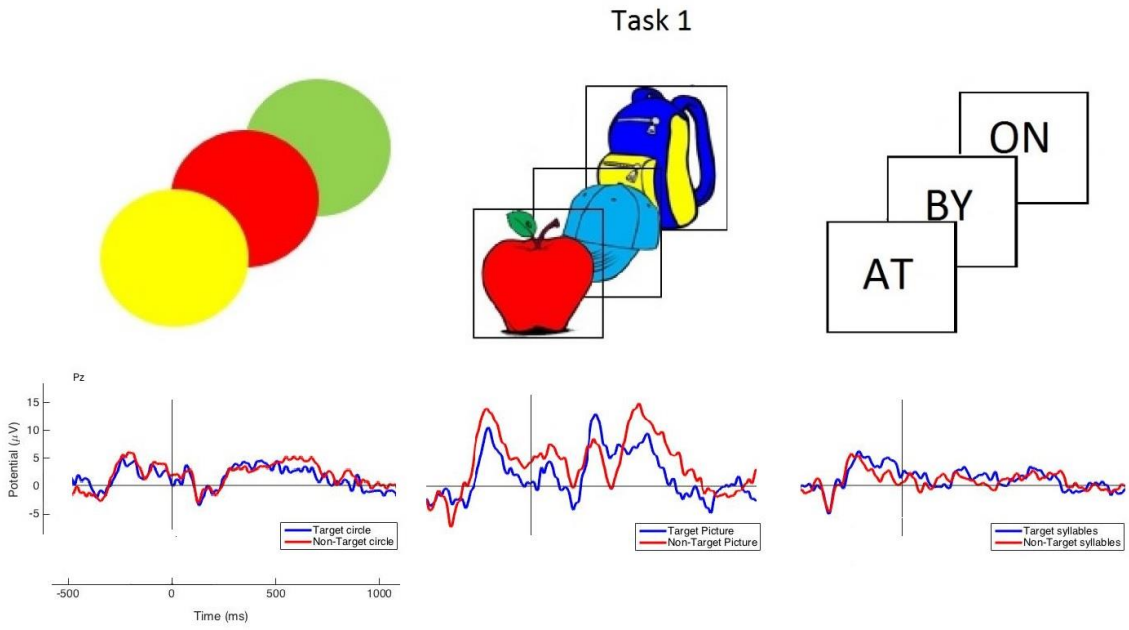
Abstract: MAS and VP contributed equally to the study.
MMVH and AS share senior authorship.
Although working memory (WM) is part of many cognitive functions, its mechanism is still elusive. A popular way to assess WM function is with the N-Back task: subjects are asked to report whether the current stimulus matches one presented N stimuli earlier. While the basic structure of the N-back task is consistent across literature, the used stimulus type and task features vary considerably, such as duration, ISI, feedback, and response contingencies, and little is known about their effect on the neural correlates of WM. To investigate the latter, 36 healthy adults (18 males, 18 females) were recruited to perform different versions of the same N-back task while simultaneously recording their EEGs: Three different experimental set-ups (stimulus durations, ISI, feedback response contingencies) and three different stimuli types (colored circles, pictures, and syllables), crossed into nine different combinations. Preliminary results suggest that neural signatures differ for different versions of the N-Back task. Within each set-up, stimuli type seems to play an independent role modulating the P300 event-related potential (ERP) component. This shows that care must be taken when comparing study outcomes in particular when it comes to stimulus type.





Disclosures: V. Pergher: None. **M. Shalchy:** None. **A. Pahor:** None. **S. Jaeggi:** None. **M. Van Hulle:** None. **A. Seitz:** None.

Poster

426. Human Cognition and Behavior: Working Memory

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Program #/Poster #: 426.24/JJJ23

Topic: H.02. Human Cognition and Behavior

Support: National Natural Science Foundation of China 81771407
National Natural Science Foundation of China 61533006
National Natural Science Foundation of China 81471653

Title: Effects of temporal lobe epilepsy on cognitive abilities: An automated memory assessment with eye tracking

Authors: *L. FENG[1], Q. WANG[2], J. WANG[1], K. YANG[1], S. HUANG[1], B. LI[3], B. XIAO[1], D. LIU[4]
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Abstract: Cognitive dysfunction is a very common comorbidity of epilepsy with a severe influence on daily living quality for epileptic patients, which calls for early neuropsychological diagnostics. To increase the robustness and reproducibility, we created an automated computer-based memory assessment with eye tracking (Tobii Glass II), which provides game-like interaction and simultaneously a visual attention tracking system. In this automated assessment, one to four memory targets is presented for 10 seconds along the middle horizontal line of the computer screen for participants to memorize. After that, the memory targets disappear and 12 potential answers are presented on the screen. The participant must select the target(s) from the previous screen in order to proceed to the next, more difficult level. We invited 31 patients (13 males, age of 30.65 ± 9.89) with temporal lobe epilepsy and 28 age-matched healthy controls (12 males, age 32.93 ±10.01). In classical assessment of Digit Span Backward of the Wechsler Memory Scale, with linear mixed model with clinical diagnosis as the factor and age as the covariate, we found that the patients have significantly less number memorized items than the control group ($F(1,40) = 7.91$, $p = .008$), covaried age is significant ($F(56)=4.91$, $p = .031$). With automated computer-based assessment with eye tracking, the patients took significantly longer time to find the memorized items ($F(1,55)= 12.03$, $p= .001$). Eye tracking data show patients have longer total looking duration ($F(1,55) = 9.86$, $p= .047$), more visit counts ($F(1,55)=5.04$, $p = .029$). The patient group showed a strong correlation between the Wechsler memory performance and the number of visit count ($r(28) = -0.41$, $p = .014$) while none of correlation in control group. There is also marginal correlation between the Wechsler memory performance and the task accomplished time ($r(28) =-0.34$, $p =.068$) and the total looking time ($r(28) = 0.34$, $p =.067$). With our automated computer-based cognitive assessment platform, even though the patients with temporal lobe epilepsy showed no sign of attentional deficits, they might demonstrate significant memory deficit than healthy controls, which was consistent as Wechsler Memory Scale. This platform demonstrated its advantage in automated assessment, quantitative analysis and controlled for visual attention. Future direction would focus on investigating the underlying electrophysiological mechanisms responsible for memory impairment in the patients with temporal lobe epilepsy.

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Poster

427. Decision Making II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 427.01/JJJ24

Topic: H.02. Human Cognition and Behavior

Support: Wabash College: Daniel F. Evans Chair

Title: Validation of a translational virtual experiential foraging task for humans

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Abstract: Foraging tasks provide valuable insights into decision-making, as animals make decisions about how to allocate limited resources (such as time). In the "Restaurant Row" task, rodents obtain food rewards at several sites. In the "Web-Surf" task, humans are offered short videos from different categories. In both tasks, rewards are available after a variable delay. While rodents physically move between reward sites, humans transition to the next video type by pressing a button. Rodents and humans show similar patterns of behavior on these tasks, supporting their use to study decision-making across species. We tested a new human task ("Movie Row"), which combines elements of the Restaurant Row and Web-Surf tasks.
In the Movie Row task, participants navigate through a 3-d virtual environment on a rounded track. A movie screen is positioned at each corner of the track, where 4-sec video clips are available from one of four categories (kittens, bike accidents, landscapes, and dancing). As participants arrive at a screen, the category and loading time (delay) is presented. Participants accept offers by stepping onto a platform and looking at the screen, or skip an offer by moving to the next reward site. Delays range from 3 to 29 seconds (though longer delays were possible in the second version).
Two versions of the task were tested, the first in a sample of 30 male undergraduates who completed the Movie Row task in a laboratory. The second version was tested in an online sample of 51 participants (30 females, mean age = 42.2 years) recruited through Amazon's Mechanical Turk.
Behavior on the Movie Row was similar to that of the Web-Surf task. Revealed video preferences, assessed by delay thresholds (the delay below which they would accept, above which they would skip an offer) were positively correlated with stated preferences, with more than 64% of participants having a correlation greater than +0.75 with the average video rankings and with post-experiment rankings of each video category. Decision consistency (the proportion of offers where participants deviated from their delay threshold) was lower on the first (in-person) version ($M = 0.12$, $SD = 0.05$) than the second (online) version ($M = 0.20$, $SD = 0.11$), higher than the reported mean for the Web-Surf task ($M = 0.07$), but similar to the Restaurant Row task ($M = 0.12$).
These data support the Movie Row task as translational tool to study foraging behavior in humans, providing convergent results with a rodent navigation task and a human stay/go foraging task. And, by including a virtual navigation component, the task may be useful in testing if behavioral measures identified in the rodent Restaurant Row task translate to human virtual navigation.

Disclosures: N.C. Schmitzer-Torbert: None. **T. Huynh:** None. **K. Alstatt:** None. **M. Trebing:** None. **S.V. Abram:** None.

Poster

427. Decision Making II

Location: SDCC Halls B-H

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Program #/Poster #: 427.02/JJJ25

Topic: H.02. Human Cognition and Behavior

Support: NIH-NEI Grant R01EY026701
NSF EPSCoR Grant GR03373

Title: Greater willingness-to-pay for real foods (but not food images) that are perceived to have higher caloric content

Authors: *C. A. ROMERO, J. C. SNOW
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Abstract: Laboratory studies of human dietary decisions have been based almost exclusively on studies of computerized two-dimensional (2-D) images. In contrast, food choices in everyday life are typically made in the context of real foods —solids that afford grasping and have actual caloric content. Contrary to the assumption that 2-D images are appropriate proxies for real foods in the study of food valuation, we recently found that willingness-to-pay (WTP) increases by ~6% for food items that were displayed as real objects versus planar high-resolution colored images. Here, we examined whether effects of display format on valuation are modulated by the actual or perceived caloric content of the foods, and whether the stimuli were physically accessible at the time of choice. Participants placed monetary bids on everyday snack food items that were displayed either as real objects or 2-D images. Critically, although all of the stimuli were presented within reaching distance of the observer, on half of the trials the stimuli were displayed behind a clear Plexiglass barrier that prevented in-the-moment access to the stimuli. Linear mixed-effects modeling analysis revealed that although the presence of the barrier reduced WTP for preferred foods, this effect was comparable for real foods and food images. However, for real foods (but not images) WTP increased for items that were perceived by observers to have greater caloric content. There were no other higher-order interactions between the factors. These results suggest that although humans place greater value on foods that are perceived to yield higher caloric return, this only occurs when the decision is made in the presence of a real food.

Disclosures: C.A. Romero: None. **J.C. Snow:** None.

Poster

427. Decision Making II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 427.03/JJJ26

Topic: H.02. Human Cognition and Behavior

Support: NIH NEI R01EY026701

Title: Similarities and differences in the representation of real objects versus 2-D planar images and 3-D augmented reality displays: Insights from inverse multidimensional scaling

Authors: *D. HOLLER[1], S. FABBRI[2,1], J. C. SNOW[1]
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Abstract: Images of objects are commonly used as proxies to access the organization of conceptual knowledge in the human brain. However, recent studies from our laboratory have highlighted differences between images and real objects at the level of their neural representation, as well as in their contribution to memory, attention and decision-making. Asking an observer to make judgments about the similarities among a set of objects can provide unique insights into the nature of the underlying neural representations of those objects in human cortex (Mur et al, 2013). Here, we used inverse multidimensional scaling (Kriegeskorte and Mur 2012) to investigate the subjective properties that observers use to characterize objects during free-sorting, when the stimuli are displayed as 2-D images of objects, 3-D augmented reality objects, and real objects. Observers were asked to arrange 21 different items so that the distances between them reflected their perceived dissimilarities. One group of participants sorted 2-D images on a computer monitor using a mouse drag-and-drop action; another group manually sorted AR displays of the same objects; the remaining group manually arranged real-world objects. Critically, participants were free to use any dimension they liked to group the items, and were asked to report their sorting principle to the experimenter prior to sorting the stimuli. By correlating models based on the various sorting criteria, and the dissimilarity matrix obtained by the behavioral ratings, we identified the properties that observers used to separate the items in each format. Using stepwise linear regression, we found that both common and different criteria were used to arrange the stimuli across formats. For example, for all formats, the location where the item is typically encountered in everyday life was a salient dimension, as was elongation. However, unlike 2-D images, real objects and AR stimuli were sorted based on their physical size. Critically, only real objects were sorted based on their weight. These results suggest that although images and real objects are represented similarly with respect to their semantic properties, images lack the representational richness of their real-world counterparts.

Disclosures: D. Holler: None. **S. Fabbri:** None. **J.C. Snow:** None.

Poster

427. Decision Making II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 427.04/JJJ27

Topic: H.02. Human Cognition and Behavior

Title: Evidence for response-boundary adaptation in two-choice decision tasks across probability conditions in three sensory modalities

Authors: *J. NADEL, P. SIMEN
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Abstract: A key aspect of cognitive tasks is the tradeoff between speed and accuracy. When faced with extraction of a stimulus in noisy environments, organisms must choose between a) increasing the certainty of the stimulus's identity and b) rapid action without full certainty. Despite being a well-documented phenomenon in speeded response tasks, the factors leading to the setting of a particular tradeoff remain unclear. Evidence from a variety of lines of research have indicated that the Drift-Diffusion Model (DDM) accurately describes human behavior in such tasks. The drift diffusion model is a continuous sequential sampling model, in which evidence is accumulated in favor of one option or the other until a boundary is reached and a decision is made.
We previously proposed a diffusion model that heuristically approximates the optimal parameter values, and that can adapt speed-accuracy tradeoffs rapidly. This model predicted specific patterns of response time autocorrelation, along with negative cross-correlations between response times and estimated reward rates. To test the predictions of the model, human participants performed two-alternative forced choice tasks in visual (n = 4 subjects, 18 sessions), auditory (n = 4, 13), and tactile (n = 4, 18) modalities. Each session consisted of ten five-minute blocks. Additionally, to examine the effects of different probabilities of one choice being correct on response behavior, two blocks of five probability conditions were included in each session. The probability conditions were 85% rightward, 70% rightward, 50%, 70% leftward, and 85% leftward. Our results were consistent with the prediction of our previously proposed model; specifically, we observed strong autocorrelations and negative cross-correlations across modalities. Additionally, HDDM (hierarchical drift-diffusion model) fitting with all parameters but starting point held constant showed that participants' starting point shifts according to probability condition, such that left-biased blocks lead to starting points closer to the left boundary, and vice versa. Interestingly, cross-correlation patterns across probability conditions displayed an inverse "U" shape, such that more biased probability conditions had weaker relationships between reward rate and response time. This suggests that increases in bias may decrease the importance of the speed-accuracy tradeoff.

Disclosures: J. Nadel: None. **P. Simen:** None.

Poster

427. Decision Making II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 427.05/JJJ28

Topic: H.02. Human Cognition and Behavior

Title: Does raising the stakes reduce mistakes? Reward affects some, but not all, suboptimalities in a perceptual evidence accumulation task

Authors: *S. M. COOK, W. KEUNG, R. C. WILSON
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Abstract: Many decisions require us to integrate evidence over time. For perceptual decisions, such evidence accumulation involves integrating noisy sensory information to make a decision about some characteristic of the stimulus (e.g. whether dots are drifting left or right). While there is strong evidence that humans and animals actually perform such evidence accumulation, this process is subject to a number of suboptimalities including: (1) sensory noise, (2) uneven integration of evidence over time, (3) order effects such as repeating the last action and (4) irrational preferences in favor of one option over another ("side biases"). In this study we asked whether any or all of these suboptimalities can be modulated by reward. That is, by raising the stakes can we reduce mistakes? To measure the effect of reward on suboptimal perceptual decision making, we used a modified version of the Poisson Clicks Task developed by Brunton et al. (Science 2013). In this task, (N = 82) participants were presented with a stream of auditory clicks in both ears, and they were asked to simply respond which side had more clicks, right or left? Critically, on some, "high stakes", trials the potential reward for a correct choice was high, and on other, "low stakes", trials the potential reward for a correct choice was low. On all trials, they were not rewarded if they answered incorrectly. A simple analysis of accuracy showed that raising the stakes did indeed reduce mistakes in this task from 21% to 19% ($p < 0.001$). To understand which suboptimalities were affected by stakes we used a simple regression model which allowed us to quantify all four suboptimalities. Results show that on high stakes trials, only the average weight given to each click was affected (by about 12%, $p < 0.001$). This suggests that only the relative effect of sensory noise is modulated by stakes such that there is a higher signal to noise ratio on high stakes trials. This further suggests that some, but not all suboptimalities can be modulated in the service of maximizing reward.

Disclosures: S.M. Cook: None. **W. Keung:** None. **R.C. Wilson:** None.

Poster

427. Decision Making II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 427.06/JJJ29

Topic: H.02. Human Cognition and Behavior

Support: ONR N00014-16-1-2251

Title: Modeling memory systems interactions during the development of decision-making expertise

Authors: *B. REUVENI, B. FEINSTEIN, P. J. REBER
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Abstract: Becoming an expert in a complex decision making task can produce a learning curve that is non-monotonic (U-shaped) as the learner switches among competing strategies. Here we observe a non-monotonic learning curve in a visual categorization task as participants are encouraged to switch strategies by model driven stimulus selection. The model, PINNACLE 2.0 (Nomura & Reber, 2012; Reuveni & Reber, *in prep*), is based on memory systems theory and provides a framework for understanding the roles of implicit / explicit memory and their interplay during learning. When initial performance is based on a partially effective explicit rule, early task accuracy will be modestly better than chance. If optimal expertise depends on a more complex representational structure gradually extracted from environmental statistics the learner must move beyond these initial rules. In this case the expert will exhibit intuitive decision making based on implicit learning. The mechanism underlying this transition is not well understood. We examined this process using a visual category learning task based on trial and error with feedback. Participants were initially taught a simple explicit rule before revealing that the true category structure required a more complex one typically learned implicitly. Computational modeling was used to provide online estimates of strategy and memory system use based on patterns of recent choice behavior. Model estimates were used to guide the participants through the strategy shift by selecting stimuli to encourage implicit learning. Successfully modeling behavior in a two memory system framework counter intuitively required hypothesizing that learning happens in parallel across systems on every trial, raising questions about the neural feedback learning mechanisms when systems compete. Conflict resolution between systems was captured with a simple mechanism, but which required a metacognitive analog to confidence, even for implicit (nonconscious) learning. The accuracy of the mechanisms used to fit human behavior can potentially be tested with neuroimaging data that connects neural activity changes to model mechanisms. Dynamic identification of strategy use over 400 trials of learning revealed a gradual shift from a simple rule to intuitive decision making punctuated by periods of rapid switching between strategies (thrashing). This process is inconsistent with

simple models of expertise that assume gradual transformation and improvement of task knowledge that would produce monotonically improving performance. Our model provides a preliminary account of the operation and interaction of these memory systems as expertise develops.

Disclosures: B. Reuveni: None. **B. Feinstein:** None. **P.J. Reber:** None.

Poster

427. Decision Making II

Location: SDCC Halls B-H

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Program #/Poster #: 427.07/JJJ30

Topic: H.02. Human Cognition and Behavior

Support: F32DA039648
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1R01DA04367601A1

Title: Physiological measures link clinical states to impulsive decision-making in opioid use disorder

Authors: *S. LOPEZ-GUZMAN[1,2], A. B. KONOVA[2], J. MESSINGER[2], N. BANAVAR[2], K. LOUIE[2], J. ROTROSEN[3], P. W. GLIMCHER[2]
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Abstract: Objective: Impulsive decision-making is characteristic of addictive disorders and is associated to changes in the brain valuation network due to chronic substance abuse. Computations in this network have been shown to be strongly context-dependent and to explain variation in decision-making behavior in healthy subjects. We hypothesized that changes in clinical states — particularly high-craving states — and other symptomatology may similarly lead to contextual changes in impulsive choice computations that could precipitate a return to drug use. However, inconsistent findings on the relation between self-reported symptom severity and relapse to drug use suggest that measuring psychophysiological manifestations may add precision to the evaluation of current clinical states. We addressed these questions in a group of opioid users receiving standard treatment in an outpatient urban clinic. We explored whether different levels in reported craving, withdrawal symptoms, and anxiety, as well as heart rate variability, skin conductance, and facial electromyography were related to changes in choice behavior in a delay discounting task.
Methods: Individuals with Opioid Use Disorder (OUD) who endorsed recent craving for heroin or other opioids were recruited to participate in 2 sessions. One was conducted before the participant received their medication and the other was conducted after. The order of the two

sessions was randomized across subjects. On each session, we employed validated instruments to assess craving, subjective withdrawal symptom severity, and current levels of anxiety. Participants then completed a 12-minute real-monetary-incentive delay discounting task. Heart rate variability (HRV), galvanic skin response (GSR), and corrugator and zygomatic surface electromyography (EMG) were measured during the task.
Results: In preliminary results (n=22), we find that there were on average no significant differences in symptomatology or impulsive choice between the 2 sessions. However, discount rates in both sessions were significantly explained by anxiety levels and the number of craving episodes in the last 24 hours, and were inversely proportional to the length of those episodes. Interestingly, HRV during the task was negatively correlated to craving intensity reported right before it, suggesting craving may have a physiologically measurable influence on impulsive decision-making. Current efforts in this study focus on increasing our sample size and establishing whether this and other psychophysiological measures are related to subjective value computations during the task.

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Poster

427. Decision Making II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 427.08/JJJ31

Topic: H.02. Human Cognition and Behavior

Title: Evidence accumulation and optimal stopping in stochastic economic choice: Challenging the DDM

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Abstract: Drift-diffusion models (DDM) have had empirical success in fitting both behavior and electrophysiological recordings during binary choice tasks. They model response times along with choice probabilities and permit insights into the processes from which (perceptual or value-based) decisions originate. In the recent theoretical literature, the DDM has been shown to arise as the optimal solution to a tightly limited class of learning problems. DDMs however are only one representative of the class of bounded accumulation or sequential sampling models. Unfortunately, based on choices and response times alone, it is almost impossible to distinguish between different models of this class, and the assumption of optimality remains untested. We address this problem in a behavioral experiment in which we use a new task to directly measure how choice accuracy evolves with time as evidence accumulates, and compare our findings to choices made under unrestricted time. Our incentive compatible paradigm attempts to, in

essence, reveal the evolving decision variable modeled by the DDM in real-time. In each of about 360 trials, subjects are shown 100 dots, some red and some blue, and are asked whether the majority of these are red or blue. In fixed-time trials the dots are masked after a given time that varies between trials (from 0.1 to 6s), and subjects then must select red or blue within 2s. After subjects choose red or blue we elicit their probabilistic beliefs (or confidence) that their previous choice was correct. Subjects thus report their best guess and their confidence in that guess. Reaction-time trials, which serve as a control, are identical but of unlimited duration and presented without a mask. As expected and previously demonstrated, the accuracy of choices increases as a function of the time during which the dots are displayed. However, our preliminary results show surprising patterns in how accuracy and confidence evolve with time that appear to be inconsistent with predictions of the DDM. The reported confidence is largely consistent with the accuracy of the decisions, and provides further empirical restrictions on how the underlying stochastic process modeled by the DDM evolves with time. While our results are preliminary, it is surprising that our measurement of the evolving decision variable in an economic task does not accord well with the predictions of the DDM in this task. This experimental methodology thus promises empirical insights that permit to refine bounded accumulation models in a way that is not possible relying on decisions and response times alone.

Disclosures: S.F. Bucher: None. **P.W. Glimcher:** None.

Poster

427. Decision Making II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 427.09/JJJ32

Topic: H.02. Human Cognition and Behavior

Title: Computational evidence for impulsive responding when expectation for inhibitory control is low in cocaine use disorder

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Abstract: Substance use disorders are among the most disabling conditions among young adults worldwide, yet their underlying pathophysiology is poorly understood. Recent computational approaches have provided new insights into cognitive and behavioral process dysfunctions in substance use disorder. Based on a Bayesian Dynamic Belief Model (DBM), we have found that individuals with stimulant use disorder show poorer tracking of the expectation to engage in inhibitory control (p(stop)). In this investigation, we combined a between-trial learning model (DBM) and a within-trial reaction time model (Hierarchical Drift Diffusion Model (HDDM)) to further delineate computational processing dysfunctions in subjects with cocaine use disorder.

We aimed to determine whether stimulant users, as we have observed in healthy comparison subjects, adjust reaction times as a function of p(stop). We applied a two-level modeling approach, in which p(stop) based on DBM determines the within-trial drift rate (rate of information accumulation) in a HDDM. 35 individuals with cocaine use disorder and 34 controls completed a stop-signal task. Trial-level p(stop) calculated using a DBM was entered as a regressor in a HDDM to examine the main effect of p(stop) on drift rate and interaction between group and p(stop). Cocaine use disorder subjects made more stop trial errors compared to controls based on LME controlling for stop signal delay ($p<.05$). There was a significant main effect of p(stop) on drift rate with a negative slope (slower drift rate when p(stop) was high) (posterior $P<.001$). There was a significant group-by-p(stop) interaction on drift rate, with a more negative slope in cocaine use disorder subjects than controls (i.e. cocaine use disorder subjects were more sensitive to p(stop)) (posterior $P<.001$). Drift rate intercept was negatively correlated with the slope of the effect of p(stop) on drift rate in both groups (cocaine use disorder: $R=-.74$, $p<.001$; controls: $R=-.53$, $p=.001$), indicating individuals who were more sensitive to p(stop) exhibited faster drift rates when p(stop) was low. The results suggest that cocaine use disorder is associated with greater behavioral sensitivity to the probability of a stop. Cocaine use disorder subjects also made more errors on stop trials, possibly due to relatively fast processing when p(stop) was low (despite relatively intact slowing when p(stop) is high). The combination of altered expectation to engage inhibitory processing and increased impulsive responding may be a critical process to target for interventions to reduce relapse in subjects with cocaine use disorder.

Disclosures: J.R. Howlett: None. **K.M. Harle:** None. **M.P. Paulus:** None.

Poster

427. Decision Making II

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Program #/Poster #: 427.10/JJJ33

Topic: H.02. Human Cognition and Behavior

Support: 2R01MH091068

Title: The impact of framing effects and ADHD on intertemporal choice in adolescence

Authors: *J. B. SCHWEITZER[1], P. MUKHERJEE[2], C. FASSBENDER[3], A.-M. IOSIF[5], M. B. MENOR[3], J. BURNS[4], A. J. ROGAWSKI[6], A. MLODNICKA[4], J. F. DIXON[4], S. M. MCCLURE[7]
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Abstract: *Objective*: To explore the effect of contextual (i.e., timing; rounded vs. decimal numbers) and individual variables (i.e., ADHD or not; IQ) on intertemporal choice for monetary outcomes in adolescents (n=129). *Rationale*: Opting for the sooner, smaller (SS) rewards over more delayed, larger (DL) rewards is associated with poor outcomes, such as substance use disorders, poorer physical health and greater economic problems. This project explores variables that might influence decision-making for monetary rewards varying in amount and delay to delivery. *Methods*: Typically developing (TD) and adolescents diagnosed with ADHD chose between 62 intertemporal choices, with half of the trials using rounded values (e.g., $11.00 today or $21.00 in 6 weeks - rounded condition) and half of the trials using decimal conditions (e.g., $10.87 today or $20.74 in 6 weeks - decimal condition). In addition, we explored the effect of the timing of the smaller, sooner reward (e.g., immediate vs sometime in the future - later) versus larger, much later in time on choice. Finally, we analyzed the effect of diagnosis (e.g., ADHD or TD) and IQ on choice. *Results*: Adolescents were more impulsive (i.e., chose SS) on the rounded versus decimal trials ($p = .007$) and when the SS trials were later (e.g., in 2 weeks) than when the SS were immediate (e.g., today; $p = .0001$). ADHD diagnosis was associated with selecting more SS over DL; $p = .05$), in the immediate condition (not the DL condition), however, choice in the TDs was more affected by the length of time to the SS than in the ADHD group ($p = .01$). There was emerging support for sex by group differences ($p = .096$) with females with ADHD demonstrating less impulsivity across conditions and their behavior approximating the TD females. As choice of the SS over the DL choices appeared to be driven by the ADHD male group (i.e., ADHD female choice approximated TD female choice) we further analyzed the effect of cognitive factors on male choice ($n = 83$). Higher IQ resulted in fewer impulsive choices (i.e., greater choice of DL over SS) in the TD group ($r=-.5$; $p = .003$), but not in the ADHD group ($r=.15$; $p = .3$). *Conclusion*: Framing effects should be considered in developing interventions to decrease impulsivity.

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Poster

427. Decision Making II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 427.11/JJJ34

Topic: H.02. Human Cognition and Behavior

Support: Bial Foundation Grant 388/14

Title: Changes in perceived time of intention and movement onset in arbitrary and deliberate decisions

Authors: *U. MAOZ[1,3,5], S. WONG[2], N. ZIARI[3], M. J. SAMAD[4], X. ZHANG[4]
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Abstract: Libet (1985) showed that information about action onset exists in the brain before participants report forming the intention to act. This led to claims that all decisions are made unconsciously, challenging pillars of human social order, like free will and moral responsibility. In that paradigm, intention onset and movement onset were timed using an external clock (W and M time, respectively). Importantly, the decisions in these studies were arbitrary (e.g., raising the left or right hand). Here we investigated the extent to which W and M time generalize to deliberate decisions.
Participants (n=31) first tasted 10 different drinks—from appetitive ones like lemonade to aversive ones like diluted soy sauce—and rated their palatability. Then, on each trial, they made a choice between two drinks by pressing a button. Our clock was a rapidly changing stream of letters (inter-letter interval 200 ms) for both W time and M time. The experiment included 3 types of decisions in a counterbalanced, blocked design (10 trials per block). In *deliberate-decision* blocks, participants selected the drink they preferred. To motivate deliberate decisions, one trial in each block was randomly selected, and participants had to sip the drink they chose in that trial at the end of the block. *Arbitrary-different* blocks were the same except that participants had to drink both drinks in the randomly selected trial at the end of each block, regardless of their selection. In *arbitrary-same* blocks, participants were presented with the same drink twice, differently motivating arbitrary selection. Randomly interleaved memory catch trials motivated subjects to pay attention throughout the experiment. Participants consistently reported earlier W times in deliberate decisions compared to both arbitrary ones, whereas M time was reported earliest for deliberate decisions, incrementally later for arbitrary-different, and later still for arbitrary-same decisions. In a follow up study of subjects prescreened for high OCI-R scores (indicative of obsessive-compulsive disorder, OCD), W time moved increasingly towards movement onset with higher OCD scores for arbitrary-different decisions. We also found that W time was consistently about 200 ms earlier for all decision types when M and W were measured in the same experimental session, than when W time alone was measured. A preliminary drift-diffusion model that we constructed explains this result via nonlinearities stemming from the number of thresholds imposed on the model. Our results challenge the generalizability of the Libet results from arbitrary to deliberate decisions and could suggest a link between OCD and the report of intention onset.

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Poster

427. Decision Making II

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Topic: H.02. Human Cognition and Behavior

Support: LABEX BIO-PSY

Title: Executive fatigue and time pressure show dissociable effects on decision-making

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Abstract: The ability to overcome time and effort costs is an important predictor of higher education and professional success. This ability has been related to the intervention of the executive control network, including lateral prefrontal and parietal regions. Recently, it has been shown that fatigue of the executive control system leads to an increased preference for immediate rewards, over larger but delayed rewards (Blain, Hollard, & Pessiglione, 2016). However, the executive control system may also be involved in the choice process itself, ensuring precise selection of the best option. We thus tested whether executive fatigue would specifically change the preference about options (i.e., exert a bias on which option is selected) or change the amount of control exerted on the choice process itself (i.e., altering the accuracy of decision-making).To better dissociate these two possibilities, we compared the effects of executive fatigue and time pressure, in two separate experiments. In both experiments, participants made choices between no-cost options (immediate, sure and free rewards) and costly options (delayed rewards, probabilistic rewards and rewards in exchange for performing an effort, either cognitive or physical). In the first experiment (n=24), we induced executive fatigue in participants by 6 hours of difficult executive control tasks (working memory and task-switching). In the second experiment (n=27), participants had to make the same choices with and without time pressure (timeout set at 70% of self-paced response time). Choice behavior was fitted with standard discounting models including 3 parameters (bias toward no-cost option, weight on cost level and choice stochasticity).Comparison of fitted parameters revealed distinct effects of executive fatigue and time pressure. Executive fatigue increased the bias towards no-cost options (without affecting choice accuracy) when opposed to delayed and effortful options. By contrast, time pressure increased the choice stochasticity (without affecting choice preference), for all types of options. Thus, we conclude that executive fatigue changes preferences about time and effort, but does not disrupt the choice process itself.

Disclosures: A. Wiehler: None. **M. Pessiglione:** None.

Poster

427. Decision Making II

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Program #/Poster #: 427.13/JJJ36

Topic: H.02. Human Cognition and Behavior

Support: ERC Grant 63829

Title: Exploring the neural basis of metacognition in perceptual decision-making

Authors: *W. RYS[1], G. LOUGHNANE[2], D. P. MCGOVERN[2], C. JUDD[2], S. KELLY[3], R. G. O'CONNELL[1]
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Abstract: Computational modelling and neurophysiological recordings suggest that perceptual decision-making involves integrating noisy sensory evidence up to an action triggering threshold. Theoretical work and single-unit recording studies have also suggested that the same evidence integration process plays a central role in facilitating graded representations of choice confidence. The present study examined this possibility in human subjects during motion discrimination judgments. Participants were presented with a patch of coherently moving dots and, at random intervals, were presented with a response cue prompting them to simultaneously indicate the dominant motion direction and their confidence in that choice. The task was administered under two separate conditions requiring either saccadic or left/right hand button press responses. Analyses centred on two recently characterised non-invasive electroencephalographic signatures of decision formation: the Centro-Parietal Positivity (CPP) which traces the accumulation of sensory evidence for perceptual decisions irrespective of the sensory or motor requirements of the task, and effector-selective premotor beta-band activity which reflects the translation of the decision into a specific motor plan. Here, the CPP exhibited a gradual build up during presentation of the coherent motion stimulus and the amplitude it reached at the time of response cue presentation exhibited a strong positive correlation with confidence reports and choice accuracy. Meanwhile, premotor beta activity indicated that higher choice confidence was associated with reduced preparation of the unchosen effector, consistent with models in which choice confidence reflects the relative activation of the chosen and unchosen response alternatives (i.e. stronger response conflict would lead to lower confidence). These results suggest the neural representation of cumulative evidence provides the necessary information for the emergence of a graded representation of choice certainty.

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Poster

427. Decision Making II

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Topic: H.02. Human Cognition and Behavior

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The McGovern Institute

Title: On the capacity of recurrent inhibition models for Bayesian updating and control of behavior

Authors: *S. W. EGGER[1], N. LE[2], M. JAZAYERI[3]
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Abstract: Humans and animals face the challenging task of producing precise motor outputs to match task demands in a dynamic environment. This problem can be formalized under a Bayesian framework in which sensory information acts to mitigate uncertainty arising from changes in external states and internal sources of noise. While experimental psychophysics support the hypothesis that the brain performs Bayesian inference, the implementation at the level of neural circuits is poorly understood. Here, we develop a model based on a simple circuit motif, mutual inhibition, that can emulate the key characteristics of Bayesian updating to control motor output. We begin by considering a simple timing task in which the model has to initiate an "action" after a specified interval. A pair of mutually inhibitory units can be configured to generate an output that ramps up at different rates depending on the strength of a common input. By applying a fixed action-triggering threshold, this model can flexibly control action initiation time. However, internal noise can lead to significant variability in threshold crossing times. This variability can be offset by integrating additional temporal cues. To test whether the model can emulate this integration, we created a cascade of mutually inhibitory modules. Modules are activated sequentially and each generates ramp-like activity between a specific pair of consecutive temporal cues. When a cue is received, the output of the current module adjusts input into the subsequent module. Iteration of this process allows each module to counter deviations due to previously accumulated noise. The final module generates ramp-like activity to threshold and controls motor initiation time. Consistent with Bayesian updating, variability of cascade output decreases with increasing number of temporal cues. Importantly, parameters of

the model are robust (i.e., do not need adjustment) to both input levels and variations in noise, allowing for near-optimal integration with fixed circuit properties. The success of this simple model motivated the development of extensions in the form of linear and circular bump attractors. Using similar updating principles, these networks represent and update beliefs about the position and speed of a moving object by integrating intermittent spatiotemporal cues. Together, these results provide a plausible neural substrate for performing complex probabilistic inferences about the dynamic states of objects and events in the environment.

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Poster

427. Decision Making II

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Program #/Poster #: 427.15/JJJ38

Topic: H.02. Human Cognition and Behavior

Support: ERC Consolidator Grant 617629

Title: Transfer of confidence in a novel observational learning task

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Abstract: Methods for measuring the confidence with which people make decisions, have traditionally relied on post-decision self-reports. We wanted to: 1) develop a new experimental paradigm that aims at measuring the confidence in a decision without participants having to explicitly rate their confidence, 2) characterize their decision using fMRI, and 3) show transfer of confidence through observation using the said paradigm.
In our experiment, the participants have to move a cursor around a circle to catch particles moving from the centre to the edge of the circle. The direction of the particles is block-wise determined by a mean and its standard deviation from that mean. The participants can change the size of the cursor, and the amount of points rewarded for each catch is inversely proportional to the size of the cursor.
The paradigm was tested in several deceit-free behavioural psychophysics experiments and analysed using computational modelling.
To test that the task actually measures confidence, we added a condition in which blocks of trials were followed by a confidence rating scale. The model estimated trial-by-trial particle variance correlated strongly with the normalized ratings given by the participants. Similarly, the computational modelling found each participant's trial-by-trial estimation of the particle variance to be correlated with the width of the cursor on the given trial.
fMRI results show strong correlations between expected decision making and learning areas

adding validity to the task.
Finally, to investigate if it is possible to transfer confidence between participants, we ran an experiment where participants could observe the cursor width chosen by previous participants. Results show that participants that observed a player with higher base confidence chose a significantly smaller catcher than the group observing a player with lower base confidence, an effect that persisted after the other player's choices were no longer displayed.
In conclusion, we have developed a novel task that allows for measuring choice confidence implicitly, and used this task to show that participants adapt an observed level of confidence to their own choices, a level that persists even after observations are no longer available.

Disclosures: T. Larsen: None. **D. Pischedda:** None. **G. Coricelli:** None.

Poster

427. Decision Making II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 427.16/JJJ39

Topic: H.02. Human Cognition and Behavior

Support: NSF Grant 5260013
NIH Grant 524294

Title: Ramping risk-taking: Progressing value function increases gambling in humans

Authors: *G. J. PAGNIER, A. WESTBROOK[1,2,3], M. J. FRANK[3]
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Abstract: Anticipating a reward is critical for guiding behavior. Recent work has hypothesized that mesolimbic dopamine (DA) levels, distinct from phasic DA reward prediction errors, encode this anticipation by signaling the value of work (Hamid et al., 2016). In an instrumental task, rats exhibit a gradual ramping up of extracellular mesolimbic DA tone in proportion to the distance to, and magnitude, of the reward. But while previous accounts suggest that such DA ramps only invigorate responding, our theoretical models of striatal DA (Collins & Frank, 2014) suggest that higher endogenous DA levels would also bias decisions toward options that have higher perceived reward even if they also have higher cost. Here, we test the prediction that humans are more likely to gamble for a potential, larger reward at the expense of losing a certain reward, the closer they are to receiving that certain reward (i.e., as the value function ramps). We developed a novel gambling task in which participants were promised a certain amount of money at the end of a fixed interval on every trial, with progress indicated by a continuous progress bar. As the progress bar was filling up, participants were sometimes presented with the choice to either stay

with the guaranteed amount, or to gamble for a potentially larger reward. We describe implications of these results and test whether increased vigor and/or modified reward and cost perception affect an individual's decision to gamble. Preliminary results indicate that participants potentially gamble more the closer they were to the end of the fixed trial period, and only when the benefit of gambling was sufficiently large. These results suggest that participants are possibly more sensitive to rewards versus costs as they approach a rewarding outcome, potentially independent of the increased vigor associated with increased DA, and are also consistent with the hypothesis that striatal DA tone ramps in humans during an instrumental task.

Disclosures: A. Westbrook: None. **M.J. Frank:** None.

Poster

427. Decision Making II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 427.17/JJJ40

Topic: H.02. Human Cognition and Behavior

Support: NIH/NIGMS Grant K12GM102773-05
NIH/NCATS Grant KL2TR001421
NIH/NIDA Grant P50 DA006634-26

Title: A pilot study of a novel reinforcement learning task: The value of cognitive control task

Authors: *S. BELL[1], K. T. KISHIDA[1,2]
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Abstract: Background: Value-based decision making is critical to adaptive behavior and is disrupted in substance use disorder and other neuropsychiatric disorders. Substance use disorder is hallmarked by exploitative reward seeking with a lack of regard for inherent cost. Computational reinforcement learning theory provides a useful framework for investigating value-based decision-making. We have designed a novel "Value of Cognitive Control (VoCC) Task," to investigate how learning about future "costs" associated with present choices guides behavior. The present study with n=35 healthy participants (16 M, 19 F, mean age = 27.8) aims to determine whether the VoCC task elicits behavior consistent with value-based decision-making that accounts for costs, represented by varying levels of required cognitive control, and monetary rewards. Methods: The VoCC task is a computer based decision-making game. At the beginning of each trial participants must choose one of three options. Each option leads, respectively, to 'low', 'medium', or 'high' difficulty problems from Cattell's Culture Fair Intelligence Test. Participants earn $1 for every problem answered correctly. If a particular option is exploited and "depleted", the task is programmed deliver the most difficult problems to

encourage exploration. The optimal strategy is to learn and exploit options that give the easiest problems in order to maximize reward. We employed binary logistic regression to examine, at each trial, predictors of switching options in the following trial. We hypothesized that incorrect responses and higher difficulty levels would be associated with a higher likelihood of subsequent switching (exploration). Analyses were performed on the full sample across both genders; gender differences will be examined in the future. Results: As hypothesized, problem difficulty positively predicted exploratory switching behavior during the task (mean coefficient = 0.74, p = 0.016) and correct responses were negatively associated with subsequent exploration (mean coefficient = -3.21, p = 0.016). Conclusions: Our results suggest that the VoCC task elicits choices that appear to be based on both rewards and cost. Future directions include employing the task in substance-using populations, modeling behavior using temporal difference reinforcement learning framework, and examining the neural substrates of optimal task performance using fMRI.

Disclosures: K.T. Kishida: None.

Poster

427. Decision Making II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 427.18/JJJ41

Topic: H.02. Human Cognition and Behavior

Title: Generalisation of human random behaviour

Authors: *S. WONG[1], U. MAOZ[2], G. MERHOLZ[3]
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Abstract: Human endogenous random-number generation (RNG) has been shown to be systematically biased towards underestimating the likelihood of long runs of repeated entries (e.g., a run of 0-0-0-0 is underrepresented in binary (0/1) random-series). However, this bias can be reduced in a competitive environment. We investigated whether participants could transfer this learning back to a non-competitive environment. Participants (n=153) carried out a 3-part experiment where they: (Part A) created random sequences of Rock (R), Paper (P), Scissors (S) to establish baseline bias, (Part B) played a competitive game of R-P-S against a computer, then (Part C) again created random sequences of R-P-S as in part A, to measure any post-learning transfer effects.
This experiment had 3 between-subjects conditions, varying by computer algorithm and instruction to the participants in part B. In conditions 1 and 2, the computer searched for patterns in each participant's response and win/loss/tie history to predict the participant's next move. Thus, a participant's game responses needed to be as random as possible to win. In condition 3,

the computer generally followed a simple R-P-S-R-... pattern, where the corresponding winning strategy for the participant was P-S-R-P-.... Furthermore, in conditions 1 and 3, participants were only instructed to play to win against the computer, while in condition 2 they were specifically informed about the computer search algorithm and that they had to be as random as possible to win.
We computed each participant's randomness score using the Wald-Wolfowitz runs test, confirming that participants indeed produced more random sequences and longer runs in part B than in parts A or C. Their patterns in part B were not statistically different than Matlab's pseudo-random number generation. While participants' randomness in part B of conditions 1 and 2 were not reliably different, only those in condition 1 showed transfer learning from part B to C, despite not being informed about the computer's algorithm. Our results suggest that humans can learn to become relatively random in a competitive situation with feedback and maintain that randomness for other tasks, but only if the ability is learned implicitly.
Future directions include putting this experiment on mTurk to collect more data for more sophisticated analysis. We will also be varying the features of the game situation to explore the extent to which improvement in random sequence generation is due to the competitive environment or feedback provided by the game.

Disclosures: S. Wong: None. **U. Maoz:** None. **G. Merholz:** None.

Poster

427. Decision Making II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 427.19/JJJ42

Topic: H.02. Human Cognition and Behavior

Support: 15/CDA/3591

Title: The neurocognitive architecture that underlies the redundant signal effect in audio-visual target detection: An EEG study

Authors: *J. M. EGAN[1], R. G. O'CONNELL[2], S. KELLY[1]
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Abstract: When two signals are redundant, such that either signal alone is sufficient to elicit a response, we can respond to their co-occurrence faster than we can respond to either of the signals presented in isolation. This is known as the 'Redundant Signals Effect' (RSE), and although many studies have inferred from response behaviour that convergent processing of the signals must be involved, the neurocognitive architecture that underlies the RSE has not been firmly established. Specifically, while studies on perceptual decision making show that we

translate noisy sensory information into appropriate responses by accumulating the information over time, it is not known whether information from redundant signals is accumulated in parallel, or whether the information first converges to a common pool and is then accumulated by a single accumulator. In order to determine the neurocognitive architecture that underlies the RSE in a simple target-detection task, we recorded EEG data from participants who were asked to report periods of coherency in otherwise incoherent auditory and visual stimuli. The participants performed two variants of this task in separate blocks: In half of the blocks participants were asked to report either visual or auditory events, or their co-occurrence [OR condition]; in the other half the participants were asked to only report the co-occurrence of auditory and visual events [AND condition]. The CPP (Centro-parietal positivity; a signal that reflects evidence accumulation), reached different peak amplitudes for auditory and visual events during the OR condition, despite the response time distributions being very similar, suggesting that auditory and visual information does not converge to a single modality-independent accumulator. However, CPPs elicited by the co-occurrence of auditory and visual events in the OR condition - which produced an RSE - did not reach the amplitude that was predicted by simulating an entirely independent race between two parallel modality-specific accumulators to initiate a response. Furthermore, CPPs were elicited by auditory or visual events alone in the AND condition, suggesting that information from each source is accumulated separately and is not bound into a distinct multisensory construct prior to accumulation. We believe that an architecture that involves parallel processing of information until at least an evidence accumulation stage, followed by online convergence of information at a later stage (eg. motor planning), provides the simplest explanation of our results, and we are currently conducting further EEG studies to test this proposition.

Disclosures: J.M. Egan: None. **R.G. O'Connell:** None. **S. Kelly:** None.

Poster

427. Decision Making II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 427.20/JJJ43

Topic: H.02. Human Cognition and Behavior

Support: NIMH grant 1R03MH101592
NSF cogneuro grant BCS-1358955

Title: Manipulation of initial dynamics of value-biased sensorimotor decision mechanisms

Authors: *K. AFACAN[1], S. KELLY[2]
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Abstract: In daily life when we must quickly respond to sensory cues, sensorimotor decisions must be biased towards more valuable options for reward maximization. We recently reported that under such circumstances, humans show behavioral and electrophysiological patterns consistent with an initially non-selective, temporally increasing evidence representation in addition to a value-based shift in "drift rate" of an accumulate-to-bound decision process, contrary to dominant, "starting-point bias" mechanisms that best explain deliberative, perceptual judgments (Afacan-Seref et al 2018). In our Dynamic Shift of Influence model, the underlying principle is that feature-tuned neural populations initially respond non-selectively, but their selectivity increases over time, and when the population tuned for the higher value feature is boosted relative to the lower-value color coding population, the differential evidence driving an evidence-accumulation process initially favors the high-value option regardless of what is presented. Thus, on presentation of a low-value cue, differential evidence (and hence drift rate) initially favors the incorrect, higher value alternative but over time switches to favoring the correct alternative, resulting in a turn-around in cumulative evidence that is reflected in choice error patterns and electroencephalographic signatures of motor preparation. Here we extend our study to investigate the hypothesis that cues with a more intense and longer-lasting initial nonselective phase will enhance this dynamic shift of influence effect. In our task, subjects perform rapid, suprathreshold color discrimination reported through left/right hand button clicks within a very strict deadline, with one alternative rewarded more points than the other if correct. In randomly interleaved half of the trials, the cue begins with a sudden bright but non-discriminating flash and then fades to one of the color alternatives in ~50ms in order to evoke a more intense and longer-lasting initial nonselective phase. Preliminary analysis of data collected so far indicate that the shift of influence from value to sensory information driving action choices is delayed in the "flash" condition compared to the regular color cue trials. Preliminary behavioral modelling suggests that drift rate bias models with increasing evidence, as previously, provided a better fit of the data for both cue conditions. We will show further results from electrophysiology and modeling examining the role of such rapid drift rate bias mechanisms in value-biased sensorimotor decisions.

Disclosures: K. Afacan: None. **S. Kelly:** None.

Poster

427. Decision Making II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 427.21/JJJ44

Topic: H.02. Human Cognition and Behavior

Support: Grant-Aid for JSPS Fellows

Title: Probability estimation and decision with limited knowledge of uncertainty

Authors: *K. OTA[1,2,3], J. PHILLIPS[1], L. T. MALONEY[1,4]
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Abstract: Bayesian decision-theoretic models predict actions maximizing expected gain in stochastic tasks. BDT presupposes (1) that the visual system has estimates of probability (density) that conform to laws of probability and (2) that it correctly combines probability with value. We test these assumptions in two visual tasks, *estimation* and *decision*. Methods: In the estimation task observers were shown a Gaussian sample of size 5 or 30 on a display. They were then shown an interval marked on the display and asked to estimate the probability p[I] that the next dot from the same distribution would fall in the interval. Observers received feedback. Correct performance in this *interval estimation* task is equivalent to correct use of sample information in estimating probability density. There were three types of intervals: symmetric S around the mean and the other two being the upper SU and lower SL halves of S. These triples allowed a test of *density additivity*:P[S] = P[SU] + P[SL]. We varied interval width so that p[I] spanned the range 0.1 to 0.9. In the decision task, observers were shown similar samples but now with a penalty boundary (PB) marked on the display. Observers moved the visible sample to any location on the screen translating the mean of the Gaussian. Then a new dot from the translated Gaussian distribution determined a score in the trial. If the point was above the PB they suffered the penalty, if below, the reward decreased with distance from the PB. There were two penalty conditions 0 and -500. We used BDT to predict the statistically optimal setting points for each condition (2 penalties * 2 sample sizes). Results: *Estimation*: observers' estimates of probability were close to accurate with a tendency for some observers to overestimate probability. Their estimates satisfied density additivity. *Decision*: the settings for the 30 dot condition were close to the optimal settings. However, in the 5 dot condition, they were too close to the PB in both penalty conditions. *Conclusion*: Although their estimates of probability were near accurate, their decision was incorrect, suggesting that observers had a problem with transforming their estimates of probability to their decision when they had limited knowledge of uncertainty.

Disclosures: K. Ota: None. **J. Phillips:** None. **L.T. Maloney:** None.

Poster

427. Decision Making II

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant DA043196
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Title: Predicting memory-based decisions using semantic fluency and preferences

Authors: Z. ZHANG[1], A. NRUSIMHA[2], M. HSU[1], *A. S. KAYSER[3]
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Abstract: The past decade has seen substantial progress in our understanding of the neural basis of economic decision making. To date, however, most existing work has focused on "stimulus-based decisions" (SB-D), in which all relevant attribute information is physically present at the time of choice. Such focus is instrumental in identifying how value is encoded and processed in the brain, but may have overlooked important constraints that other cognitive processes, such as memory, impose on decisions. To fill this gap, we examine an important class of so-called "memory-based decisions" (MB-D), such as choosing a dinner restaurant from memory. In contrast with SB-D, in which an externally defined set constrains choices, MB-D require decision makers to recall the set of relevant choice alternatives. We hypothesized that to make MB-D, decision makers must first construct an internal choice set based on semantic memory, after which they make a decision based on valuation of the options in the choice set. We collected independent datasets for brands from two product categories (fast food restaurants and running shoes) in order to define three measures: (1) semantic memory, measured by a classic semantic fluency task (i.e. name as many brands in a category as possible; sample size N = 240); (2) valuation, measured by choices in SB-D with a list of brands in a category; N = 1405); and (3) MB-D, measured by choices of brands in a category without a menu (N = 1405). We then used the semantic fluency and SB-D data as the input to a two-stage model embodying our hypothesis, and used its output to predict MB-D. This model improves substantially over the baseline model that only uses preference (SB-D) information (likelihood ratio test $p < 0.001$ for both categories), implying the critical role of memory in MB-D. It also outperforms models predicting MB-D based on semantic memory only, or on simple additive effects of memory and preference, lending strong support to the proposed mechanism involving the intermediate construction of an internal choice set through recall. These findings reveal an important cognitive mechanism through which semantic memory influences and constrains value-based decision making. They also bear important clinical implications for better characterizing suboptimal decision making in pathological conditions impacting semantic memory, such as Alzheimer's disease and semantic dementia.

Disclosures: Z. Zhang: None. **A. Nrusimha:** None. **M. Hsu:** None. **A.S. Kayser:** None.

Poster

427. Decision Making II

Location: SDCC Halls B-H

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Topic: H.02. Human Cognition and Behavior

Support: European Union-funded FP6 Integrated Project IMAGEN (Reinforcement- related behavior in normal brain function and psycho- pathology), Grant/Award Number: LSHM-CT- 2007- 037286
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Title: Dissecting the decision to respond provides insight into the processes driving successful inhibition between good and bad inhibitors

Authors: *S. ADISE, N. D'ALBERTO, B. CHAARANI, S. HIGGINS, H. GARAVAN
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Abstract: **Introduction:** Deficits in inhibitory control are observed in populations that exhibit maladaptive behavior (e.g., substance abuse, overeating). This suggests that unwanted responses are due to a failure of inhibitory control. The stop signal task (SST) assesses inhibitory control behaviorally by calculating a stop-signal reaction time (SSRT) (i.e., the time needed to stop a response once already initiated). Yet, the ability to stop depends on the decision to respond. Thus, understanding the mechanisms driving go processes in relation to successful inhibition is important.
Objective: The objective of the current study was three-fold: 1) to apply a drift-diffusion model (DDM) to the reaction time of go processes in the SST to parse out different decision-making components, such as response caution (i.e., bias to go/stop), motor execution and stimulus processing time, and speed; 2) to determine if these components relate to the SSRT of good (GI) and poor inhibitors (PI); 3) to examine how these components relate to blood-oxygen-level-

dependent (BOLD) responses in the stopping network.
Methods: Secondary data analysis was conducted on 210 adolescents (n = 95 male; 17-21-years-old) who underwent functional magnetic resonance imaging (fMRI) while performing the SST. SSRT was used to classify subjects as GI and PI; these groups showed differences in BOLD response in the bilateral inferior gyrus (regions associated with stopping behavior). Multiple ANCOVAs were run to assess differences between groups in components of the DDM and BOLD responses to stop trials. Regions of interest were defined based on the stop contrast for all subjects. Covariates were age, sex, handedness, and data collection site.
Results: Preliminary results show that when controlling for covariates, GIs were more cautious (m = 0.69; SD = 0.13) than BIs (m = 0.62; SD = 0.10; $F(1,198) = 7.5$, $p = 0.007$). In addition, motor execution and stimulus processing time was shorter in GIs (m=0.31, SD=0.4) than PIs (m = 0.33, SD = 0.04, $F(1,198) = 14.9$, $p < 0.001$). No significant differences were observed between groups in the speed of the go process. BOLD responses for stop trials between groups did not relate to these decision-making components.
Conclusion: These preliminary results suggest that drift diffusion modeling may provide insight into the decision-making processes contributing to successful inhibition in good and poor inhibitors. However, how these components relate to BOLD activity needs further exploration. Understanding the mechanisms of both go and stop processes in relation to successful inhibitory control may have implications for interventions that target decreasing maladaptive behaviors.

Disclosures: S. Adise: None. **N. D'Alberto:** None. **B. Chaarani:** None. **S. Higgins:** None. **H. Garavan:** None.

Poster

427. Decision Making II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 427.24/JJJ47

Topic: H.02. Human Cognition and Behavior

Title: How do analogies happen in the brain?

Authors: *S. R. DEISS
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Abstract: This theoretical research seeks to identify the brain mechanisms of analogical reasoning which produce and then use concepts arrived at through perceptual and higher order induction using similarity comparison. Long thought by many to be the "foundational substrate" of "higher" intelligence, analogical reasoning remains an "opaque" subject the "insight" into which should likely "stem" from deep cortical "folds" and be represented by yet unknown encodings of "spiking" activity. All our concepts, just a few of which were highlighted in quotes in that last sentence, derive from analogical similarities just as our conscious perceptions require

recognition of objects, actions, and processes in terms of their similarities to past experiences. From these acts of recognition, relevant decisions and predictions can be made. For general artificial intelligence (GAI), and especially for neuromorphic engineering (NME), lack of understanding of the underlying brain processes is a serious stumbling block. Is there anything yet that can usefully be said about how this happens in the brain? At the very least we can start listing the features such a model of brain activity must have to suggest where we might look among the spikes and waves to provide some guidance for GAI and NME engineers as well as neuroscience experimentalists.

Disclosures: S.R. Deiss: None.

Poster

427. Decision Making II

Location: SDCC Halls B-H

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Program #/Poster #: 427.25/JJJ48

Topic: H.02. Human Cognition and Behavior

Support: NSF DMS1724240
NSF DMS1516288

Title: Dopamine-related changes in striatal pathway competition modify specific decision parameters

Authors: *J. E. RUBIN[1], K. DUNOVAN[2], C. VICH[3], M. CLAPP[4], T. VERSTYNEN[2]
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Abstract: Introduction: Mammals selecting actions in noisy contexts quickly adapt to unexpected outcomes to better resolve uncertainty in future decisions. Such feedback-based changes in behavior rely on plasticity within cortico-basal-ganglia-thalamic (CBGT) networks, driven by dopaminergic (DA) modulation of cortical inputs to the direct (D) and indirect (I) pathways of the striatum. DA error signals favor the D pathway over the I pathway for rewarding actions with the opposite tendency for aversive ones, effectively encoding the values of alternative actions. It remains unclear how changes in action value influence the mechanisms of the action selection process itself.
Results: (1) Our simulations of a biologically plausible spiking model of CBGT networks illustrate that feedback-driven DA signals lead to asymmetrical weights in the D and I pathways within a given action channel and the ratio of these weights effectively encodes the action's expected value. (2) Simulations of the full CBGT network in the context of a simple 2-choice value-based decision task under different weighting schemes for cortical inputs to the D and I

pathways (high, medium, and low weight ratio) for one of the action channels yield simulated response times that were fit with variants of a drift-diffusion model (DDM), leaving parameters such as the drift-rate or the boundary height free to vary with the weight ratio. As the ratio increases, the speed of information accumulation in the decision process also increases, providing a direct mapping between network level properties of CBGT systems and cognitive decision processes. (3) Finally, we have incorporated the corticostriatal plasticity module into the CBGT network model to form an integrated learning and decision-making network. Fits of the DDM to integrated network outputs provide novel predictions about the mapping between CBGT and DDM parameters -- drift-rate, boundary height, accumulation onset time, bias, and others -- that best captures RTs associated with variable reward schedules in human experiments performed in our lab. This framework also allows us to explore how particular basal ganglia network features, such as tonic dopamine levels and changes in synaptic connection strengths, relate to changes in decision-making strategies, including those driven by behavioral parameters such as expectation and motivation.

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Poster

427. Decision Making II

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Topic: H.02. Human Cognition and Behavior

Support: CRCNS/NIMH grant 1R01MH112166-01
NSF grant EEC-1028725

Title: Partially observable Markov decision processes explain probabilistic reasoning in social decision making

Authors: *K. KHALVATI[1], S. A. PARK[3], J.-C. DREHER[4], R. P. RAO[1,2]
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Abstract: When decisions are made in a social context, humans often rely on a model of others to infer their intentions and predict their future actions. This ability can also take into account the fact that others have the same ability for inference and prediction. This human capability, known as Theory of Mind (ToM), involves reasoning about others' mental states and decision making under uncertainty. Here we model this ability using the normative computational framework of Partially Observable Markov Decision Processes (POMDPs). POMDPs provide a formal

framework for combining Bayesian reasoning with long-term utility maximization for optimal decision making under uncertainty.
We show that POMDPs can predict human behaviour in a complex social decision making task that requires accurate predictions about the intentions of others. Specifically, we explore an instance of the Volunteer's Dilemma. In a volunteering situation, a few individuals must bear a cost in order to deliver a greater benefit to all members of the group they belong to. The complexity of the task arises from the fact that while the lack of a sufficient number of volunteers results in a failure to achieve the group's goal, ending up with too many volunteers results in a waste of resources. In such a scenario, a precise estimation of others' intentions can be extremely beneficial for each group member. In our experiment, 30 human subjects played a binary version of a multi-round Public Goods Game (PGG) in a group of 5 players. The number of required volunteers to produce the "public goods" in each PGG was fixed to be either 2 or 4. We compared the predictions of the POMDP model in the PGG task to Q-learning, a reinforcement learning approach that computes actions based on the history of rewards without using a model of others. We found that the POMDP model explained the actions of subjects significantly better than Q-learning: while the POMDP fitted human choices in the PGG task with an accuracy above 80%, Q-learning gave a poor fit (less than 60% accuracy). Additionally, the POMDP model outperformed state-of-the-art descriptive models for predicting actions in the PGG task.
The POMDP framework selects actions by combining probabilistic reasoning about hidden state based on a model of the environment with utility maximization. Q-learning on the other hand is model-free and selects the action having the highest expected reward based on past rewards. Our results demonstrate a greater similarity in behavior between the POMDP model and humans compared to Q-learning, and suggest a mechanism for human social decision making that incorporates probabilistic reasoning about others' intentions when selecting actions.

Disclosures: K. Khalvati: None. **S.A. Park:** None. **J. Dreher:** None. **R.P. Rao:** None.

Poster

427. Decision Making II

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Topic: H.02. Human Cognition and Behavior

Support: Grant W911NF-16-1-0474 from the US Army Research Office.

Title: Cardiac sympathetic dynamics of optimal choice in context

Authors: *N. M. DUNDON[1], V. BABENKO[1], M. CIESLAK[1], N. GARRETT[2], N. D. DAW[2], S. T. GRAFTON[1]

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Abstract: Many choice tasks, such as foraging, require organisms to accept or reject options without directly observing the alternatives. In such situations, optimal choice (given by the marginal value theorem) requires appraising options relative to the average reward rate of the environment. We hypothesized that such context-appropriate evaluations are supported by autonomic mechanisms. Our task emulated a formal sequential foraging task under a time constraint; participants performed a simple video game in which they were travelers on a space ship that needed refueling to persist in play. Fuel was acquired by capturing serially approaching invaders, whose identities mapped onto a profitability rank (1 to 4) depending on whether they provided a hi or low fuel reward, and a hi or low capture time cost. Participants performed two 12-minute blocks, one with a disproportionately high concentration of rank 1 invaders (hi reward environment) and one with a disproportionately high concentration of rank 4 (low reward environment). Impedance cardiography and electrocardiography were used to measure beat to beat changes of sympathetic tone as measured by the duration of the pre-ejection (PEP) and left ventricular ejection time (LVET). We hypothesized that more demanding environment (low reward) with greater foraging demands would require increased sympathetic drive. Behavioural results demonstrate that in the high reward environment, participants quickly learned the optimal strategy: to only take rank 1 invaders and to pass ranks 2 to 4. In the low reward environment, participants selected more rank 2 and 3 invaders - again the optimal strategy predicted by the marginal value theorem. We further observed shorter PEP and longer LVET in the low reward environment, pointing to greater sympathetic drive when participants foraged in a scarce context. These findings are the first to demonstrate the sensitivity of sympathetic measures acquired over a short time period to the influence of a context defined by reward scarcity and support a contributory role of cardiac sympathetic drive in optimal decision making.

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Poster

427. Decision Making II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 427.28/JJJ51

Topic: H.02. Human Cognition and Behavior

Title: Evidence for hierarchically-structured reinforcement learning in humans

Authors: *M. ECKSTEIN[1], A. G. COLLINS[2]
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Abstract: Flexibly adapting behavior to different contexts is a critical component of human intelligence. It requires knowledge to be structured as coherent, context-dependent action rules, or task-sets (TS). For example, a computer user might possess different TS that specify the rules for using Windows, Mac, and Linux. This person needs to select the appropriate TS based on the current context, such as selecting the Mac TS in the presence of an Apple symbol. Inferring which TS is optimal in a given context is computationally complex, but can be approximated by a model that employs hierarchically-structured reinforcement learning (RL) (Collins & Frank, 2013). The model is based on classical RL algorithms (Sutton & Barto, 2017), but encompasses two levels. A higher-level RL process learns values for each TS in different contexts. These TS values guide the selection of appropriate TS based on context clues. A second, lower-level RL process learns values for each action in response to different stimuli, within TS. These action values guide the selection of appropriate actions based on stimuli, conditional on the currently selected TS. The model is inspired by the hierarchical organization of cortico-basal-ganglia loops, which underlie RL in the brain. Here, we test specific predictions of this model, specifically that learning TS involves RL values across two levels of abstraction. We tested 51 participants in a novel task and found strong evidence for this model. Participants' behavior showed sensitivity to both TS values and action values, such that higher-valued TS and actions were selected preferentially. Specifically, participants showed a preference for contexts that had been associated with higher-valued TS compared to contexts associated with lower-valued TS. Participants also preferred higher-valued TS in a generalization test, such that higher-valued TS were more often applied to novel contexts. In addition, TS values influenced participants' error patterns, such that more errors were intrusions from higher-valued TS. Furthermore, TS values were associated with learning speed, such that higher-valued TS were acquired faster than lower-valued ones. All of these results were obtained in regression models that also controlled for action values, and were replicated in a second, independent dataset (n=31).Taken together, these results support the claim that human action selection is guided by hierarchical RL mechanisms that involve the acquisition of abstract, context-dependent TS. This work has implications for research on learning and decision making, as well as for clinical conditions that are associated with problems of abstract reasoning.

Disclosures: M. Eckstein: None. **A.G. Collins:** None.

Poster

427. Decision Making II

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 427.29/JJJ52

Topic: H.02. Human Cognition and Behavior

Support: Faculty of Business and Economics Strategic Initiatives Fund grant, The University of Melbourne

R@MAP grant, The University of Melbourne

Title: Where the really hard decisions are - A general framework to quantify decision difficulty

Authors: P. FRANCO, N. YADAV, P. BOSSAERTS, *C. MURAWSKI
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Abstract: Current models of decision-making more often than not ignore the level of difficulty of decisions. They assume that the decision-maker is always able to identify the best option - whether it is a choice between two flavours of ice cream or a choice of investment option for a retirement portfolio from thousands of available options. Where decision difficulty has been taken into account, it has been done either informally or in a highly domain-specific way. We propose instance complexity (IC) as a generalisable mathematical framework to quantify difficulty of a decision based on a small number of properties of the decision.
The aim of IC is the characterisation of the computational complexity of individual instances of a computational problem, based on an instance's properties. The main advantage of IC compared to other measures of difficulty is fourfold. Firstly, it is based on the theory of computation (computational complexity theory), a rigorous mathematical framework. Secondly, our measure captures complexity that is intrinsic to a decision task, that is, it does not depend on a particular solution strategy or algorithm. Thirdly, it allows computation of difficulty of a decision task ex-ante, that is, without knowing the solution of the decision task. And finally, it does not require any knowledge of the decision-maker's attitudes or preferences.
We tested the relation between IC and (i) decision quality and (ii) effort exerted in a decision using two canonical variants of the 0-1 knapsack problem, a canonical and ubiquitous computational problem. We show that participants exerted more effort on instances with higher IC but that decision quality was lower in those instances.
We also acquired 7-Tesla functional magnetic resonance imaging data to characterise the neural circuits that code IC and are involved in the allocating of neural resources in response to varying levels of IC.
Together, our results suggest that IC can be used as a general framework to measure inherent complexity of decision tasks and to quantify computational resource requirements of decisions. The latter is particularly relevant for models of resource allocation in the brain (meta-decision-making/cognitive control).

Disclosures: P. Franco: None. **N. Yadav:** None. **P. Bossaerts:** None. **C. Murawski:** None.

Poster

427. Decision Making II

Location: SDCC Halls B-H

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Program #/Poster #: 427.30/JJJ53

Topic: H.02. Human Cognition and Behavior

Support: NSF 1719130: PFI:BIC- Unobtrusive Neurotechnology and Immersive Human-Computer Interface for Enhanced Learning

Title: Stability of EEG dynamics in transient cortical networks during decision making

Authors: H. COURELLIS[1], J. R. IVERSEN[3], D. A. PETERSON[4], T. MULLEN[5], *G. CAUWENBERGHS[2]
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Abstract: Computation in the brain is driven by the transient dynamical interactions of distal brain regions fulfilling various different functions. During network-level computation, active brain regions exhibit oscillatory activity which, when projected into a phase space, allows for the quantification of attractor states that individual neurons or the region as a whole might tend towards or away from depending on the nature of the computation being conducted. Such descriptions of the brain's dynamics are often conducted on the scale of individual neurons or neuronal ensembles using firing rate information, and are less frequently considered based on population measures of activity such as neural oscillations. We sought to interrogate the transient connectivity and associated non-linear dynamics exhibited by regions of the human cortex in electroencephalographic (EEG) recordings in the context of a reward-based decision making task. Participants were administered a standard multi-arm bandit task with an added probabilistic reward rate reversal during each participant's recording session. Through application of convex-optimization and dynamical systems characterization techniques, we find that the transient connectivity exhibited a number of cortical regions relevant to decision making strongly correlates with dynamical system instability quantified by the largest Lyapunov exponent. Additionally, when transient connectivity information is used to inform the construction of phase spaces during decision making, we find that there are cases in which the relationship between a region's transient connectivity and instability reverses polarity depending on task demands. The results reveal different cortical regions that exhibit either transient-network stabilizing or destabilizing behavior as a function of their participation in transient network dynamics.

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Poster

428. Human Cognition and Behavior: Decision Making and Reasoning: Value, Gains, and Loses

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 428.01/JJJ54

Topic: H.02. Human Cognition and Behavior

Support: ARC Grant DP160103353
EPS Study Visit Grant

Title: Decoding changes of mind in voluntary decisions - Dynamics of choice representations in a fronto-parietal network

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Abstract: Many previous studies have investigated the neurocognitive mechanisms underlying the generation of voluntary decisions. However, little is known about how these decisions change over time. In particular, changes in the external environment can cause a Change of Mind regarding ongoing actions. In the current fMRI study, we investigated how the neural patterns associated with initial voluntary choices change as new information can trigger decision reversals. In a novel task, participants made free choices between images of faces and houses, and then had to navigate a manikin to the chosen image on the screen. On some trials, the images changed their location after action onset. Participants could then decide to either pursue their initial choice to earn a higher reward, or switch to the alternative image to save time – depending on the remaining travel distance. Using multivariate pattern analysis, we decoded participants' initial face/house choices from visual areas (inferior occipital lobe and fusiform gyrus), precuneus, angular gyrus and medial prefrontal cortex (mPFC). In visual areas, the decoder trained on initial choices could also classify final face/house choices after image relocation, suggesting that the neural representations of choice options in these areas did not substantially change throughout the decision process. Conversely, cross-classification was not above chance in fronto-parietal areas, indicating changes in neural patterns over time. Further analyses suggested that representations in precuneus and angular gyrus might have been updated to incorporate image distance, which informed participants' Change of Mind decisions. By contrast, mPFC encoded Changes of Mind in participants who did not base their final decisions exclusively on external information about image distance. This suggests an endogenous component of Changes of Mind decision, for which information is present in this area. In conclusion, we found that the fronto-parietal network not only generates initial voluntary

decisions, but continuously integrates new decision-relevant external and internal information over time, allowing agents to dynamically reverse their own choices.

Disclosures: A. Loffler: None. **P. Haggard:** None. **S. Bode:** None.

Poster

428. Human Cognition and Behavior: Decision Making and Reasoning: Value, Gains, and Loses

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 428.02/JJJ55

Topic: H.02. Human Cognition and Behavior

Support: The Israel Science Foundation (ISF) Grant 1798/15

Title: Functional changes in perceptual, memory and value-related regions underlie non-reinforced behavioral change in the short and long term

Authors: *R. BOTVINIK-NEZER[1], T. SALOMON[2], T. SCHONBERG[3]
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Abstract: Understanding how preferences are constructed and modified is a major challenge in the research of human behavior with broad implications, from basic science to interventions for long-lasting behavioral change. Cue-approach training (CAT) is a novel and unique paradigm for non-reinforced behavioral change. During CAT, some items are presented one by one on the screen and participants are instructed to press a button as fast as they can whenever they hear a neutral cue, consistently paired with some of the items (Go items) but not with others (NoGo items). Results over multiple samples show that participants significantly choose Go over NoGo items immediately as well as months after CAT. The neural underpinnings of this behavioral change are still unclear. Here, we introduce a novel passive viewing task, whereby participants were scanned with fMRI while snack food items were presented individually before, after and one-month following CAT. We hypothesized and pre-registered (https://osf.io/yu3tw/) that the underlying mechanism will involve attentional, memory and value-related processes. Thirty-six healthy participants completed the experiment. A subset of 27 participants returned to a one-month follow-up session. We compared fMRI activity in response to Go vs. NoGo items in three time points: before, immediately after and one-month following CAT, using a multi-stage GLM approach with whole-brain GRTF cluster correction (z&gt2.3, p&lt0.05). We also performed small volume correction (SVC) on preregistered regions: ventromedial prefrontal cortex (vmPFC), hippocampus and superior parietal lobule.We revealed enhanced fMRI response to Go items following CAT in bilateral high level visual regions and in the vmPFC. In the one-month

follow-up, fMRI response to Go items in the left orbitofrontal cortex and right hippocampus was modulated by the choice effect across items. Our results suggest that preferences can be changed for the long term through perceptual processing enhancement without external rewards, context change or self-control. Neural findings demonstrating modification in brain response during passive viewing, indicate a multi-level change in the representation of the items from visual to memory-related and prefrontal regions.

Disclosures: R. Botvinik-Nezer: None. **T. Salomon:** None. **T. Schonberg:** None.

Poster

428. Human Cognition and Behavior: Decision Making and Reasoning: Value, Gains, and Loses

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 428.03/JJJ56

Topic: H.02. Human Cognition and Behavior

Title: Distinct neural patterns of effort-cost valuation: Evidence from prospective choices

Authors: *N. ARIDAN, N. J. MALECEK[1], R. A. POLDRACK[2], T. SCHONBERG[3]
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Abstract: We often consider a compromise between effort and reward in pursuit of our goals. Intuitively, effort requirements impose a cost upon associated rewards, as it makes them more difficult to obtain. Compared to other costs present in common choices, such as risk and delay, the neural basis of effort-based valuation remains poorly understood. Animal models and human neuroimaging studies have primarily linked the anterior cingulate cortex (ACC) and ventral striatum (VS) to the integration of effort and reward. However, studies have also demonstrated their role in salience detection and invigoration to effort demands. This suggest that neural activity observed during anticipation or performance of effort might be separate from cost value. To examine the interaction of value and effort separated from these processes, we asked 40 participants to accept or reject monetary gambles that could be resolved by the future performance of a familiar dynamometer grip-force effort or a fixed risk prospect during fMRI. Participants' willingness to accept prospective gambles reflected discounting of value by effort and risk. Choice-locked neural activation in vmPFC and contralateral primary-sensory cortex tracked the magnitude of prospective effort-cost participants faced, independent of choice time, monetary stakes and risk. Estimates of subjective value discounted by effort were tracked by the activation of a network of regions common to valuation under other costs such as risk and delay, including vmPFC, ventral striatum and sensorimotor areas. Together, our findings support differentiation between neural activity of effort-cost valuation and invigoration within effort-based decisions.

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Poster

428. Human Cognition and Behavior: Decision Making and Reasoning: Value, Gains, and Loses

Location: SDCC Halls B-H

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Topic: H.02. Human Cognition and Behavior

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Title: Episodic memory contributions to value-based decisions: Evidence from amnesic patients

Authors: *A. BAKKOUR[1], D. J. PALOMBO[3], A. ZYLBERBERG[2], M. N. SHADLEN[4], M. H. VERFAELLIE[3], D. SHOHAMY[1]
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Abstract: Memory is central to adaptive behavior, allowing past experience to guide decisions and actions. However, the neurobiological mechanisms by which memory guides decisions and the consequences for behavior remain poorly understood. Here we test the idea that episodic memory contributes to value-based decisions by providing samples of evidence relevant to choice options. This idea may explain why it takes more time to decide between options of similar value: such decisions require more memory-guided evidence and therefore take more time. To examine the role of episodic memory in value-based decisions patients with anterograde amnesia due to medial temporal lobe damage (n=6) and age-matched healthy controls (n=15) participated in a value-based decision task. The task involved choosing between pairs of familiar foods. On each trial, two foods were paired such that the difference in their values (ΔRating; based on participant-specific ratings) differed from trial to trial. We predicted that amnesic patients, compared to controls, would show a different relationship between ΔRating and reaction time (RT). As a control condition, both groups performed a perceptual decision task that involved decisions based on external rather than internal samples of evidence. The perceptual task required choosing the predominant color of a mixture of yellow and blue random dots. On each trial, the stimulus was assigned a color coherence that determined the color category and its

ambiguity. For the food task, controls chose the higher-rated food more often and their RT decreased as |ΔRating| increased. Choices and RTs were well characterized by a drift diffusion model (DDM), which relies on principles of sequential sampling and optional stopping to reconcile choice and RT. The patients, by contrast, showed a different pattern: their choices and RTs were governed more weakly by ΔRating and the DDM was worse at explaining their behavior. The findings from the perceptual task instead revealed similar performance among patients and controls; both groups showed the typical effect of difficulty on RT and accuracy and their DDM fits were similar. The findings point to a critical role for the medial temporal lobe memory system in supporting value-based decisions about highly familiar items. We suggest that deliberation during certain value-based decisions involves episodic memory processes, which contribute to assembling or accumulating evidence bearing on preference, a process that is disrupted in amnesic patients.

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Poster

428. Human Cognition and Behavior: Decision Making and Reasoning: Value, Gains, and Loses

Location: SDCC Halls B-H

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Program #/Poster #: 428.05/JJJ58

Topic: H.02. Human Cognition and Behavior

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Title: Comprehension as Bayesian decision-making: Neural computations of inferring what is meant from what is said in language games

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Abstract: Communication is a ubiquitous feature of social interactions across multiple species. A cornerstone of effective communication is the ability to recognize the intended message of a speaker for a listener (a.k.a., speaker meaning) even though that message is often not coded in the utterance directly. A number of theoretical and behavioral models of pragmatic reasoning have been proposed, in particular the rational speech act, which connects pragmatic inferences with probabilistic inferences by drawing on formal decision models such as Bayesian theory. Here, we investigate the cognitive and neural substrates of pragmatic reasoning by exploring

brain regions that encode model-derived inference signals used to decipher speaker meaning in a stylized communicative setting. Specifically, we examined the fMRI data of a language game where a listener needs to infer a target object in a given context based on a message received from an anonymous speaker. Behavioral analyses show that the process of inferring a speaker's intended referent can be characterized as a Bayesian decision process, which integrates the prior information with a mentally simulated likelihood for speaker's actions. Consistent with the behavioral model, imaging data reveal that the latent likelihood signal derived from the model is expressed in the ventromedial prefrontal cortex (vmPFC), even when Bayesian reasoning is unnecessary for discerning speaker meaning (e.g., when speaking meaning is explicitly coded in the utterance). Interestingly, this region also demonstrates model-dependent connectivity with a number of brain regions, including the dorsomedial prefrontal cortex and temporoparietal junction, which are known to be involved in theory-of-mind, and the left inferior frontal gyrus, which has been repeatedly implicated in language processing. The robustness and specificity of the observed behavioral and vmPFC response patterns are further demonstrated in two additional experiments. Together, these results provide a neuromechanistic account of pragmatic reasoning where effective language interpretation arises from a Bayesian decision process, and vmPFC plays a central role in inferential computations critical for Bayesian reasoning. The data point to a new avenue that bridges the literature of model-based decision neuroscience and that of language and social communication.

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Poster

428. Human Cognition and Behavior: Decision Making and Reasoning: Value, Gains, and Loses

Location: SDCC Halls B-H

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Program #/Poster #: 428.06/JJJ59

Topic: H.02. Human Cognition and Behavior

Support: NSERC PGSD3-471313-2015

Title: Representation of subjective value for self and other agents in the dorsal anterior cingulate cortex is consistent across tasks and predicts social attitudes

Authors: *M. R. PIVA[1], K. R. VELNOSKEY[2], R. JIA[3], A. NAIR[2], I. LEVY[4], S. W. CHANG[2]
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Abstract: While making decisions on behalf of others is ubiquitous in daily life, few studies have addressed the underlying neural processes. One key aspect in decision-making is the

similarity in subjective value between options. We investigated the neural correlates of subjective value disparity and proximity during choice for self and other in two independent behavioral tasks using fMRI. Behavioral paradigms included intertemporal choice (n = 20), in which participants chose between options of smaller amounts of money sooner or larger amounts of money later, and risk (n = 21), in which participants chose between options of smaller amounts of money with larger probabilities or larger amounts of money with smaller probabilities. Behavioral modeling indicated that participants distinguished between themselves and other individuals with dissimilar preferences. We then quantified the subjective value of each option in each trial. Trials in which the value of the two options were similar constituted high-proximity trials, while trials in which the value of the two options were dissimilar constituted high-disparity trials. Univariate neural analyses indicated that while the dorsal anterior cingulate cortex (dACC) responded to high-proximity trials, the ventromedial prefrontal cortex (vmPFC) responded to high-disparity trials, and these results were consistent for both self and other trials and across both behavioral tasks ($P < 0.05$, corrected). However, multivoxel pattern analysis indicated that the dACC, but not the vmPFC, selectively decoded high-proximity versus high-disparity trials, and these results were again consistent across self and other and were replicated in both tasks ($P < 0.01$, permutation). The code for high-proximity versus high-disparity trials in the dACC was generalizable across self and other, as classifiers trained on data from self trials were able to categorize subjective value in other trials and vice versa ($P < 0.01$, permutation). Notably, this neural code in the dACC was generalizable across tasks, as classifiers trained on data taken from the dACC during intertemporal choice were able to predict subjective value in trials from the risk paradigm ($P < 0.01$, permutation). Finally, the level of classification accuracy in other relative to self trials correlated with self-reported social attitudes ($P = 0.03$, Spearman). Together, these results indicate the importance of subjective value disparity and proximity signals in the human dACC, arising during decision-making across different perspectives and contexts.

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Poster

428. Human Cognition and Behavior: Decision Making and Reasoning: Value, Gains, and Loses

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 428.07/JJJ60

Topic: H.02. Human Cognition and Behavior

Title: Architecture of representations in pre-frontal cortex during credit assignment

Authors: *P. WITKOWSKI[1], S. A. PARK[2], E. D. BOORMAN[3]
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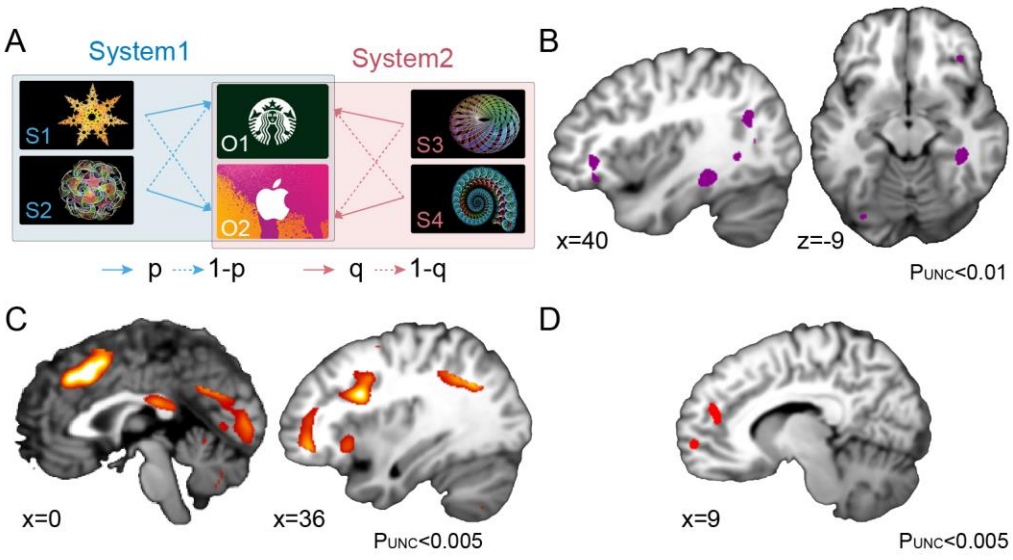
Abstract: Representations of the causal relationships between behavior and outcome are essential to implementing adaptive goal-directed behavior. The structure of causal relationships in the environment are not always apparent, yet they often must be used to infer relationships from experienced outcomes. In this study we show how the brain represents latent causal relationships when assigning credit for an outcome to its causes and how the brain flexibly updates these representations to maximize reward. Subjects (N=22, 14 Female, Median age = 21) participated in a reward-learning task where they tracked two systems of stimulus-outcome associations. Each system comprised two interrelated stimuli that always led to opposite outcomes, allowing subjects to make inferences about one stimulus from observing the other. In this way subjects learned about directly experienced and inferred associations through knowledge of these interrelations. Behaviorally, we found evidence subjects learned from both directly experienced and inferred past outcomes (experienced: $t(21)>6.95, p<0.001$; inferred: $t(21)>3.73$, $p<0.001$). We analyzed the fMRI data using a combination of univariate and multivariate approaches. Specifically, we used a support vector machine to decode representations of the recently chosen stimulus and the interrelated but unchosen stimulus at the time subjects received feedback for their choices. Our results show that areas in the lateral orbitofrontal cortex and ventrolateral prefrontal cortex, lateral occipital cortex, and hippocampus code for the specific chosen stimulus at outcome time (**fig.1A**). Updates to these representations recruited a network including lOFC, lateral prefrontal cortex, and pre-SMA **(fig.1B**). Finally, medial prefrontal cortex simultaneously coded for a representation of the latent cause – the abstract knowledge about the interrelations between sets of stimuli and outcomes**(fig.1C**). These findings suggest a representational architecture of prefrontal representations for credit assignment.



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Poster

428. Human Cognition and Behavior: Decision Making and Reasoning: Value, Gains, and Loses

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Title: The role of uncertainty in curiosity about wins versus losses

Authors: *L. L. VAN LIESHOUT[1,2], F. P. DE LANGE[1], R. COOLS[1,2]
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Abstract: Curiosity is a basic biological drive, but little is known about its behavioral and neural mechanisms. In a previous study, we have demonstrated that curiosity is a function of information uncertainty, indicating that we are particularly curious when information provides us with a substantial update of what we know. This is the case even though receiving information is not instrumental, meaning that it will not help us to improve performance or to maximize rewards. It is unclear, however, whether and if so, how this effect interacts with our automatic bias to approach information about positive events. To assess this, we designed a lottery task in which we independently manipulated outcome uncertainty as well as the (reward/punishment) valence of trial outcomes. In contrast to our previous study, participants could either win or lose an uncertain amount of money in different blocks of the task. This allowed us to disentangle the desire for information about positive (win) and negative (loss) events. Results showed that curiosity robustly increased with increasing outcome uncertainty, in both the win as well as in the loss context. In addition, curiosity was overall higher for the win context compared with the loss context. However, there was no interaction between outcome uncertainty and outcome valence, indicating that these two factors have distinct effects on curiosity. These results suggest that curiosity is monotonically related to the uncertainty about one's current world model and that people are driven to improve this model, regardless of outcome valence. Independent of this uncertainty effect, participants exhibit a (Pavlovian approach) bias towards information gain about positive compared with negative events. These findings provide novel insights into the behavioral mechanisms of curiosity and suggest that curiosity is a function of multiple

independent factors, one of which is related to information updating and the other to reward maximization.

Disclosures: L.L. Van Lieshout: None. **F.P. de Lange:** None. **R. Cools:** None.

Poster

428. Human Cognition and Behavior: Decision Making and Reasoning: Value, Gains, and Loses

Location: SDCC Halls B-H

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Program #/Poster #: 428.09/JJJ62

Topic: H.02. Human Cognition and Behavior

Support: NIGMS/NIH P20GM103645 Center of Biomedical Research Excellence

Title: Sum before difference: Differential temporal contributions of overall set value and value difference in value based decisions as revealed by ERPs

Authors: *R. FRÖMER, A. SHENHAV
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Abstract: Previous research has characterized the neural dynamics underlying perceptual decisions (e.g., whether some dots are moving leftward or rightward), yet major gaps remain in our understanding of the dynamics underlying value-based decisions (e.g. which of a set of options we want most). Research on perceptual and value-based decision-making alike has demonstrated that a centro-parietally distributed positive event-related potential (CPP) tracks the amount of evidence in support of a given choice option, until that evidence reaches a putative decision threshold and a response is made. However, the limited research into the dynamics of value-based choice has focused on relatively short decisions and often specifically on the dynamics associated with the relative subjective value of one option over another (value difference [VD]), which typically serves as a proxy for the decision evidence. Less is known about the neural dynamics associated with the overall value (OV) of a choice set, despite recent findings that OV and VD contribute in different ways to decision time and to the emotional experiences associated with decision-making. To better characterize these dynamics in more realistic value-based choices, we measured EEG while participants (N=39) made a series of choices between pairs of consumer goods that they had the opportunity to receive. Choices were individually tailored to vary in OV and VD (based on prior ratings of each item), and participants were given up to 4s to make each choice. Using a MASS-univariate approach with cluster-based permutation tests, we found that OV and VD modulated event-related potentials (ERPs) with differential topographies and timing: whereas OV was associated with a CPP locked to stimulus onset (peaking 700ms post-onset, much earlier than most decisions were made, median RT=1.7s), VD was associated with a pronounced frontocentral negativity and posterior positivity

locked to the response (peaking ~ 500 ms pre-response). The timing of the stimulus-locked CPP effect and the lack of a response-locked CPP - including when using RT as a proxy for evidence accumulation - challenge the claims that CPP reflects a signature of a supramodal decision variable. Our results are in line with certain biophysically-inspired computational models of decision-making that predict sequential encoding of OV and VD while suggesting that different patterns of brain activity might underlie the accumulation of additive value (OV) and relative value (VD).

Disclosures: R. Frömer: None. **A. Shenhav:** None.

Poster

428. Human Cognition and Behavior: Decision Making and Reasoning: Value, Gains, and Loses

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 428.10/JJJ63

Topic: H.02. Human Cognition and Behavior

Support: R01DA038063

Title: The neural signature of risk in gains and losses underlying bundle valuations

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Abstract: The neuroscientific study of decision making has revealed insights about human cognition leading to advanced theories of human behavior. Here, we focus on the neural underpinnings of choice behavior in both the gain and loss domain. Standard theories of rational decision making assume context-independent valuations. However, a stark context-dependency on gain versus loss contexts have been widely observed. To account for this, Kahneman and Tversky (1979) proposed prospect theory; a utility/value function with an inflection point separating concave utility for gains from convex utility for losses (diminishing sensitivity). Paradoxically, prospect theory predicts even more anomalous behavior when subjects choose over riskless losses; bundles of goods encountered in the loss domain. Specifically, prospect theory's value function shape predicts that in the loss domain people prefer losing all of one type of good to losing some of each of the goods in a bundle. To test this prediction, we designed risky and riskless choice tasks in both gain and loss domains using real consumption goods - food bundles - while imaging subjects to investigate neural mechanisms. In both tasks, goods varied from trial to trial, visual stimuli were identical and participants were instructed similarly in all tasks to make a series of choices between two options based on their own preferences in an incentive compatible manner. Behavioral data confirms our previous finding that prospect theory

appears to make inaccurate predictions about the nature of valuation when subjects choose over what are called “riskless bundles” in the loss domain. They showed consistent preferences across gain and loss domain in riskless choices. This was true even while they showed gain-loss-dependent changes of risk attitudes when choosing over simple lotteries of these same goods. Ongoing imaging of 30 subjects should allow us to rapidly test neural representation similarity (discrepancy) in the valuation of food bundle in different contexts. Taken together, these findings point toward the importance of interdisciplinary approaches to better understand the neural circuits and human behavior.

Disclosures: P.W. Glimcher: None. **H. Chung:** None. **J. Zimmermann:** None. **A. Tymula:** None.

Poster

428. Human Cognition and Behavior: Decision Making and Reasoning: Value, Gains, and Loses

Location: SDCC Halls B-H

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Topic: H.02. Human Cognition and Behavior

Support: NIH grant R01MH104251
NIH grant R01DA038063

Title: Efficient coding and the adaptation of human choice behavior to the statistics of recent rewards

Authors: *C. K. STEVERSON[1], H.-K. CHUNG[2], J. ZIMMERMANN[3], P. W. GLIMCHER[5], K. LOUIE[4]
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Abstract: It is widely assumed that economic decision making is driven by value encoding in the brain. Efficient coding theory suggests that the representation of value, and hence choice behavior, should adapt to features of the anticipated distribution of values. For example, adaptation to the anticipated range of values is necessary to make use of the full set of possible neuronal firing rates in encoding value. Without adaptation to the range, encoding values for highly valuable items (e.g., houses, cars etc.) unnecessarily degrade coding performance when making choices in a much lower value context (e.g., choosing which coffee to order). It has been hypothesized that the distribution of recently encountered values, which we refer to as the temporal context, drives what is anticipated (encoded) in the next decision. In studies of non-human primates, adaptation to the value range of the temporal context has been previously observed in both neural encodings of value as well as in choice behavior. In this study, we tested

whether human choice behavior also adapts to the distribution of recently encountered values. Subjects performed a two-alternative forced-choice task over monetary lotteries. Choices were divided into two blocks: in one block subjects encountered a larger range of values and in one block they encountered a smaller range of values. About half the choices were "test choices" that appeared in both blocks. We measured how behavior adapts to the range of values by testing how measures, such as choice stochasticity, differ on the test choices across the two blocks. We also designed our experiment to distinguish whether behavior is sensitive only to the shape of the cumulative distribution function of value, as has been proposed in certain classic efficient coding theories, or depends on the cardinality of the values as would be suggested by a model such as normalization. How human choice behavior adapts to the statistics of recently encountered values is a growing but still understudied area. Our works seeks to add this literature in a way that advances our understanding of the influence of the temporal context and the role of efficient coding in human choice behavior.

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Poster

428. Human Cognition and Behavior: Decision Making and Reasoning: Value, Gains, and Loses

Location: SDCC Halls B-H

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Program #/Poster #: 428.12/JJJ65

Topic: H.02. Human Cognition and Behavior

Support: MOST 104-2410-H-010 -002 -MY3

Title: The parietal cortex dynamically integrates the cost and benefit of time to inform decision timing

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Abstract: When making a decision, gathering more information about the options under consideration in principle can never hurt. However, collecting information takes time that can be allocated to other potentially rewarding events. Knowing that information is helpful but time is costly, the decision maker not only has to decide which option to choose but when to choose it. Regarding the latter decision problem, little is known about how and how well the brain dynamically evaluates the cost and benefit of time in order to solve the "when" problem. Methods. In a perceptual decision task, subjects were instructed that a box consisted of a total of 100 red and green balls. Unbeknownst to the subjects, on each trial, either there were more green

balls or more red balls. The subjects' task was to guess which ball - red or green - had more in number in the box. In order to provide information about the box, we sampled from the box and *sequentially* presented the samples as red or green dots on the screen. Here, we introduced time cost by implementing a decreasing reward function. That is, on each trial, the gain associated with a correct decision decreased over time. The subjects can freely choose when to make a response. The question is, can he or she balance the benefit of time - accumulating more dots over time leads to better performance - against the cost of time? Results. In order to maximize reward earned, an ideal decision maker in the decision task would choose a time to make a decision that would lead to the maximization of expected gain. Compared with an ideal decision maker, subjects' decision time were typically slower. To explain the suboptimal pattern, we tested 3 models: risk aversion (RA), loss aversion (LA), and probability weighting (PW). Both RA and LA models failed to consistently describe the data. In contrast, a probability weighting function that was convex in moderate to large probabilities successfully described suboptimal patterns across subjects. This indicates that subjects underweight moderate to large probabilities and that the marginal gain in probability of success increased as a function of time. This made waiting become extremely attractive, even though the benefit of waiting was rather limited. With fMRI, we found that activity in the precuneus represents the probability of making a correct decision over time within a trial. By contrast, the inferior parietal lobule dynamically represent the expected gain - the integration of cost and benefit associated with time - of making a decision. Together, these results suggest distinct regions in the parietal cortex involved in information accumulation over time and in the dynamic integration of cost and benefit of time.

Disclosures: M. Wang: None. **S. Wu:** None.

Poster

428. Human Cognition and Behavior: Decision Making and Reasoning: Value, Gains, and Loses

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Topic: H.02. Human Cognition and Behavior

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Title: Flexible concept representation for value-based decisions

Authors: *G. CASTEGNETTI, M. ZURITA, B. DE MARTINO
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Abstract: A distinctive characteristic of human cognition is the ability to interact flexibly with the environment by using objects as tools to achieve goals. The usefulness of any particular

object depends on the nature of the current goal; in other words, objects acquire value in a context-dependent manner. In the brain, this process is likely to involve construction of conceptual representations of the objects at hand, which are then carved into value representations. This process is likely to be hippocampal dependent (i.e. involving the retrieval of episodic information about previous experiences with similar objects). The medial prefrontal cortex (mPFC) has been shown to play a key role in extracting regularities present in the retrieved information (i.e. schema formation), and has recently been proposed to perform dimensionality reduction to extract goal relevant features. In this study, we sought to investigate how conceptual representations morph into value representations, with particular focus on the interaction between hippocampus (HPC) and mPFC. To do this, we developed a novel task in which volunteers interacted with set of objects in the context of different goals. We first acquired a number of behavioural measures including goal dependent value estimates, confidence estimates and familiarity measures. Pilot data show that our manipulation resulted in markedly different scores when the same object was evaluated in relation to different goals. From these scores we built conceptual similarity matrices, which were then used to detect changes in the fMRI signal when participants imagined how to use objects to achieve a particular goal. Our preliminary data suggest that HPC appears to be preferentially implicated in context representation, whereas mPFC builds value representations in a goal-dependent fashion. In summary, this study provides mechanistic insights into the interplay between HPC and mPFC during the construction of conceptual knowledge, and shows how identical stimuli elicit different neural representations when evaluated in the context of different goals.

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Poster

428. Human Cognition and Behavior: Decision Making and Reasoning: Value, Gains, and Loses

Location: SDCC Halls B-H

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Program #/Poster #: 428.14/JJJ67

Topic: H.02. Human Cognition and Behavior

Support: US Army Research Office Grant W911NF-16-1-0474

Title: Painstaking choices: Distinguishing neural representations of subjective value, conflict,magnitudes,andcontextualbiasesin deterministic decision making with mixed outcomes

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Abstract: In a world with few free lunches, most choices entail mixed outcomes of both costs and benefits. Decision making requires careful accounting of the positive and negative attributes

of each option to determine its overall value and whether it's worth pursuing. Behavioral economists and psychologists have typically modeled valuation of mixed outcomes using rewards paired with probabilistic risks of loss. However, the subjective value of a probabilistic outcome often diverges substantially from its measurable objective value, making it difficult to parse preference from errors in statistical reasoning. Relatively less work has surveyed models of deterministic decisions. Moreover, the neural representations of subjective value, conflict, magnitudes, and contextual biases in such choices have been left largely unexplored. We were particularly interested in how subjects establish preference between offers of equivalent subjective value and whether normalization models commonly applied to risks in probabilistic decisions would generalize to outcome magnitudes in deterministic decisions. We measured BOLD responses in 24 human participants who made choices about monetary rewards paired with pain stimuli. Experiment 1 was a single-offer approach-avoid task in which participants accepted or rejected individual offers of monetary rewards ranging from $0.01-$1.50 contingent on receiving a cutaneous shock that ranged from minimally to maximally painful. We independently fit each individual's choice outcomes with logistic regression to estimate their cost/reward preference structure and perceived subjective value throughout the decision space. Choice outcomes predicted activity in the caudate, vmPFC, and OFC. Subjective value predicted activity in the anterior insula and dACC. Choice conflict predicted activity in posterior insula, posterior cingulate, cingulate motor area, and sgACC. Value models from Experiment 1 were used to identify subject-specific trial parameters for Experiment 2, a two-offer free choice task in which participants chose between two cost/reward offers with equivalent subjective value (P(Accept)=.5) but different magnitudes (distance along the decision boundary, ranging from min. reward /min. cost to max. reward / max. cost). Rather than normalizing magnitudes to determine choice, subjects formed individualized preferences for a unique magnitude and their choice behavior was largely determined by this preference. Preferred magnitudes selectively predicted activity in vmPFC, OFC, posterior cingulate, and the basal ganglia.

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Poster

428. Human Cognition and Behavior: Decision Making and Reasoning: Value, Gains, and Loses

Location: SDCC Halls B-H

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Program #/Poster #: 428.15/JJJ68

Topic: H.02. Human Cognition and Behavior

Support: Inserm Grant C12-69

Title: The effort of choosing: Neural correlates of deliberation during value-based decision-making

Authors: *N. CLAIRIS, M. PESSIGLIONE
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Abstract: When deciding about which action to take, people must weigh the costs against the benefits associated with alternative options. FMRI studies have identified a brain network whose activity correlates positively with the appetitive value of potential outcomes. This network, coined Brain Valuation System (BVS), mainly includes the ventromedial prefrontal cortex (vmPFC), the ventral striatum and the posterior cingulate cortex. Less is known about the existence of an opponent brain system that would correlate positively with the aversive value of effort costs, although some brain regions, such as the dorsomedial prefrontal cortex (dmPFC) have been repeatedly implicated. One critical issue is to disentangle between brain activity linked to effort associated with the envisaged options and to effort invested in the decision-making process. To tease apart these neural representations, we recorded the brain activity from healthy participants (n=38) with fMRI and from epileptic patients (n=12) implanted with intra-EEG electrodes while they both performed tasks involving valuation of virtual reward and effort items. More precisely, they performed 3 tasks that required 1) rating of reward appetitive value and effort aversive value on an analog scale, 2) choosing the most appetitive of two rewards, or the least aversive of two efforts, 3) deciding whether or not to make a given effort for a given reward. Reward appetitive values and effort aversive values were simply the ratings assigned by participants to the different items. Response time was taken as a proxy for the amount of effort invested in every task trial. We verified that response time was correlated to the amplitude of pupil dilation, which is considered a valid marker of mental effort. As expected, the appetitive value of reward items were signaled in all tasks by increased activity in the BVS, including the vmPFC. Activity in this brain region was also sensitive to the aversive value of effort items, but with a negative correlation, consistent with the idea of a net value representation. Activity in the putative opponent regions, notably the dmPFC, did not correlate with aversive value but with response time, whatever the task. Thus, we established dissociation between effort costs attached to choice options, which are (negatively) integrated in the vmPFC, and the effort cost entailed by the very process of decision-making, which is (positively) reflected in the dmPFC. Further research is needed to precise whether this activity participates to the decision-making process or simply signals the amount of resource that is consumed by the process.

Disclosures: M. Pessiglione: None.

Poster

428. Human Cognition and Behavior: Decision Making and Reasoning: Value, Gains, and Loses

Location: SDCC Halls B-H

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Topic: H.02. Human Cognition and Behavior

Support: UCSD CRES
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Title: Neural coding in macaque monkeys of the facial features underlying human social perception of faces

Authors: *J. HUANG[1], J. LIU[2], D. GUO[1], C. K. RYALI[2], J. GUAN[3], A. J. YU[3]
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Abstract: Humans readily infer social traits (e.g. attractiveness, trustworthiness, and intelligence) from a stranger's face. Little is known about the neural encoding of the facial features underlying such social judgments. Indeed, up until now it has not been clear whether facial features contributing to human perception of social traits are encoded by face processing neurons of other primates such as monkeys, since it is difficult to elicit social judgments (especially about human faces) from non-human primates. In this work, we overcome this difficulty by using a sophisticated computational modeling and analysis framework, which allows us to accurately predict human rating of different social traits for any novel human face image presented to an experimental subject. Combining these predictions with recordings of neural activities of face-sensitive neurons in the macaque monkey while viewing human face images (Freiwald & Tsao, 2010), we can therefore quantify whether an individual neuron encodes facial features contributing to the (human) perception of a social trait, or a combination of social traits. Specifically, we represent the space of all faces using the Active Appearance Model, which has recently been shown to have latent features encoded by face patch neurons in the macaque monkey, and use linear regression to finding linear combinations of facial features that maximally account for human perception of 20 social traits (e.g. attractiveness, trustworthiness, typicality), 3 demographic traits (race, age, gender), and 7 emotional expressions (e.g. disgust, anger, fear, happiness). We first train AAM on two publicly available datasets of face images, then linearly regress neural responses against human ratings of those images, to predict social and emotional judgments as a function of the AAM latent features. We find a significant number of neurons in the macaque face patches significantly encode the facial features that drive the human perception of all three categories of facial traits that we

investigated, including some that specialize in encoding facial features specific to each of the three categories of traits, and others that encode facial features common to all three categories. Despite these findings, our results do not suggest that the monkey brain encodes social judgment percept analogous to human percept, or that monkeys care about the social dimensions of humans faces. However, they do suggest that the facial featural information necessary for "high-level" human social judgments are already encoded in the macaque monkey brain, in the sense that it is readily decodable using a linear decoder.

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Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

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Program #/Poster #: 429.01/JJJ70

Topic: H.02. Human Cognition and Behavior

Support: Irish Research Council GOIPG/2015/1700

Title: A neurally-informed approach to modelling learning in human perceptual decision making

Authors: C. A. DEVINE[1], D. P. MCGOVERN[2], C. GAFFNEY[3], S. P. KELLY[3], R. G. O'CONNELL[3]
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Abstract: Despite the well-established benefits of training for perceptual decision-making, there is still considerable uncertainty regarding the precise stages of information processing that are altered by learning. The present study seeks to develop a neurally-informed model of how learning affects perceptual decision making. To this end, we isolated distinct electrophysiological signatures of the three key stages of information processing necessary for simple sensorimotor transformations - sensory evidence encoding, evidence accumulation and motor preparation - while subjects (N=22) were trained to perform a two-alternative contrast discrimination task over five days. The stimulus consisted of two overlaid left- and right-tilted gratings each of which underwent subtle antithetical changes in contrast. Separate steady-state visual evoked potentials (SSVEPs) provided a direct read-out of the neural representation of each stimulus while the centroparietal positivity (CPP) and beta-band activity respectively indexed motor independent and effector-selective decision formation. Significant improvements in accuracy and response time were observed throughout training. The neural data suggest that training-related improvements in behaviour arise principally from a progressive boosting of

sensory evidence representation, which had a knock-on effect on the build-up rate of the CPP and beta band activity. Consistent with this, preliminary modelling using the standard drift diffusion model (DDM) attributed the learning effects to increases in the rate of evidence accumulation (drift rate). However, our neural data also suggest that training leads to changes in the amount of accumulated evidence required to trigger a response - a finding that was not predicted by our initial modelling attempts. This neural data will be used to further inform the development of a model that fully characterises the effects of learning on both behavioural and neural signatures of perceptual decision making.

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Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 429.02/LLL1

Topic: H.02. Human Cognition and Behavior

Support: The Unviersity of Alabama Research Grants Committee

Title: Law enforcement officers' neural processing of intentions of suspects during virtual high threat scenarios

Authors: *R. COOK[1], R. A. HOUSER[2], D. FONSECA[3], W. B. WEBBER[1], I. HEIM[3], D. DOLLIVER[4]
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Abstract: This project was designed to identify neural correlates of law enforcement officers' assessment of the intentions of others in virtual high threat situations. Specifically, we focused on law enforcement officers (LEOs) decision to *correctly* utilize deadly force. We collected EEG data to understand the active neural correlates in the moments leading up to the decision to utilize lethal force. Participants were five local-level LEOs from a small sized city in the Southern who, on average, had 5.75 years of experience (*SD* = 6.75). The results are a part of a larger data collection project. We used five simulation scenarios that all four participants completed that necessitated the participants to fire their weapon. All scenarios were of situations that LEOs might experience (e.g., DUI traffic stop, hostage situation, etc.). In the scenarios, the virtual suspect directed a weapon at the officer or a bystander, thereby justifying the legal use of deadly force by the officer (Broomé, 2011). We initially collected baseline data of brain activity (a two minute EEG recording) followed by presentation of the six scenarios). We collected EEG

data using a 64-channel mobile EEG amplifier, EEGO Sports (Advanced Neuro Technology; Zanow & Knösche, 2004). Data was collected at a sampling rate of 500 Hz in an ambient temperature room. To examine the neural correlate activity for each EEG epoch, we used sLORETA (standardized low-resolution electromagnetic tomography). sLORETA is a method of creating 3D images depicting the neurological activity and is a useful approach to reduce localization errors and eliminate erroneous sources (Pascual-Marqui, 2002). Source data were based on the heads models from the Montreal Neurological Institute (Oostendorp & van Oosterom, 1989). We created 2s epochs from data up to 10 seconds prior to the participants discharging their weapon, signaling the decision to utilize deadly force. Using sLORETA, we found activation in the frontal lobe (BA 8, 9, 10, 47), the occipital lobe (BA 19), and the temporal (BA 21, 39). We found that emotional recognition and cognitive empathy may be important in LEO decision-making during high threat situations. Our findings provide an opportunity for future research in understanding the neural processing in high threat situations as well as implications to inform LEO training.

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Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

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Program #/Poster #: 429.03/LLL2

Topic: H.02. Human Cognition and Behavior

Title: A neuroeconomic study of impaired interpersonal decision making in anorexia nervosa

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Abstract: Anorexia nervosa(AN) is a severe eating disorder that characterized by a high mortality rate, restricted eating, and disturbed body image. One of neurocognitive phenotypes of eating disorder is impaired decision making. Previous decision making studies of AN focus on the intrapersonal side. However, interpersonal factors play significant roles in many pathological aspects of the disorder, for example the onset, maintenance, and remission. Considering interpersonal hypersensitivity observed in eating disorder, impaired decision making would manifest in the presence of social peers. However, there are few studies that focus on social interaction, and the neural mechanisms remain unclear. The aim of this study is to investigate the

neural correlates of impaired interpersonal decision making in AN combining structural MRI and economic games, which can quantify interpersonal processes. Ten patients with AN and 18 age and gender-matched healthy controls were studied. As behavioral task, we used two economic games, the Ultimatum Game(UG) and Third Party Punishment(TPP). In MRI scans, participants underwent T1-weighted three dimensional magnetization-prepared rapid gradient-echo(3D-MPRAGE). 3D-MPRAGE data were processed by the Statistical Parametric Mapping 12 software package. Using voxel-based morphometry, we investigated the relationship between regional gray matter volume and the economic game scores. In behavioral results, patients with AN rejected unfair offers in UG more frequently than healthy controls. No significant difference was found between patients and healthy controls in TPP. In brain imaging results, acceptance rate of unfair offers in UG had a positive correlation with the volumes of left medial orbitofrontal cortex and left anterior insula in AN. The typical behavior tendency in AN was observed in the situation where patients with AN were treated unfairly by others. Our findings suggest that two particular brain regions might be important for the impaired social decision making in AN.

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Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 429.04/LLL3

Topic: H.02. Human Cognition and Behavior

Support: MRC Fellowship MR/P014097/1
Wellcome Trust WT100973AIA
Wellcome Trust 106164/A/14/Z

Title: Associative learning of self and other ownership

Authors: *P. L. LOCKWOOD[1], M. WITTMANN[1], M. APPS[1], M. KLEIN-FLUGGE[1], M. J. CROCKETT[2], G. W. HUMPHREYS[1], M. F. S. RUSHWORTH[1]
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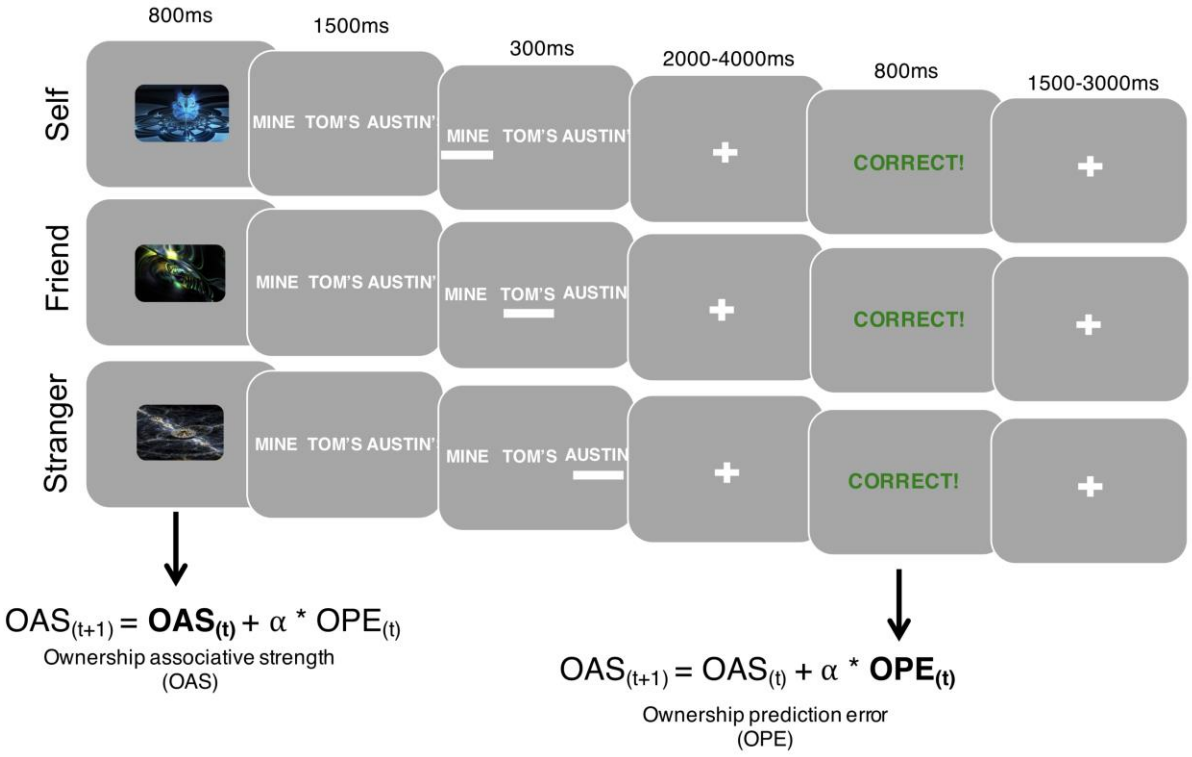
Abstract: Sense of ownership is such a fundamental aspect of human cognition that it influences the grammar of many human languages. For example, words are changed and used in special ways such as the genitive case or the construct state to indicate the ubiquitous association that constitutes the ownership relationship. More specifically, social environments often require that we distinguish what in the world is “ours” and what belongs to other people. However, the

mechanisms that underpin how we acquire a sense of ownership over objects that are ours and others is unknown. Here we used an associative learning task, model-based functional magnetic resonance imaging and a minimal ownership paradigm to probe the behavioural and neural mechanisms underpinning ownership acquisition for ourselves, friends and strangers.
We find a self-ownership bias at multiple levels of behaviour from initial preferences to reaction times and computationally defined learning rates. Several areas within medial prefrontal cortex tracked ownership associative strength between objects and agents. Ventromedial prefrontal cortex and anterior cingulate (ACC) sulcus responded more to self vs. stranger associations but despite a pervasive neural bias to track self-ownership, no brain area tracked self-ownership exclusively. However, ownership prediction errors for strangers were specifically coded in ACC gyrus and value representations in this area were tracked only for friends and strangers but not oneself. Core neural mechanisms for associative learning are biased to learn in reference to self but also engaged when learning in reference to others. In contrast, ACC gyrus exhibits relative specialization for learning about others.



Disclosures: P.L. Lockwood: None. **M. Wittmann:** None. **M. Apps:** None. **M. Klein-Flugge:** None. **M.J. Crockett:** None. **G.W. Humphreys:** None. **M.F.S. Rushworth:** None.

Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 429.05/LLL4

Topic: H.02. Human Cognition and Behavior

Title: Neural dynamics of performance during virtual battlespace cooperative teaming

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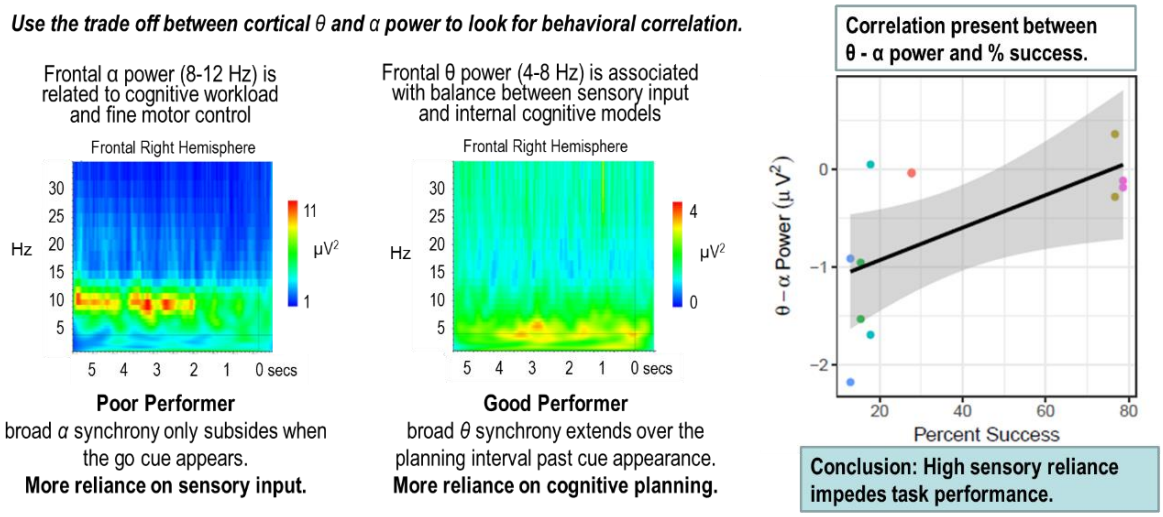
Abstract: This study investigated the behavioral relationships and physiological correlates of partner interactions as participants performed cooperative missions while walking in a virtual battlespace. Subjects (N=18) navigated through a simulated cargo ship searching for enemies in an immersive VR motion-enabled environment while measuring brain activity (EEG). The goal of the study was to determine if differences in cortical activation patterns during cooperative teaming could be detected based on partner behaviors and if the extent of these differences would correlate with team performance. Our initial findings suggest that we can measure neurophysiological correlates of performance that cannot be understood from behavioral measures alone. Specifically, we observed a wide range of success at the task, with little to no correlation with traditional behavioral measures such as movement speed, reaction time and partner coordination. However, we found that performance was correlated with suppression of cortical alpha power (8-13 Hz) in favor of theta power (4-7 Hz) (see attached Fig). This result is particularly intriguing given that frontal theta power is thought to be related to cognitive workload and motor control, while alpha power is implicated in controlling the balance between sensory input and internal cognitive models of the world. This work is critical to understanding and predicting our Warfighters' ability to interact and make decisions in complex, stressful scenarios.
Figure caption: Cortical results for two representative participants. Data are time-locked to the go cue at t = 0. The poor performer in the left plot shows a wide α synchronization that persists up to the go cue. While the good performer in the middle plot has prominent θ band power that increases as the go cue is anticipated. The right plot shows the difference between θ and α over the last second of the planning interval. The good performer is the top-right points and the poor performer the left-bottom points.



Disclosures: J. Snider: None. **M. Alam:** None. **J.R. Lukos:** None.

Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 429.06/LLL5

Topic: H.02. Human Cognition and Behavior

Support: Hong Kong Research Grants Council(15603517)
The Hong Kong Polytechnic University

Title: The casual role of dorsolateral prefrontal cortex in multiple option decision making

Authors: *T. WOO[1], C.-K. LAW[1], K. TING[2], C. C. CHAN[3], N. KOLLING[4], K. WATANABE[5], B. K. CHAU[1]
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Abstract: Activity in dorsolateral prefrontal cortex (dlPFC) is sometimes reported in decision making experiments. However, the reason why dlPFC is only involved in a subset of decision

making experiments is largely unclear. DlPFC is also implicated in cognitive control and working memory, which are important cognitive processes especially in decisions where there is a large number of alternatives. It is possible that when decisions involve a large set of choices, dlPFC is particularly important to store and manipulate decision information for guiding decision making. The current study examined the roles of dlPFC in information sampling and decision making in choices with multiple options. We recruited healthy human participants to perform a multiple option decision making task in which they chose between choice sets of two, four or sixteen food options. To test the causal role of dlPFC in multiple option decision making, we applied transcranial direct current stimulation (tDCS) over the right dlPFC. In a double-blinded crossover design, participants performed the same task in two separate sessions in which they received either an anodal tDCS to enhance dlPFC or sham tDCS as a control. To test the process of information sampling, we recorded the duration of gaze fixation on each option using an eye tracker. To estimate the decision accuracy of choosing a good food option, participants performed an additional Becker-DeGroot-Marschak (BDM) auction task and indicated their subjective preference to each food option. Our results showed that, in the control sham tDCS session, participants' decisions were accurate when there were longer fixation durations with the better options and shorter fixation durations with the poorer options. These effects were comparable in the anodal tDCS session when dlPFC was enhanced and were also comparable across two-, four- and sixteen-option trials. This suggested that dlPFC was unrelated to how information was sampled. Next, we investigated how the sampled information was used to guide decision making. In the sham session, less accurate decisions were made when there were longer fixations at the poorer options. Interestingly, after anodal tDCS was applied over dlPFC, such effect was attenuated, especially on trials with sixteen options. Furthermore, better decisions were made when there were longer fixations at the better options and this was comparable in the sham and anodal session. Overall, these results suggested that dlPFC did not have a particular role in how decision information was sampled. However, when there was a large number of options dlPFC was important to filter out the decision information of the poorer options.

Disclosures: T. Woo: None. **C. Law:** None. **K. Ting:** None. **C.C. Chan:** None. **N. Kolling:** None. **K. Watanabe:** None. **B.K. Chau:** None.

Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 429.07/LLL6

Topic: H.02. Human Cognition and Behavior

Title: Constructing single-neuronal representations of another's beliefs in the human prefrontal cortex

Authors: *M. JAMALI, B. L. GRANNAN, R. BAEZ-MENDOZA, Z. WILLIAMS
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Abstract: Humans have the remarkable ability to build a detailed understanding of the world and, based on this, reason about the hidden beliefs and states-of-mind of others. While this capacity to form a complex model of reality and to infer the beliefs of others is a major cognitive milestone in human ontogeny, the process by which these computations are formulated by individual neurons in the human brain is unknown. The human prefrontal cortex has been implicated in inferential processing and the ability to reason about others, and is broadly connected with limbic and temporal-parietal areas thought to be involved in social behavior. Here, we acutely followed the activity dynamic of single prefrontal cells (n = 212), as 11 participants were given detailed story narratives and then required to formulate ideas about them. Using this approach, we show how individual neurons (1) respond to distinct features describing social agents and (2) encode the relation between those agents and specific objects or events in their worlds. When later reasoning about these events, largely distinct neurons become predictive of the other's beliefs and also distinguish another's false beliefs from the participant's own understanding of reality. Taken together, these cellular representations reflect the other agent's own awareness of events and are predictive of variations in their content, further providing detail about the other's beliefs and their relation to experiences in their worlds. Collectively, these findings suggest a detailed cellular process in the human prefrontal cortex that may allow us to construct complex mental representations of others and make inferences about their beliefs. From this perspective, these neurons may be good candidates within the frontal-parietal network for supporting human theory-of-mind.

Disclosures: M. Jamali: None. **B.L. Grannan:** None. **R. Baez-Mendoza:** None. **Z. Williams:** None.

Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

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Program #/Poster #: 429.08/LLL7

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01NS084948

Title: Striatal prediction errors in a decision-making task are modulated by action execution failures

Authors: *S. D. MCDOUGLE[1], D. E. PARVIN[2], P. A. BUTCHER[1], F. MUSHTAQ[3], Y. NIV[1], R. IVRY[2], J. A. TAYLOR[1]

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Abstract: Decisions must be implemented through actions, and actions are often prone to error. As such, when an expected outcome is not obtained, an individual should not only be sensitive to whether the choice itself was suboptimal, but also whether she successfully performed the action required to indicate that choice. To explore this scenario, we used a modified version of a classic reinforcement learning task, manipulating the feedback to indicate if negative prediction errors were or were not associated with execution errors. Using fMRI, we asked if prediction error computations in the human ventral striatum, a key substrate in reinforcement learning and decision making, are sensitive to this manipulation. Behaviorally, participants were more tolerant of negative outcomes when they were the result of an execution error compared to when execution was successful, but reward was withheld. A model-driven analysis of the fMRI data revealed an attenuation of negative reward prediction error signals in the striatum following execution errors. These results converge with other lines of evidence suggesting that prediction errors in the mesostriatal dopamine system integrate high-level information with instantaneous reward outcomes. In the present case, cues concerning action execution inform striatal computations.

Disclosures: S.D. McDougle: None. **D.E. Parvin:** None. **P.A. Butcher:** None. **F. Mushtaq:** None. **Y. Niv:** None. **R. Ivry:** None. **J.A. Taylor:** None.

Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

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Program #/Poster #: 429.09/LLL8

Topic: H.02. Human Cognition and Behavior

Support: European Research Council Advanced Grant
Postdoctoral Fellowship of the British Academy

Title: Neural correlates of subjective and objective freedom of choice

Authors: *L. CHARLES, P. HAGGARD
Univ. Col. London, London, United Kingdom

Abstract: What do we know about the factors that influence our decisions? Are we aware of the true reasons of our ‘free’ choices? The ability to introspect the origin of our choices constitutes a key metacognitive function but little is known of the cognitive process and neural substrate that underlie our sense of freedom of choice.

It has been proposed that when faced with a free choice in the absence of external evidence, the brain might use endogenous noise to break the symmetry between different options, and decide how and when to act. However, the specific mechanisms that underlie such free decisions remain unclear. Which cognitive processes determine the influence of endogenous and exogenous signal used to form free decisions? And can we actually introspect how much our decision are based on each of these two sources, evaluating our true freedom of choice?
In this study, we investigated the brain mechanisms underlying objective and subjective freedom of choice in decision making. In particular, our aim was to determine what are the neural substrates enabling us to detach from sensory affordances and rely on internal signal to make free choices. To do so, we used a novel paradigm that estimated how human decisions were influenced by minor fluctuations in a visual signal that either cued a decision, or left participants free to decide for themselves. By varying the clarity of these cues, we were able to investigate both how fluctuating sensory information could influence action selection processes, and also participants' awareness of such influence.
Confirming previous findings, we observed that when participants were instructed to act freely, they were nevertheless biased in their choice of action by current sensory input. However, they nevertheless experienced their decisions as free. Brain activity recorded with fMRI seemed to confirm this dissociation. While subjective freedom of choice was associated with brain activity in a broad network of brain regions involving pre-motor areas and prefrontal cortex, neural correlate of objective freedom of choice, defined as the ability to detach from sensory affordances, only partially overlapped with these regions. In particular, greater influence by sensory fluctuations seemed to correlate with activity in parietal cortex and posterior cingulate region.
Taken together, these results provide evidence that brain regions involved in actual sensory detachment and the sense of freedom of choice partially dissociate, providing neural evidence consistent with limited ability to introspect the origin of our actions.

Disclosures: L. Charles: None. **P. Haggard:** None.

Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 429.10/LLL9

Topic: H.02. Human Cognition and Behavior

Support: Friends University Internal Grant

Title: Insight into the before and after of problem solving using event related potentials

Authors: T. BURNHAM, 67213, S. OESER, A. LAMP, *J. D. HALONEN
Psychology, Friends Univ., Wichita, KS

Abstract: Individuals regularly make a myriad of choices based on information presented to them. We aim to identify if the underlying neural processes for different modalities of conscious decision-making are temporally similar. The readiness Potential (RP) is an indicator of conscious decision-making processes (Fifel, 2018; Harim, 2018). Here we use the RP to investigate if fundamental differences between language- and visual-based information decision processing exist. Investigations into neural correlates of problem solving have used Chinese characters, riddles, or other puzzles in conjunction with various forms of neural imaging or recording techniques (for review see Sprugnoli et al, 2017). To gain insight about how the brain processes different information, we performed this experiment using riddles in English and Object Rotation Tasks (ORT). Electroencephalographic data was collected using iWorx (Dover, NH) IX-EEG hardware using a 10/20 Electro-Cap (Eaton, OH). After obtaining informed consent and calibrating procedures were performed, participants were exposed to a series of seven ORTs alternating with six written riddles. A total of 20 participants were instructed to press a button held in their right hand when they believed they had the answer. We hypothesized that RP and Event-Related Potentials (ERP) measures would be different between the tasks. Data for RP from electrodes FP1, F3 and F7 is currently under analysis. Repeated measures general linear model analysis of the ERP data indicates significant voltage differences at Fz, Pz, & Cz electrodes.

Disclosures: T. Burnham: None. **S. Oeser:** None. **A. Lamp:** None. **J.D. Halonen:** None.

Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

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Program #/Poster #: 429.11/LLL10

Topic: H.02. Human Cognition and Behavior

Support: This work was supported by the Australian Research Council (ARC) Centre of Excellence for Integrative Brain Function (ARC Centre Grant CE140100007). JBM was supported by an ARC Australian Laureate Fellowship (FL110100103).

Title: Perceptual decision-making depends on feature-based attention

Authors: *D. RANGELOV, R. WEST, J. B. MATTINGLEY
The Univ. of Queensland, St Lucia, Australia

Abstract: Researchers have had great success in understanding the neural processes responsible for simple, perceptual decisions such as discriminating the direction of motion of a single patch of moving dots. Most such studies of visual decision-making have employed a single stimulus (e.g., a dot patch) which is, by implication, always relevant to the task at hand. In everyday life, however, decision-making typically involves prioritizing goal-relevant stimulus properties and ignoring irrelevant ones. To date, it remains unclear whether task-irrelevant stimulus properties influence decision-making. To investigate this issue, we used electroencephalography (EEG) to measure neural activity as participants monitored two spatially overlapping patches of moving dots, each in a unique colour and each moving in a different direction. Participants (N = 25) were instructed to monitor the motion direction of one patch of dots (target coloured) and to ignore the other patch (distractor coloured). The overlapping motion patches were presented twice per trial, and participants were required to reproduce the average motion direction of the two target signals. This averaging task enabled us to dissociate decision-related processes associated with the motion stimuli from response selection processes which, by design, could occur only after both signals had been processed. To compute the relative contributions of target and distractor motion to the final decision, we used linear regression modelling with complex-valued data. The regression weights for target signals were several times higher than those for distractors, suggesting that only task-relevant stimulus properties contribute to decision-making. Next, we used forward-encoding modelling of the EEG data to characterise feature-specific brain activity evoked by different motion signals. These analyses revealed strong, direction-specific responses to target signals, starting from 200 ms after motion onset. Remarkably, there was no motion-direction-specific neural response to distractor motion. Thus, even though target and distractor stimuli were physically matched and overlapped completely in space and time, there was virtually no evoked neural activity associated with the distractor dots. Taken together the findings suggest that task-relevance plays a critical role in complex perceptual decision making.

Disclosures: D. Rangelov: None. **R. West:** None. **J.B. Mattingley:** None.

Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 429.12/LLL11

Topic: H.02. Human Cognition and Behavior

Support: DFG Grant TR-SFB134 C05 to J.P. ans L.S.

Title: Neural and hormonal basis of sleep deprivation-induced impairments in reward-based decision making

Authors: *J. RIHM[1], L. SCHILBACH[2], S. SCHMID[3], J. PETERS[1]
[1]Dept. of Psychology, Biol. Psychology, Univ. of Cologne, Cologne, Germany; [2]Max Planck Inst. for Psychiatry, Munich, Germany; [3]Univ. Hosp. Schleswig-Holstein, Luebeck, Germany

Abstract: Dysbalanced energy levels, e.g. due to sleep deprivation or fasting, alter valuation and reward representations in the midbrain (Menz et al., 2012, Gujar et al., 2011). Previous literature found that sleep deprivation prior to a risky monetary choice task resulted in more inconsistent choices and attenuated brain valuation signals compared to habitual sleep (Menz et al., 2012). However, an open question is if different homeostatic interventions (e.g. fasting) similarly affect risky monetary decision making and if hormones involved in hunger and satiety signaling, such as ghrelin, modulate possible effects. To answer this question, we tested homeostatic modulations of reward processing in risky monetary decision making in two studies using high-resolution fMRI, a classic probability discounting task, blood concentrations of ghrelin, leptin, insulin, glucose, and cortisol, and short-term fasting (study 1) or sleep deprivation (study 2) as homeostatic manipulations. The studies comprised thirty (study 1) or thirty-six (study 2) lean, healthy men and used randomized, within-subject designs. In both studies, a non-linear probability weighting model (Lattimore et al., 1992) fitted the choice behavior best. In addition, model comparison revealed a superior fit of models allowing decision noise levels (i.e. softmax slope parameter) to change according to a power function over trials. Following sleep deprivation, noise levels increased more over trials (study 2), whereas fasting increased the intercept of the noise power function (study 1). Neither effect was correlated with hormonal changes from the control to intervention sessions. Consistent with previous studies (Menz et al., 2012, Peters & Büchel 2009), parametric modulation of risky choices by subjective values of the probability weighting model revealed a main effect of subjective value in the ACC, lateral PFC, PCC, precuneus, striatum and SN/VTA after both interventions. These effects were not correlated with hormonal changes. Our results indicate that different homeostatic interventions can impact risky decision making via a modulation of decision noise levels, but do not suggest a prominent role for hormonal changes in these effects.

Disclosures: J. Rihm: None. **L. Schilbach:** None. **S. Schmid:** None. **J. Peters:** None.

Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 429.13/LLL12

Topic: H.02. Human Cognition and Behavior

Support: NWO Grant 400-03-392

Title: An ERP study of the determinants and dynamics of reactive cognitive control

Authors: *K. L. MANSFIELD[1,2], M. W. VAN DER MOLEN[3], G. J. M. VAN BOXTEL[2]
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Abstract: The current study used laplacian transformed ERPs to investigate how the dynamic interplay between proactive and reactive control (c.f. Braver et al., 2007) varies with task predictability during mixed stimulus-response (SR) mapping tasks. Seventeen participants (1 male) took part in a mixed SR-mapping task, in which they were required to give a spatially *compatible* or *incompatible* response to left/right gaze direction stimuli, such that the SR mapping rule depended on the blue/green color of the eyes (counter-balanced between participants). We manipulated the predictability of the SR-mapping by varying the probability of *compatible* vs. *incompatible* SR-mappings (80/20, 50/50, 20/80). We assessed reactive control mechanisms using response-locked midline N-120 (c.f. Mansfield et al., 2012) and lateralized motor activity (c.f. Vidal et al., 2003). We anticipated that participants prepare (proactively) for the *expected* SR-mapping, predicting that on *unexpected* trials reactive control corrects the mapping, appointing inhibition of the incorrect response, indexed by ipsilateral positivity, and activation of the correct response, indexed by contralateral negativity. In line with previous behavioral studies that manipulated the probability of SR-mappings, SR-mapping effects were robust with 80%, eliminated with 50%, and reversed with 20% compatible trials. Midline N-120 was most enhanced with *unexpected compatible* trials, suggesting that reactive control was needed most in correcting SR-bindings after participants had proactively prepared for an *incompatible* SR-mapping. However, ipsilateral positivity between N-120 and execution of the response was enhanced for all *unexpected* trials, and mostly for *unexpected incompatible* trials, suggesting that inhibition of the incorrect response tendency might be recruited by reactive control reflected at N-120 or earlier. Differences in activity over motor areas contralateral to the response hand were unreliable. The demand for and mechanisms of reactive control reflect both the predictability and the difficulty (SR-mapping) of the prepared tasks, which we assume to be mediated by strategic performance adjustments associated with proactive control.

Disclosures: K.L. Mansfield: None. **M.W. van der Molen:** None. **G.J.M. van Boxtel:** None.

Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 429.14/LLL13

Topic: H.02. Human Cognition and Behavior

Support: UC Berkeley Graduate Division Mentored Research Award
NIH Grant EY024554

Title: Evidence accumulation in abstract decisions cued by varying perceptual information

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[1]Helen Wills Neurosci. Inst., Univ. of California Berkeley, Berkeley, CA; [2]Neurol., Univ. of California San Francisco, San Francisco, CA

Abstract: Perceptual decision making is thought to depend on the accumulation of sensory evidence in the intraparietal sulcus (IPS), independent of specific motor effector. However, whether similarly general mechanisms apply to evidence supporting abstract decisions is unknown. Under this framework, evidence used to drive both concrete (1st order) and abstract (2nd order) stimulus-response (S-R) relationships should accumulate in the same area of IPS. In contrast, progressively higher-order policy requires progressively more anterior regions of the lateral frontal cortex, each of which is most strongly connected to different parietal regions. Under a network-based hypothesis, evidence used to drive concrete (1st order) and abstract (2nd order) S-R relationships should thus accumulate within different areas of IPS. To evaluate these competing hypotheses, we designed a task in which the coherence of a perceptually graded stimulus (motion, color, or shape) and the level of policy abstraction (1st or 2nd order) were independently varied. Behavioral and fMRI data have so far been collected from 8 human subjects (3 male, ages 18-46). Following training to ensure stable performance, subjects each completed 5 fMRI sessions, for 660 total trials. Within a trial, subjects viewed 3 separate, sequentially presented stimuli - dot motion (up/down), dot color (blue/white), and shape (circle/triangle) - before making a button press. Prior to each run, subjects were informed that one stimulus (e.g. color) would represent the 2nd order cue. Based on the 2nd order percept for each trial (e.g. blue/white color), subjects then made a button press response based upon one of the other features (the relevant feature - e.g. motion) while ignoring the third (the irrelevant feature - e.g. shape). Accuracy was significantly greater on high vs low coherence trials for both the 2nd order and relevant 1st order stimuli, but no such difference was seen for irrelevant stimuli. When both 1st order relevant and irrelevant stimuli cued the same button response, accuracy was significantly greater than when different button responses were cued. This result reflected a strategy shift supported by a significant interaction between 2nd order coherence (high/low) and 1st order response (same/different): subjects were significantly more accurate in the low*same vs low*different conditions, but not in the high coherence comparison. These behavioral results demonstrate not only that coherence and abstraction are processed independently, but also that we can systematically manipulate strategy within a trial subtype. Ongoing imaging analyses will evaluate the neural correlates of these findings.

Disclosures: M. Newton: None. **A. Kayser:** None.

Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 429.15/LLL14

Topic: H.02. Human Cognition and Behavior

Support: Stanford Neuroscience Institute NeuroChoice Grant
NIH 2T32MH020006-16A1

Title: Neural affective predictors of engagement with online videos

Authors: *L. TONG, M. Y. ACIKALIN, B. KNUTSON
Stanford Univ., Stanford, CA

Abstract: Neural circuits implicated in affect, including the nucleus accumbens (NAcc), anterior insula (AIns), and medial prefrontal cortex (MPFC) have been robustly implicated in guiding decisions involving how to spend money. Less is known, however, about the role that these circuits play in decisions about spending time. We conducted two fMRI studies to test whether neural and affective responses predicted (1) choices to engage with watching YouTube videos, and (2) how much time subjects subsequently spend watching the videos before deciding to disengage. In both studies, subjects viewed 64 YouTube splash screens and were asked whether they wanted to watch the depicted video. In study 2, subjects were then asked to watch 32 of these videos, with the option of skipping any video at any time. Consistent with previous research in the financial decision-making domain, NAcc and MPFC activity in response to its splash screen predicted whether a subjects accepted to watch a video (NAcc: b=0.60, $p<0.05$; mPFC: b=0.54, $p<0.001$; AIns: b=0.324, *ns*). Furthermore, Nacc activity was a significant predictor of video choice controlling for self-reports of positive arousal (b=0.88, $p<0.05$). This pattern of results was replicated using a different set of stimuli in study 2 (NAcc: b=0.58, $p<0.05$; mPFC: b=0.45, $p<0.01$; AIns: b=0.55, $p=0.09$). Moreover, mPFC activation was associated with increased time spent watching the videos, controlling for video length (b=0.046, $p<0.05$). Interestingly, anterior insula responses were most (negatively) strongly associated with time that other subjects spent watching the videos (b=-0.047, $p<0.05$). Our results converge with prior research, indicating that anticipatory NAcc and MPFC signals guide subsequent choice not only in monetary domains, but also in choices about how to spend time. Interestingly, while MPFC signal was the strongest factor associated with subjects' own subsequent engagement with the videos, AIns signal was most strongly related to other subjects' engagement. These results are consistent with the view that different neural circuits carry signal about distinct affective components of a decision, which can vary in the extent to which they generalize across people (Knutson & Genevsky, 2018).

Disclosures: L. Tong: None. **M.Y. Acikalin:** None. **B. Knutson:** None.

Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 429.16/LLL15

Topic: H.02. Human Cognition and Behavior

Support: NSF Grant 1658303

Title: The time course of endogenous brain signals reflect different cognitive processes during human decision making

Authors: *M. D. NUNEZ, K. A. SCAMBRAY, K. K. LUI, J. VANDEKERCKHOVE, R. SRINIVASAN
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Abstract: Electrophysiological research has shown that electrical signals recorded from the human brain track evidence accumulation during perceptual decision making. We have previously found evidence that figure-ground segregation of a visual stimulus before evidence accumulation, dubbed Visual Encoding Time (VET), can be tracked by the latency of a visual evoked potential (a negative peak occurring between 150 and 275 milliseconds after stimulus presentation). In this study we sought to elucidate the contribution of different neural networks as recorded by EEG oscillations (in particular the theta, alpha, and beta bands) on the cognitive components of decision-making that occur after VET (i.e. after the N200 peak-latency). In a preregistered study, we confirm previously found 1-to-1 relationships between N200 peak-latency and non-decision time as estimated by cognitive models. We also found evidence that beta desynchronization reflects a neural network involved in motor execution. In an exploratory analysis we found evidence that posterior theta band power increases during evidence accumulation, in support of published research. We also explore evidence that alpha band power desynchronization reflects internal noise suppression during evidence accumulation. These hypotheses were tested directly by estimating parameters of novel neuro-cognitive models of decision making, fitting both human behavior (reaction time and accuracy distributions) and scalp-recorded EEG simultaneously. A hierarchical Bayesian fitting procedure was used to ensure variance between subjects and conditions was accounted for by cognitive models. A cohesive neuro-cognitive theory of quick human decision making is proposed based on these results.

Disclosures: M.D. Nunez: None. **K.A. Scambray:** None. **K.K. Lui:** None. **J. Vandekerckhove:** None. **R. Srinivasan:** None.

Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 429.17/LLL16

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01MH095894
NIH Grant R01MH108627
NIH Grant R37MH109728
SFARI Grant 304935 MLP

Title: Modeling behavior of rhesus macaques and humans in an iterative chicken game

Authors: *S. MADLON-KAY[1], W. S. ONG[2], M. L. PLATT[3]
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Abstract: Competitive opponents today might be cooperative allies tomorrow. Navigating social environments requires understanding strategies of others, and how their actions may change across different situations. To study how the brain generates predictions about others' strategies, and updates those predictions across changing environments, we devised a variant of the "chicken" game that alternates between competitive and cooperative scenarios that is played iteratively, over multiple sessions, by pairs of rhesus macaques or pairs of humans. Two individuals (M1&M2) face each other across screen(s) showing 2 colored annuli and 4 response targets. On some trials, the larger reward (denoted by visual tokens) lies opposite M1 behind the opponent (M2)'s annulus; smaller rewards lie to the left (see figure). To obtain the larger reward, M1 goes straight, but if M2 also goes straight the annuli collide and neither monkey gets reward. On some trials, a "cooperation bar" allows both monkeys to obtain larger rewards if and only if both choose to go left; if only one yields he receives a smaller reward. 4 trained monkeys maximized juice intake by attending to the reward tokens as well as the choices of their opponent, while 55 human pairs played cooperative strategies with far less regard to the reward amounts. To describe choice behavior, we constructed a learning model where the agent, M1 uses his beliefs about the strategy of his opponent, M2, to predict M2's action on each trial. M1's beliefs about M2's strategy are represented as a logistic regression relating the potential payoffs of a trial to the probability of M2 swerving versus going straight. M1 learns M2's strategy by updating his beliefs using a strategic prediction error, the mismatch between his expectation about M2's actions and what M2 did. We find that the belief updating model fits choice behavior best, over the reinforcement-learning model, while the intermediate model with static beliefs regarding M2's strategy also improved but to a lesser extent in both human and non-human primate plays. We also collected eye-tracking data on one or both of the players. Surprisingly,

the monkeys spent more time looking at their opponents, while humans rarely did.
Our results indicate that while rhesus macaques played strictly in accordance to the payoffs, humans cooperate more than is conventionally considered rational, and as a whole, have similar gaze and joystick movements. Both are able to learn about the strategies of others, and are able to perform predictions based on at least one level of reasoning.

Disclosures: S. Madlon-Kay: None. **W.S. Ong:** None. **M.L. Platt:** None.

Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 429.18/LLL17

Topic: H.02. Human Cognition and Behavior

Support: NIH R01 Grant 108627

Title: Integration of social information and value by superior temporal sulcus (STS) neurons in monkeys trading in a simulated stock market

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Abstract: People have a keen ability to act on the basis of inferences made about others' intentions, an ability referred to as theory of mind (ToM). However, individuals in stochastic and complex environments, especially financial markets, often maladaptively employ ToM when forecasting others' value estimations, leading to herding and bubble markets. Prior research supports the hypothesis that connections between brain areas associated with value judgements (e.g. ventromedial prefrontal cortex; vmPFC) and those associated with theory of mind (e.g. temporal parietal junction; TPJ) underlie suboptimal decision making due to artificial inflation of stock values in 'bubble markets.' Current work in our lab demonstrates that macaque middle superior temporal sulcus (mSTS), the putative homolog of human TP, is engaged during strategic social decision making, highlighting a potential role for STS in social biasing of decisions in financial markets. In order to further explore the evolutionary roots and neural circuit mechanisms underlying these behaviors, we developed a rudimentary 'stock market' task for rhesus macaques (*Macaca mulatta*), which was also validated in humans. Multichannel electrodes recorded electrophysiological activity from mSTS while monkeys made investment decisions on a touchscreen computer for juice reward in 4 conditions: 1) computer opponent, 2) replay opponent, 3) dummy opponent, and 4) live opponent. Preliminary results indicate that macaque mSTS neurons differentially signal the value of actions in social and non-social

contexts, and that firing rates are modulated by the outcome value of others' choices. Furthermore, we observed an overall increase in engagement of mSTS neurons during the formation of bubble markets compared to non-bubble markets. This research suggests that mSTS neurons encode a social information signal that is likely integrated into downstream valuation computations (e.g. in vmPFC). Ultimately, the spontaneous inclusion of social information signals, which is helpful in most social situations, leads to suboptimal decisions in bubble markets.

Disclosures: A.W. Huttunen: None. **M.L. Platt:** None.

Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 429.19/LLL18

Topic: H.02. Human Cognition and Behavior

Support: NSF-NCS 1533623
NIH T32 NRSA

Title: Tonic pupil dynamics predict individual differences in the complexity of mental models used for adaptive decision-making

Authors: *A. L. FILIPOWICZ[1], C. M. GLAZE[2], J. W. KABLE[3], J. I. GOLD[2]
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Abstract: To make good decisions in uncertain environments, humans build mental models of relevant environmental statistics and adapt these models when environments change. Despite the importance of adaptive behavior in our everyday lives, there are substantial gaps in our understanding of how this model-building process differs between individuals and the brain networks underlying these differences. We have recently demonstrated that the complexity of a mental model, measured as the size of the space over latent variables to which it is sensitive, is an important source of individual differences in adaptive decision-making. Here we present evidence that individual differences in adaptive behavior linked to mental model complexity are reflected in pupil-linked physiological arousal, a proxy for system-wide levels of norepinephrine. Concepts such as 'mental effort' and 'cognitive abilities' have been identified as a key, albeit loosely defined, factors related to pupil dynamics. We propose that this effort is more precisely defined in terms of the complexity of the mental model used to solve a given task. We measured the pupil diameters of human subjects performing an auditory adaptive decision-making task. The task required them to predict which of two sources would generate a tone on each trial. The source generating the tone sometimes switched, with the probability of a source switch, or

'hazard rate', also changing at unannounced points throughout the task. Mental model complexity was measured using a novel information-bottleneck method that calculates the extent to which participant responses encode past information about both the source generating tones and the environment's hazard rate. According to this metric, more encoded information corresponds to more complex models. Consistent with our previous results, participants with more complex mental models adapted better to changes in the task environment but produced noisier responses than participants with simpler models. Pupil analyses revealed that low frequency tonic pupil dynamics were modulated by different task hazard rates, behaving differently on trials where switches occurred rarely (low hazard rate) compared to environments in which switches occurred more frequently (high hazard rate). Moreover, the strength of this modulation was related to mental model complexity, such that subjects with more complex models showed stronger hazard rate-dependent modulation of tonic pupil dynamics. These results suggest that the neurobiological mechanisms that govern tonic pupil dynamics contribute to individual differences in the complexity of mental models used for adaptive decision-making.

Disclosures: A.L. Filipowicz: None. **C.M. Glaze:** None. **J.W. Kable:** None. **J.I. Gold:** None.

Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 429.20/LLL19

Topic: H.02. Human Cognition and Behavior

Title: Integrating abstract structures and constructing cognitive maps about social hierarchies

Authors: *S. A. PARK[1], D. S. MILLER[2], E. D. BOORMAN[1]
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Abstract: During navigation, animals' hippocampal-entorhinal circuit integrates path information including location, distance and direction. Recent findings suggest that this circuit may serve a more general mechanism for constructing the cognitive maps of task environments beyond spatial navigation. Here we test whether human brains follow the same principles underlying path integration while integrating social hierarchical structures and building a cognitive map of social networks. Participants first learnt the social hierarchies of two groups on two independent social dimensions separately. Second, under each dimension, participants learnt the relative hierarchy of one individual in a group against another in the other group, which created a unique associative path across groups per individual in the social network. Last, in the fMRI, participants inferred the relative hierarchy between pairs who had not been compared in the given dimension. We examined whether participants recalled specific individuals whose hierarchy had been compared between groups and used these relevant representations as "hubs"

between the two networks to enable the transitive inferences. Behaviorally, we found that the reaction time for inferences not only depends on the different levels of hierarchy between the pairs, but also the within-group Euclidean distance from the hub in the two-dimensional social space. Neurally, both the Euclidean distance and the within-dimension difference between individuals in hierarchies were encoded in the entorhinal cortex (EC). During inference decisions, the medial prefrontal cortex (mPFC) encoded the Euclidean distance from the hub. To test for neural representations of the hub, we adopted trial-by-trial fMRI suppression. While subjects performed a cover task to detect the gender of suppression images, we found suppression in hippocampus and retrosplenial cortex (RSC) for the specific trials where the pair was followed by their hub compared to other matched hubs that were not theirs. Our findings provide preliminary evidence that the human EC integrates the relationship between abstract and discrete entities into a cognitive map. They further suggest the mPFC retrieves relevant representations from prior experiences to meet current demands and infers social relationships across groups based on the spatial map. These results shed light on how abstract and discrete structures are combined and represented in the human brain, suggesting that general mechanisms in the human EC can be extended to map abstract social networks and used by the mPFC to guide goal-directed inferences, supporting their roles in higher social-cognitive functions.

Disclosures: S.A. Park: None. **D.S. Miller:** None. **E.D. Boorman:** None.

Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 429.21/LLL20

Topic: H.02. Human Cognition and Behavior

Support: National Center for PTSD
NIMH Grant R21MH102634

Title: vmPFC activity during decision making under uncertainty predicts trauma-related symptoms in combat veterans

Authors: *R. JIA[1,2], L. RUDERMAN[1], C. GORDON[3,4], M. HORVATH[3,4], C. DEFONTES[3,4], S. MIRCHANDANI[3,4], I. HARPAZ-ROTEM[3,4], I. LEVY[1,2,4]
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Abstract: Combat soldiers face high levels of uncertainty in the battlefield, yet the role of individual uncertainty attitudes in the development of trauma-related psychopathology has hardly been examined. We have recently used a paradigm inspired by behavioral economics, and

identified variations in decision making under uncertainty that were associated with posttraumatic stress disorder (PTSD) (Ruderman et al., 2016). Here, we use the same task to explore neural markers of trauma-related symptoms.
We used a monetary task to assess risk and ambiguity attitudes of 78 combat veterans. Subjects chose between a certain win (or loss), and playing a lottery which offered a larger gain (or loss) but also chance of zero outcome. Outcome probabilities for half of the lotteries were precisely known, and were ambiguous for the other half. fMRI was used to track neural activation while subjects completed 240 decisions. One choice was randomly picked for payment to ensure task engagement. We evaluated PTSD symptoms by CAPS (Clinician-Administered PTSD Scale), and used additional measures to assess trauma exposure and other psychiatric symptoms including depression, anxiety and addiction.
Behaviorally, we replicated our recent result using a dimensional approach, and found that veterans with more severe PTSD symptoms (higher CAPS) were more averse to ambiguous losses (Pearson's correlation $r=0.25$, $p<0.05$), but not gains. Veterans with higher CAPS were also more averse to risk under gains ($r=0.3$, $p<0.05$), but not losses. A whole-brain analysis revealed that overall activation in vmPFC, an area involved in both value-based decision making and fear learning, was negatively correlated with CAPS ($p<0.05$). Interestingly, when controlling for correlations between symptom clusters, emotional numbing remained the only significant cluster. Severity of this symptom cluster predicted activation under gains, but not losses ($p<0.05$), emphasizing the significance of studying reward processing in PTSD.
Our results demonstrate the potential of neuroeconomics techniques for studying psychopathology, and for devising objective diagnostic tools that compensate the insufficiency of DSM based categorical diagnoses.

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Poster

429. Human Cognition and Behavior: Decision Making and Reasoning: Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 429.22/LLL21

Topic: H.02. Human Cognition and Behavior

Support: National Institute on Drug Abuse grant R03DA040668

Title: Model-based inference involves interactions between orbitofrontal cortex and hippocampus in humans

Authors: *F. WANG[1], G. SCHOENBAUM[3], T. KAHNT[1,2]
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Abstract: Model-based inference requires using prior knowledge about the associative structure of the task to infer outcomes. Such model-based reasoning has been associated with neural processing in the orbitofrontal cortex (OFC) and hippocampus. However, the specific contributions of these two regions to model-based behavior, and how they interact during inference, has remained unclear. To address these questions, we used functional magnetic resonance imaging (fMRI) and multivoxel pattern analysis in the context of a sensory preconditioning task. Participants (N=23) first encoded pairs of visual cues (A->B, C->D; preconditioning). Subsequently, they learned associations between the second cue of each pair and monetary outcomes (B->$1, D->$0; conditioning). Finally, participants were probed to predict the outcome associated with all cues (A, B, C, D; probe test). Of note, because cues A and C were never directly paired with the outcome, successful outcome prediction required participants to retrieve and combine cue-cue (A->B, C->D) and cue-outcome associations (B->$1, D->$0). Behavioral responses during the probe test showed that participants were able to predict the correct outcomes in response to cues A and C. To test for neural representations of value-neutral cue-cue associations during preconditioning, we used a searchlight-based fMRI pattern-similarity analysis. We found that activity patterns in the OFC and hippocampus elicited by the two cues of each pair became increasingly similar over multiple presentations, suggesting that these regions encode the associative task structure in the absence of value. Furthermore, we conducted a pattern-based classification analysis and tested whether inference in response to A and C in the probe test involved the reactivation of cues B and D. Specifically, we trained a support vector classifier on activity patterns evoked by cues B vs D during conditioning, and tested it on activity patterns evoked by cues A vs C in the probe test. This analysis revealed significant reactivation of B vs D in response to cues A vs C in the OFC, but not the hippocampus. However, functional connectivity between the OFC and hippocampus increased during inference trials, suggesting that reinstatement of learned associations in the OFC could be mediated by enhanced OFC-hippocampus connectivity. Collectively, these findings show that the OFC, and its functional interactions with the hippocampus, play an important role in representing the associative structure of the task environment to support model-based inference.

Disclosures: F. Wang: None. **G. Schoenbaum:** None. **T. Kahnt:** None.

Poster

430. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Electron Microscopy and Novel Probes

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 430.01/LLL22

Topic: I.03. Anatomical Methods

Title: Electron microscopy and Golgi-staining for tracing entire neuron in 3D

Authors: *N. V. GOUNKO, K. VINTS, P. BAATSEN, D. VANDAEL, N. CORTHOUT, B. PAVIE, F. VERNAILLEN, S. MUNCK
KU Leuven, Dept. of Neurosciences, VIB Ctr. For Brain & Dis. Res., Leuven, Belgium

Abstract: Light/fluorescence microscopy allows to visualize cells and tissue and can provide information about the location of specific molecules by tagging them with genetically encoded markers or other molecular probes or by labeling with fluorescent antibodies. However, this information is constrained by the limited resolution of the fluorescence microscope as well as by the absence of cellular context on the nano-scale. The Golgi staining method is based upon the deposition of silver or mercury particles within a seemingly random small set of isolated neurons. These particles can be easily visualized by both light and electron microscopy. We developed a method based on the old Golgi staining technique that allows merging electron and light-microscopy based images from the same object, and adapted electron microscopy visualization protocols. This novel approach allows to trace neurons over the entire length of their projections throughout the brain, while still revealing to see ultrastructural details, which is in stark contrast to the currently used Golgi protocols. We optimized Golgi staining for use in block face scanning electron microscopy and developed an algorithm for automated neuronal tracing. Successful implementation of our novel approach will significantly contribute to the study of functional connections in the brain.

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Poster

430. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Electron Microscopy and Novel Probes

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 430.02/LLL23

Topic: I.03. Anatomical Methods

Support: Howard Hughes Medical Institute

Title: Large volume 3d fib-sem imaging for connectomics and cell biology

Authors: *C. S. XU[1], K. J. HAYWORTH[1], S. PANG[1], Z. LU[2], H. F. HESS[1]
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Abstract: Focused Ion Beam Scanning Electron Microscopy (FIB-SEM), developed for materials science and the semiconductor industry, has been applied to biological imaging for over a decade. Conventional FIB-SEM systems offer superior isotropic resolution, yielding data that needs minimal image registration and post processing. However, deficiencies in its imaging speed and long-term system stability limit the maximum imaging volume. I will present advancements that accelerate image acquisition and markedly improve reliability of conventional FIB-SEM, expanding the imageable volume by more than four orders of magnitude to beyond 10^7 μm^3 at 8 x 8 x 8 nm^3 voxel resolution. These large volumes are ideal for connectomics, where the excellent z resolution can help in tracing of small neuronal processes of small insects and minimize the time-consuming human proofreading effort. Even higher resolution is achievable on smaller volumes, generating ground truth for connectomics and providing important insights into cell biology. In our largest connectomic study to date, we have acquired a *Drosophila* hemi-brain dataset spanning the entire central complex, a complete unilateral mushroom body, and partial optic lobe. The next grand project, a complete *Drosophila* central nerve system (CNS) acquisition, is currently in progress.

Disclosures: C.S. Xu: None. **K.J. Hayworth:** None. **S. Pang:** None. **Z. Lu:** None. **H.F. Hess:** None.

Poster

430. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Electron Microscopy and Novel Probes

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 430.03/LLL24

Topic: I.03. Anatomical Methods

Title: Gas cluster ion milling of serial thick sections for connectomics

Authors: *K. J. HAYWORTH, D. PEALE, Z. LU, C. XU, H. F. HESS
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Abstract: Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) has a demonstrated ability to generate high contrast, high-resolution (<10nm) isotropic datasets for connectomics, however samples must typically be restricted to <50 microns in the direction of the FIB beam as glancing-angle milling results in artifacts over longer distances. Removal rate is also limited due to a current/spot size tradeoff. These are not serious limitations when using standard SEMs, but they are major obstacles to integrating FIB with multibeam SEMs. Wide area samples such as serial sections collected flat and side-by-side on a common substrate are better suited. We have begun exploring Gas Cluster Ion Beam (GCIB) milling of serial thick sections as an alternative to glancing-angle FIB milling. GCIB is a technique that has been used in the semiconductor industry to polish surfaces at the nanometer scale and to produce nanometer scale depth profiling

of polymer films for mass spectroscopy. We attached a GCIB-10s gun from Ionoptika to a Zeiss Ultra SEM creating the first combined GCIB-SEM system. We verified that smooth, <10nm sequential surface removal and SEM imaging could be achieved for large area 100nm thick brain sections. To do so we used a 10kV beam of Ar2000 (clusters of 2000 argon atoms) directed such that the beam made an angle of 30 degrees to the rotating tissue's surface. To apply this technique to larger sections we developed a high voltage electron irradiation technique that pre-cooks thick sections making them conductive prior to GCIB-SEM imaging. As a demonstration we collected three sequential 1 micron sections of Drosophila brain on silicon and performed ~250 mill/image cycles completely imaging through the depth of all three sections. We found that under these conditions there is ~10% variance in milling rate that had to be computationally 'flattened' prior to stitching the volume images of the sequential sections together. The resulting dataset appears suitable for connectomic tracing and is similar in quality to FIB-SEM but produces somewhat rougher surface images under secondary electron detection. Since GCIB can achieve high removal rates and is not limited in area we think it represents a promising path for integration with multibeam SEMs.

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Poster

430. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Electron Microscopy and Novel Probes

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 430.04/LLL25

Topic: I.03. Anatomical Methods

Title: Mitochondrial size gradients in cortical neurons revealed by 3D electron microscopy

Authors: *A. FORYCIARZ[1], N. TURNER[1], K. LEE[1,2], W. SILVERSMITH[1], W. WONG[1], J. WU[1], S. DORKENWALD[1], T. LEWIS, JR[3], Y. HIRABAYASHI[4], F. POLLEUX[5], N. M. DA COSTA[6], R. REID[7], H. SEUNG[1]
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Abstract: Neurons are among the most highly polarized cells in our body and this includes compartmentalized organelle structure and function. Mitochondria play a crucial role in the functioning of neurons by synthesizing ATP and buffering intracellular Ca^{2+}, both necessary for synaptic function. They are highly dynamic organelles, are constantly changing through fusion and fission events to adapt to changing environment in the cell (Youle et al. 2012), moving across cells (Narayanareddy et al. 2014) and often forming long filaments (Popov et al. 2005,

Amchenkova et al. 1988). The morphology and location of mitochondria can provide insights into specialized function they perform in different parts of the neuron. Observations of neuronal mitochondria thus far have mainly been in dissociated neurons in vitro, single neurons in vivo with light microscopy, or small volumes (< 1000 um^3) with 3D electron microscopy (EM). Recently Dorkenwald et al. (2017) demonstrated automated reconstruction of mitochondria from 3D EM of millions of um^3 of avian brain. Such large-scale reconstructions open up the possibility of obtaining more detailed and quantitative information about either cell-type specific and/or compartment-specific mitochondrial morphology and distribution. We acquired 3D EM images of mouse primary visual cortex (V1). All neurons with somas in the imaged volume were reconstructed using a semiautomated pipeline described elsewhere. We applied a 3D convolutional network to classify voxels as either mitochondrion or background. The mitochondrial voxels were grouped into over 900,000 mitochondria using connected components. For subsequent analyses, we focused on the mitochondria that were contained in neurons with somas in the volume. Each mitochondrion was assigned to either axon, dendrite, or soma. We calculated the distance from soma centroid to each mitochondrion centroid. It is well-known that axonal mitochondria are typically shorter and smaller than dendritic mitochondria. Our data show that somatic mitochondria are intermediate in size between axonal and dendritic mitochondria. This comparison may be simplistic, as mitochondria vary in size systematically with distance from the soma. We found that the size (length and volume) of axonal mitochondria decreases with distance from the soma. In contrast, the size of dendritic mitochondria increases with distance from the soma up to almost 100 microns. Our data so far strongly suggest that the balance between mitochondrial fission and fusion must be compartment-specific with fusion dominating over fission in dendrites and vice versa in axons.

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Poster

430. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Electron Microscopy and Novel Probes

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Topic: I.03. Anatomical Methods

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Title: A new genetic probe system for visualizing glutamatergic synapses and vesicles at high resolution by correlated light and electron microscopy

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Abstract: Communication between neurons relies on the release of chemical neurotransmitters, which represent a key-defining feature of a neuron's chemical and functional identity. These are packaged into vesicles for release at the synapse by specialized vesicular transporters. However, imaging tools designed to determine the neurochemical identity of synapses and synaptic vesicles *in vivo* are quite limited. We developed a genetically-encoded probe to identify glutamatergic synapses at both the levels of light and electron microscopy (EM). More specifically, we generated a fusion of VGLUT2 and miniSOG (mini singlet oxygen generator), a fluorescent protein that generates singlet oxygen when activated by blue light to locally catalyze the polymerization of diaminobenzidine (DAB) into an osmiophilic reaction product, easily detected by EM. We designed the new glutamatergic synapse EM probe to concentrate and accumulate DAB signal inside synaptic vesicles, so they can be easily distinguished from the unlabeled vesicles. To extend this to animals, we generated and injected an adeno-associated virus into Cre-driver mouse lines to allow Cre-conditional expression of VGLUT2-miniSOG in specific cell types. Importantly, VGLUT2-miniSOG is functional, because it can rescue Channelrhodopsin-2 (ChR2)-assisted glutamate co-release from dopamine neurons from which VGLUT2 had been knocked out. Immunostaining for miniSOG indicates that VGLUT2-miniSOG can be efficiently detected in synaptic terminals where it localizes with other synaptic markers. Furthermore, following photooxidation and EM processing, DAB staining confirmed synaptic vesicle labeling consistent with VGLUT2-miniSOG successfully trafficking to these organelles, with pristine preservation of the ultrastructure. Using a 3D imaging method, serial block-face scanning EM (SBEM), we assessed the subcellular distribution of transporter-defined vesicles at nanometer scale. To confirm the specificity of the VGLUT2-miniSOG labeling within the lumen of the vesicles, we used DAB conjugated with a lanthanide (cerium) chelate and implemented a method called multicolor-EM. This allowed us to uniquely differentiate the spectral signal of the cerium in the DAB deposits by imaging electron energy-loss spectroscopy (EELS) in an energy-filtered transmission EM. Finally, we developed a novel deep learning approach to perform automatic segmentation of the labeled vesicles. These tools should be very useful to the neuroscience community for work ranging from the subcellular structural/molecular biology of neurotransmission to long-distance tracing of neural network connectivity.

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Poster

430. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Electron Microscopy and Novel Probes

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 430.06/LLL27

Topic: I.03. Anatomical Methods

Support: NIH NINDS SNRP 5U54NS083924-03
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Title: A novel method for the quantitative analysis of ultrastructure in astrocyte monolayers after an ischemic stroke-like insult

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Abstract: Astrocytes the most abundant glial cell type in the brain, are key participants in the complex cellular response following injury to the central nervous system, and an important component of their intermediate filament system is glial fibrillary acidic protein (GFAP). In prior fluorescent microscopy studies of astrocytes, we show that following ischemic-like insults, nicotinic acetylcholine receptor blockage decreases astrocyte reactivity. Now, we extend those studies using fluorescence and immuno-electron microscopy to quantitatively characterize structural changes of reactive astrocytes and to study changes in GFAP filament immunoreactivity, respectively. After stroke, astrocytes around the affected area undergo astrogliosis that results in increased reactivity, proliferation, and the formation of a glial scar. Following neurotoxic insults, the 4R-cembranoid molecule (4R) has been shown to decrease astrocyte reactivity in vivo and to have neuroprotective effects against ischemic stroke. Previous studies have shown that 4R neuroprotective signaling is mediated via nicotinic acetylcholine receptors (Ferchmin et al; J Neurosci. Res. 2013;91:416-425; Ferchmin et al; Neurotoxicology 2014; 44: 80-90) and data suggest that this compound decreases astrocyte reactivity after ischemic-like insults such as oxygen-glucose deprivation (OGD). Building on those prior studies and using light and immuno-electron microscopy of cultured astrocytes, we characterize 4R-neuroprotection occurring via astrocyte-specific signaling, and we quantitatively characterize astrocyte reactivity and morphological changes. Following an ischemic-like insult, we analyze changes in anti-GFAP intermediate filament immunoreactivity using fluorescence and immuno-electron microscopy. Isolated murine cortical primary astrocytes (Lonza, M-ASM- 330) were

grown to confluency and underwent OGD for 3 or 6 hours. Afterwards, astrocytes were treated for 24 hours with 4R or vehicle. Astrocyte monolayers were fixed and processed for correlative light and immuno-electron microscopy to identify and quantify GFAP immunoreactive astrocytes. Analyses suggest anti-GFAP intermediate filament immunoreactivity decreased in OGD astrocyte cultures treated with 4R, in contrast to those treated with vehicle. These results not only increase understanding of nicotinic modulation following ischemia, but also demonstrate how correlative light and immuno-electron microscopy studies may advance quantitative characterization of proteins.

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Poster

430. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Electron Microscopy and Novel Probes

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 430.07/LLL28

Topic: I.03. Anatomical Methods

Title: Regulation of calcium dynamics due to intracellular ultrastructure: Insights from high resolution reconstruction and computational modeling

Authors: *M. HABERL[1,2], J. LAUGHLIN[3], E. P. CAMPBELL[4], M. BELL[3], T. DEERINCK[1,2], P. RANGAMANI[3], M. H. ELLISMAN[1,2], B. L. BLOODGOOD[4]
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Abstract: The endoplasmic reticulum (ER) of neurons extends from its' origin at the nuclear envelope throughout the cell body continuing into the axonal and dendritic arbors, including small branches within dendritic spines. It is well known that the ER is critical for the regulation of intracellular calcium, and that its limiting membrane is enriched in Ca channels (IP3-activated and Ca-activated (aka., ryanodine receptors), which in concert with Ca pumps, binding proteins and plasma membrane channels, modulate the spatiotemporal dynamics of calcium signaling. What remains unknown is how the precise intracellular positioning and 3D fine structure of the ER, in concert with these channels, regulates calcium dynamics and its signaling. We have employed a combination of large-scale automated methods for high resolution reconstruction and segmentation to build accurate models of the entire ER within whole neurons; and are now using simulation strategies to explore how intracellular ultrastructure regulates calcium dynamics in the context of these accurate geometries. Reconstructing the ER is a multiscale problem spanning from the few nm-scale of ER diameter to the mm-scale of neuronal projections. Tracking the ER

into dendrites and axons requires wide-field data acquisition but must retain nanometer scale for accurate 3D EM-based models. We have used serial block-face-scanning electron microscopy (SBEM) to generate high-resolution 3D EM volumes of the rodent cerebellum and applied deep learning-based computational algorithms for automated reconstructions of these intracellular details together with many other intracellular structures (eg., mitochondria and Golgi apparatus). These reconstructions then serve as geometric domains for computational modeling of the spatio-temporal dynamics of calcium to study the influence of ER shape and proximity from the plasma membrane on neuronal Ca signaling. Predictions from simulations are expected to help address how the neuronal fine structure, across large somatic areas and in local domains such as dendritic spines, branch points as well as at axon initial segments and at nodes of Ranvier may contribute to local and long-distance signaling. This work not only forms the basis for next generation simulation studies but also points to very specific localization experiments that remain to be carried out to determine the identities of molecular specializations at numerous sites where the ER is found to make close associations with the neuronal plasma membrane.

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Poster

430. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Electron Microscopy and Novel Probes

Location: SDCC Halls B-H

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Program #/Poster #: 430.08/LLL29

Topic: I.03. Anatomical Methods

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Pathology, Developmental Origins, and Prevention of Pediatric Dysphagia, P01 HD083157

Title: A harmonious image analysis workflow for large format electron microscopy: Web-based collaboration and local processing enabling rigorous GABA-post-embedding immunogold quantification

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Abstract: Electron microscopy (EM) image segmentation and analysis are difficult due to the complexity of shapes and intensities presented in single data sets. Thus, many of the classical image analysis approaches relying on thresholding, spatial filtering and object separation cannot

be reliably employed. The most critical step in structuring an EM data set is the segmentation and sampling of analogous objects of interest. This is routinely done by hand-navigated outlining on a single machine by one individual at time, which imposes time constrains for segmenting larger data sets. Our interests are in the hypoglossal nucleus and the surrounding areas intimately connected with the XII motoneurons (i.e., reticular formation and nucleus of the solitary tract). We employ large ultrathin sections containing the entire brainstem of a mouse, offering a robust map to navigate and focus for sampling at very specific areas of interest. The sections were mounted on silicon and processed for post-embedding immunogold with antibodies against GABA. Secondary antibodies conjugated to 10 nm gold particles were used for immuno-detection. We utilized Helios NanoLab 660 SEM (Thermo Fisher, FEI) with concentric backscattering detector in immersion mode to detect elastically backscattered electrons by landing low voltage (2kV) electrons. This approach allowed for the generation of high-quality, large area data sets by fusing large number of adjacent fields (MAPS, FEI). Individual image HFV measured 6.38µm (3072x2048px). These sets contain the structural information of similar quality as transmission electron microscope data, but allowed us to sample from a much larger sample in a cyto-architecturally justified way. Gold particles encoding GABA are readily distinguishable from the uranium stain. To increase the number of sampled GABA positive and negative synapses, a data set representing large area of hypoglossal nucleus was uploaded to arivis WebView. Synaptic terminal segmentation was crowd sourced using this platform, representing a flexible and modular commercial solution with collaborative Web tools, allowing multiple investigators to segment synaptic terminals at the same time. We built a workflow to convert image data into a pyramidal redundancy-free format and transfer it to a Web server, perform collaborative segmentations, and used our Web-derived results directly in optimized particle-counting and distribution analysis. Data transfer, processing, results analysis are all completely self-contained on the platform. Using this approach, we quantified GABA differences in concentration and localization in presynaptic terminals.

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Poster

430. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Electron Microscopy and Novel Probes

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Program #/Poster #: 430.09/LLL30

Topic: I.03. Anatomical Methods

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Title: Effect of melatonin and 1-n-substituted analogue substance (M3C) in rat testes. Immunohistochemical study

Authors: D. MARTINEZ-MEENDEZ[1], B. HERNÁNDEZ-TÉLLEZ[2], A. CASTELL-RODRÍGUEZ[2], *E.-B. NARANJO-RODRIGUEZ[4], A. LIRA-ROCHA[3]
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Abstract: Melatonin (MT) (N-acetyl-5-methoxytryptamine) regulates the biological, neuroimmunological, antioxidant, gastroprotective, anti-inflammatory, anxiolytic-antidepressant, antiproliferative and reproduction rhythms. Currently, there is a therapeutic use with administration of exogenous MT as a sleep inducer, without knowing the toxicity and side effects that may occur. Debeliuk et al., showed that 300 μg of MT (30 days), decreased the weight of the testicles and affected the production of testosterone in rats. Redins et al., reported that 100 μg of MT (22 days), injured the ultrastructure of Leydig cells in mice and Tuncer et al., found a tubular degeneration, edema and obstruction of seminiferous tubules with the administration of 3 mg / kg of MT (30 days) in rats. With the intraperitoneal administration of 300 μg of both MT and M3C substances (30 days), macroscopic and immunohistochemical studies were carried out to determine if the testicular cell structure of the rat seminiferous tubule (TS) presents any change with chronic administration of them. Post-treatment, were anesthetized the rats and with intracardiac perfusion of SSI (0.9%) and buffered formaldehyde, the testes were extracted for histopathological study (haematoxylin-eosin) and immunolocalization of MT with the anti-melatonin specific antibody. The MT and the M3C were located in ERA.

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Poster

430. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Electron Microscopy and Novel Probes

Location: SDCC Halls B-H

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Program #/Poster #: 430.10/LLL31

Topic: I.03. Anatomical Methods

Support: NSF Grant 1746511
Florida High Tech Corridor Grant

Title: Golgi stereology: A novel and efficient method for quantification of neurons, dendritic fibers and spines on Golgi-stained sections using unbiased stereology

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Abstract: For more than a century, Golgi staining has been an effective histology method for understanding the microscopic morphology of individual brain cells, dendritic spines, and patterns of fiber branching and interconnectivity. Previous attempts to quantify these microstructures using camera lucida-type drawings have relied on semi-quantitative approaches using public-domain (free) software such as Fiji (Fiji Is Just Image J) or costly neuron tracing programs from commercial vendors. Well-known problems include projection bias, e.g., fiber measurements from projected images (2-D Sholl analysis); and low precision and high labor and opportunity costs from excessive time and effort spent tracing a negligible fraction of neurons in each region of interest (reference space). Here we propose the use of unbiased methods to quantify global stereology parameters for neurons through entire reference spaces of standard Golgi-Cox stained sections. The required equipment is a computerized stereology system with an ordinary light microscope equipped with a motorized XYZ stage and a high-resolution digital camera interfaced to a PC and monitor. Neocortex and CA1 molecular layers from eight mice were outlined at low power (4x) on a systematic-random sample of 6-10 sections per reference space to estimate total volume (Cavalieri point counting). Second, thin focal-plane Z-axis scanning at high magnification (60x, n.a 1.4) was done at a minimum of 200 systematic-random locations to estimate total length and length density of fibers (space balls probe) and total numbers, volume densities of neurons and dendritic spines (optical disector probe). Among the findings are a mean total length of 25.3E+3 mm and length density of 3562 mm/mm3 for Golgi-stained fibers in neocortex. About 2 hours or less was required to achieve a high level of sampling stringency as evidenced by a low mean coefficient of error (CE) of about 0.10. Benefits of Golgi Stereology include high accuracy and precision due to unbiased probe sampling through entire reference spaces; and more than 10x efficiency compared to the fastest tracing methods. Though the present studies were done using the Stereologer system (SRC Biosciences, Tampa, FL), the same approach is theoretically possible using any comparable-equipped stereology system. Golgi Stereology may provide the most user-friendly and versatile method to date for quantifying the structural basis of neurological diseases. Also, the approach could be useful for preclinical safety and efficacy testing of drug strategies for the therapeutic management of afflicted patients.

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Poster

430. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Electron Microscopy and Novel Probes

Location: SDCC Halls B-H

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Program #/Poster #: 430.11/LLL32

Topic: I.03. Anatomical Methods

Support: Hungarian Academy of Sciences Momentum Program LP-54/2013
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Title: PharmacoSTORM: A powerful approach for antibody- or fluorescent protein-free super-resolution imaging

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Abstract: A major challenge for contemporary neuroscience is to uncover the nanoscale molecular changes underlying neuropsychiatric diseases. Super-resolution imaging techniques, such as Stochastic Optical Reconstruction Microscopy (STORM) or Photo-Activated Localization Microscopy (PALM) are the methods of choice for visualization of nanoscale protein distribution, but these approaches suffer from the lack of selective antibodies against important molecular targets or from the various pitfalls associated with overexpression of tagged proteins, respectively. Here we introduce PharmacoSTORM, which uses specific ligands labeled with proper fluorescent dyes to measure the nanoscale distribution of signaling molecules. We first overexpressed hemagglutinin (HA)-tagged α7-nicotinic acetylcholine receptor in HEK293 cells and simultaneously performed immuno- and ligand stainings using antibodies and Alexa647-labeled α-bungarotoxin, respectively. Notably, the signals acquired by different labeling procedures showed high degree of correlation. In agreement with the basic pharmacological principles, the signal intensities originating from fluorescent ligand binding were saturable. The superior sensitivity of super-resolution imaging was reflected by the fact that low concentration of Alexa647-α-bungarotoxin was not visible with confocal microscopy, but was detectable with STORM imaging with unprecedented localization precision. Since ligand-based staining lacks amplification steps, this approach can more precisely determine molecular quantity. PharmacoSTORM could also be applied to tissue preparations, and visualized the nanoscale density of nicotinic receptors at neuromuscular synapses. Finally, to demonstrate the general applicability of the PharmacoSTORM approach, we have also developed novel compounds, which enabled the nanoscale visualization of the CB_1 cannabinoid and the D_3

dopamine receptors, two G protein-coupled receptors widely implicated in many neuropsychiatric disorders. PharmacoSTORM by these compounds allowed the qualitative anatomical and the quantitative pharmacological profiling of nanoscale receptor distribution. In summary, we propose that PharmacoSTORM is an efficient new tool for super-resolution imaging of difficult and low-copy number molecular targets.

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Poster

430. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Electron Microscopy and Novel Probes

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 430.12/LLL33

Topic: I.03. Anatomical Methods

Title: Enhancing structural contrast in t1 mri protocols using multimodal targeted tissue probes

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Abstract: The imaging of soft tissue is important in both the research and clinical diagnosis of damage and disease. Magnetic resonance imaging (MRI) utilizes the electromagnetic energy released from altering the proton spins of hydrogen under changing magnetic fields to image these soft tissues. Contrast agents are often necessary to improve the resolving capability of MRI to detect subtle changes in tissues that occur. However, most of the common contrast agents provide only non-specific contrast enhancement and are generally toxic in low concentrations. New nanoparticles have recently been developed, one gadolinium based (GdNP) and one gold based (AuNP) that can function as contrast agents with the ability to target specific tissues. The GdNP has been previously shown to provide ten times the signal as typical contrast agents at equal concentrations. This allows for a much lower concentration of contrast agent to be needed to create similar contrast enhancement. These nanoparticles are capable of targeted contrast enhancement by conjugating them to dyes or antibodies that can bind to specific tissues. This also serves to further reduce the necessary concentration needed to visualize changes to specific tissues under MRI. Both nanoparticles have been conjugated to bind to serum albumin (SA) in the neurovasculature of the brain. This conjugation provides a multimodal approach to imaging by binding the nanoparticles to these fluorescent dyes allowing for contrast enhancement under in vivo MRI and fluorescent staining under microscopy. Utilizing a known model for Blood Brain barrier permeability, cuprizone, we can image the vasculature under healthy conditions as well as look for signs of BBB hyper-permeability using these nanoparticles. These probes allow

for enhanced detail of soft tissue alterations in disease states or damage for both diagnosis and research.

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Poster

430. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Electron Microscopy and Novel Probes

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Program #/Poster #: 430.13/LLL34

Topic: I.03. Anatomical Methods

Support: (Lead Agency DACH 2OOO21E-166809

Title: In silico electromagnetic and electrophysiological modelling of neuronal current imaging using ultra-low field nuclear magnetic resonance

Authors: E. NEUFELD[1], *A. M. CASSARA[1], J. H. STORM[2], N. HOEFNER[2], R. KOERBER[2], N. KUSTER[1]
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Abstract: The feasibility of non-invasive imaging of neuronal currents (NCI) in the brain by means of Nuclear Magnetic Resonance (NMR) techniques has been debated for more than a decade. NCI cannot be easily applied at high Larmor fields (>1T) due to (i) the relative weakness of the magnetic fields generated by neurons in the brain (NMFs), and (ii) the concomitant and dominant NMR signal contribution by the metabolic blood oxygen level dependent (BOLD) effect. The implementation of NCI techniques using low field (LF) NMR (<50uT, corresponding to Larmor frequencies <5kHz), has recently been explored in experimental and computational studies using simplified phantoms mimicking the brain to assess the theoretical sensitivity of NCI, with promising results. Current research combines experimental and computational approaches to allow realistic *in-silico* NCI simulations in order to (i) optimize LF-NMR hardware, (ii) optimize NCI sequences, (iii) estimate sensitivities of the techniques, and (iv) design experimental strategies in preparation of *in vivo* human trials. The identification, separation and quantification of the different deteriorating (e.g. field inhomogeneities, partial volume effects, etc.) or interesting (weak, transient and distributed NMFs) physical contributions to the NMR signals is the primary purpose of *in silico* modelling. The approach required the development of a flexible NMR solver within the Sim4Life platform (ZurichMedTech,Zurich,CH) for life sciences investigations to be used in combination with i) validated electromagnetic models of magnetic resonance imaging (MRI) hardware and of

experimental phantoms; ii) validated and iii) realistic computational models of extended neuro-electric sources (e.g. computed from electrophysiological models of neural microcircuits) positioned within high-resolution anatomical human head models to provide realistic estimations of *in vivo* NMFs. Experimental work included i) inverse source reconstruction (e.g. equivalent current dipole position and orientation) of magnetoencephalographic (MEG) signals of long-lasting human neuronal activities subsequent to median nerve stimulation to calibrate experimental and numerical head phantoms and sources; ii) the construction of test phantoms; iii) NMR-modeling validation experiments; and iv) LF-MRI experiments with novel NCI sequences. The computational modeling is capable of reproducing different physical and physiological factors involved in the generation/alteration of the NCI signal. Uncertainty assessment is currently being performed to quantify the reliability of the modeling predictions against measurements.

Disclosures: E. Neufeld: None. **A.M. Cassara:** None. **J.H. Storm:** None. **N. Hoefner:** None. **R. Koerber:** None. **N. Kuster:** None.

Poster

430. Anatomical Methods: Staining, Tracing, and Imaging Techniques: Electron Microscopy and Novel Probes

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NIH S10RR019356
US Army DBK39-03-C-0075

Title: Targeted noninvasive delivery of novel clathrin-based superparamagnetic iron oxide nanoparticles for magnetic resonance imaging of dopamine transporters in mouse brain

Authors: *J. K. KIM[1], D. MINTZOPOULOS[1], C. W. ADAM[1], S. E. LUKAS[1], M. J. KAUFMAN[1], F. VITALIANO[2], G. VITALIANO[1]
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Abstract: Objective: Magnetic Resonance Imaging (MRI) offers high spatial resolution but has poor sensitivity for visualization of molecular targets. Superparamagnetic iron oxide (SPIO) contrast agents along with antibodies are used to improve MRI sensitivity and molecular targeting, but they cannot cross an intact blood-brain-barrier (BBB) limiting their use. Our goal was to enable MRI molecular imaging using novel clathrin-based nanoprobes carrying SPIO and

antibodies, which noninvasively pass an intact BBB, to target dopamine transporters (DATs). **Methods**: Clathrin triskelia (CT)-nanoprobes were synthesized by conjugating anti-DAT antibody and SPIO to CT using polyethylene glycols (PEGs) at 1:1:1 molar ratio. Adult male mice or rats were given saline or CT-nanoprobes intranasally (68 pmol, 50 µL). Four hours later, their brains were collected for iron staining or *ex-vivo* MRI. Voxel-wise R_2* relaxation rates were obtained using a series of gradient-echo images (TR=1.5 s, TE=3.2, 4, 5, 6, 7, 8, 9, 10 ms; 128x128 in -plane matrix; 0.2 mm resolution; 64 slices at 0.5 mm thickness; 7 averages). R_2* values in the striatum (STR), substantia nigra (SN) and visual cortex (vCTX), a control region with low DAT expression, were calculated. **Results**: The iron stained brain slices showed an accumulation of CT-nanoprobes in brain regions rich in DAT (e.g., STR). MRI studies revealed that R_2* values were significantly higher in the STR ($p=0.0010$) and SN ($p=0.0007$) compared to vCTX in animals that received CT-nanoprobes, but not in saline treated animals. CT-nanoprobes significantly increased R_2* in the STR ($p<0.0001$) and SN ($p=0.0002$) compared to saline without significantly altering R_2* in the vCTX. **Conclusions:** CT-nanoprobes noninvasively delivered SPIO contrast agents along with anti-DAT antibody to the mouse brain, enabling detection of DAT using MRI. These preliminary results merit further investigation into the use of clathrin as a new theranostic for noninvasive molecular brain imaging and targeted drug delivery.

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Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

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Topic: I.04. Physiological Methods

Support: Startup Funds from West Virginia University

Title: Transparent nanoporous gan for optoelectronic neural interface to the brain networks

Authors: *J. LEE[1,2]
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Abstract: Over the past decade, advances in optogenetic techniques have opened new possibilities for studying a wide range of neuroscientific problems and brain functions. While recently, a number of devices have appeared for advancing optogenetic methods, researchers are

still unable to simultaneously apply spatio-temporally controlled multispectral optical stimulation with electrophysiology. The major challenge is finding a material with the appropriate electrical conductivity and optical transparency (or active light source).Here, we report the optical and electrical characteristics of a nanoporous GaN layer as a new class of optoelectronic neural probe. A fully transparent and electrically conductive n-GaN layer was epitaxially grown by MOCVD and various nanoporous structures were prepared by an electrochemical etching process in acid (e.g., oxalic or nitric acid). While the optical refractive index is precisely controlled by changing the pore size and ratio with doping concentration and applied voltage, the electrical property remains conductive. Compared to the unmodified n-GaN process, NP-GaN shows improved electrical properties in electrical impedance spectroscopy and cyclic voltammetry measurements. We demonstrated its multiple optical and electrical functionality for compact multisite optical/electrical stimulation and simultaneous electrical recording of neural activity in in-vivo mouse model.

Disclosures: J. Lee: None.

Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

Location: SDCC Halls B-H

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Program #/Poster #: 431.02/DP15/LLL37

Topic: I.04. Physiological Methods

Support: HFSP-CDF fellowship
NIH NINDS RO1NS095959
RettSyndrome.org
RSRT (https://reverserett.org)
www.simonsfoundation.org

Title: Transparent microelectrode arrays for simultaneous high-density electrophysiology, electrical stimulation and two-photon imaging

Authors: *P. ARTONI[1], Y. QIANG[2], K. SEO[2], S. CULACLII[3], V. HOGAN[1], W. LIU[3], H. FANG[2], M. FAGIOLINI[1]
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Abstract: Electrophysiology and Calcium (Ca^{++}) imaging are two complementary physiology techniques heavily adopted *in vivo* to study brain activity. While the first has great temporal precision, the latter has excellent spatial and cellular resolution. Significant attention has been given to the study of LFP/EEG oscillations in the time domain as different frequency bands of

the LFP/EEG reflect the interaction between different neuronal populations. Two-photon imaging, on the other hand, allows the study of Ca^{++} transients of large networks with cell resolution capabilities. Combining these two powerful techniques would represent a significant advance in our ability to dissect how network activity is generated and propagates across brain region in health and disorders. To achieve such goal transparent microelectrodes arrays (transparent MEAs) would be ideal. However, scaling down the size of the electrodes in a microelectrode array to allow dense spatial recordings is very challenging when the electrodes and the interconnections have also to be transparent for imaging. Here we developed a nanomesh composite structure with bilayers of Au/PEDOT:PSS to allow high transparency for imaging while with low impedance for high-performance LFP/single-unit recordings, by leveraging the existing from multi-decade-long development and in *vivo* application of commercial MEAs. We were able to generate microelectrodes with 130 kΩ at 1 kHz for 20-μm-diameter microelectrodes, which are comparable to the performance of microelectrodes in non-transparent, Michigan-style arrays. This approach also allowed high transparency of the film and high conductance on the wiring itself between each electrode and the recording system. The electrodes were tested for stimulation and for online artifacts rejection, together with a state-of-the-art wireless recording. The newly generated transparent MEA were implanted in adult mice and LFP, multi-unit, and single-unit recordings were acquired simultaneously with epifluorescence or 2-photon imaging in the awake animals. We measured visual evoked responses using a 32-channel flexible and transparent device, along with the concurrent 2-photon imaging of single neurons in the layer 2-3 in the mouse visual cortex. We then evaluated the biocompatibility of the MEAs by ex vivo staining of both the implanted and control cortex for the marker for microglia activation IBA. We did not detect any significant inflammation up to 3 weeks after the surgery. Together our results indicate that these electrodes are scalable to high density, are reliable and can be coupled with the Ca^{++} imaging across different neuronal populations.

Disclosures: P. Artoni: None. **Y. Qiang:** None. **K. Seo:** None. **S. Culaclii:** None. **V. Hogan:** None. **W. Liu:** None. **H. Fang:** None. **M. Fagiolini:** None.

Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

Location: SDCC Halls B-H

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Topic: I.04. Physiological Methods

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National Science Foundation (Grant ECCS-1542148)

Title: Ultra-low impedance graphene microelectrodes with high optical transparency for simultaneous deep two-photon imaging in transgenic mice

Authors: *Y. LU[1], X. LU[2], R. HATTORI[2], C. REN[3], T. KOMIYAMA[4], D. KUZUM[5]
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Abstract: The last decades have witnessed substantial progress in optical technologies revolutionizing our ability to record and manipulate neural activity in genetically modified animal models. Meanwhile, human studies mostly rely on electrophysiological recordings of cortical potentials, which cannot be inferred from optical recordings, leading to a gap between our understanding of dynamics of microscale populations and brain-scale neural activity. By enabling concurrent integration of electrical and optical modalities, transparent graphene microelectrodes can close this gap. However, the high impedance of graphene constitutes a big challenge towards the widespread use of this technology. Here, we experimentally demonstrate that this high impedance of graphene microelectrodes is fundamentally limited by quantum capacitance. We overcome this quantum capacitance limit by creating a parallel conduction path using platinum nanoparticles. We achieve a 100 times reduction in graphene electrode impedance, while maintaining the high optical transparency crucial for deep 2-photon microscopy. Using a transgenic mouse model, we demonstrate simultaneous electrical recording of cortical activity with high fidelity while imaging calcium signals at various cortical depths right beneath the transparent microelectrodes. Multimodal analysis of Ca^{2+} spikes and cortical surface potentials offers unique opportunities to bridge our understanding of cellular dynamics and brain-scale neural activity.

Disclosures: Y. Lu: None. **X. Lu:** None. **R. Hattori:** None. **C. Ren:** None. **T. Komiyama:** None. **D. Kuzum:** None.

Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

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Program #/Poster #: 431.04/LLL39

Topic: I.04. Physiological Methods

Support: Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan

The Commissioned Research of National Institute of Information and Communications Technology (NICT)
JSPS KAKENHI Grant Number 15H03049

Title: Different strategies for designing ECoG electrode arrays for functional mapping and brain decoding

Authors: *T. KAIJU[1], M. HIRATA[2], T. SUZUKI[1]
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Abstract: Recently, electrocorticography (ECoG) is being used to map cortical functions in clinical situations as well as for decoding brain signals to build brain-machine interface (BMI) systems. An ECoG electrode array contains multiple channels on its surface; however, consensus regarding their ideal designs, such as the optimal diameter and spacing of these channels, has not been reached yet. To use ECoG for functional mapping, it is important to delineate potential distribution over the electrode surface; however, to use ECoG for decoding brain signals, it is more important to accurately estimate variables of interest (e.g. specific joint angle). These two purposes are qualitatively different; thus, the optimal design of an ECoG array might not be the same for both. In this study, we investigate optimal electrode design for both purposes. Somatosensory evoked potentials (SEP) elicited by electrical stimulation of different body parts were simulated in an area of 10 × 10 mm^2 by an evoked potential model and additive noise. We compared decoding accuracy of stimulated body parts under the assumption that the SEP signal was recorded by the electrodes which had different sizes of channels (500 × 500 μm^2 to 10 × 10 mm^2). We also recorded SEPs from monkeys, elicited by electrical and vibrational stimulation of the fingers by using a high-density ECoG array (96 channels, channel size: 350 μm × 350 μm; inter-electrode distance: 700 μm). The simulation results show that if the number of channels and the surface area of an array are fixed, smaller channel sizes will allow for more accurate decoding of brain signals. On the other hand, this improvement in accuracy depends on the amplitudes of the simulated evoked responses. It was also found that the recorded amplitude of the evoked responses become larger when using electrodes with smaller channel sizes. The actual data derived from monkeys support these results.The results suggest that a smaller channel size may result in an increased signal-to-noise ratio of cortical responses and an improvement in prediction accuracy. However, in an electrode array with small channels, the channels are normally placed further apart, and this makes it difficult to delineate global responses over the array. We conclude that in our research setting, a relatively large channel size (e.g. 2 × 2 mm^2, if there are 25 channels in 10 × 10 mm^2) is optimal for mapping cortical topography, but a smaller channel size (e.g. 500 × 500 μm^2) is optimal for brain-signal decoding if an electrode is optimally placed.

Disclosures: T. Kaiju: None. **M. Hirata:** None. **T. Suzuki:** None.

Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

Location: SDCC Halls B-H

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Program #/Poster #: 431.05/LLL40

Topic: I.04. Physiological Methods

Support: KAKENHI Grant Number JP15J02011)

Title: Organic microelectrodes as chronic brain interface

Authors: *Y. GUO[1,2,4], S. JIANG[5], B. GRENA[6], I. KIMBROUGH[7], E. G. THOMPSON[8], Y. FINK[6], H. SONTHEIMER[9], T. YOSHINOBU[3], X. JIA[5]
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Abstract: Microelectrodes provide a direct access to study brain activities electrically from the external world, which has advanced our fundamental understanding of brain functions. However, they, typically based on micro-wires or silicon, inevitably trigger tissue response and fail to perform during the long-term implantation, due to huge mechanical mismatch with delicate brain tissues and biocompatibility issues. Therefore there is a pressing need to transform the materials from traditional inorganic materials toward biocompatible and functional organic ones. In the previous studies, we have pioneered to leverage thermal drawing process to fabricate flexible and biocompatible polymer fibers, which can be utilized as neural interfaces. These fibers are entirely based on polymers, integrated with multi-functionalities and have demonstrated stable chronic performance over the extended period of implantation. However, due to the low electrical conductivity of polymer materials we chose as electrode, i.e., carbon loaded polyethylene (CPE), the recording site had to be sufficiently large to maintain the moderate impedance for capturing single-neuron activities. Therefore, there has been ongoing push for developing new polymer composite materials with improved electrical performances. In the meantime, they should be much reduced in size, more flexible and also reasonably robust. Here we report the development of a new composite electrode with carbon nano fibers(CNF) doping into CPE. We demonstrated that in situ CNF unidirectional alignment can be achieved during the thermal drawing, which contributes to a drastic improvement of electrical conductivity by 2 orders of magnitude compared to a conventional polymer electrode, while still maintaining the mechanical compliance with brain tissues. The resulting fiber has a miniature

footprint, including a recording site with a reduced size comparable to a single neuron and maintained impedance that was able to capture neural activities. Its stable functionality as a chronic implant has been demonstrated with the long-term reliable electrophysiological recording with single-spike resolution and the minimal tissue response over the extended period of implantation in wild-type mice. We can further integrate these new organic microelectrodes with optical waveguides and microfluidic channels within a single fiber to realize multimodal study of brain activities. In addition, this organic electrode could also be further developed for biomolecule sensing. Technology developed here can be applied to basic brain studies as well as clinical implementation for neuro-rehabilitative applications.

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Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

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Topic: I.04. Physiological Methods

Support: NIH NINDS NS054894
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Title: More precise and complex braided microelectrodes for neural interfaces

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Abstract: Since SFN 2008, we have tested the concept of highly flexible braided microelectrodes which comprise interwoven ultra-fine microwires (9.6μm diameter wire) in a tubular braid. These form a microelectrode array for electrophysiological applications such as neural interfaces. Due to highly flexible mechanical properties and the open diamond shaped windows on the braid wall, braided microelectrodes allowed us to record neural signals from the spinal cord in freely moving animals and showed less chronic immune responses and more neural survival in the vicinity of implant. To build more precise and complex micro-braid structures, we have now developed an advanced micro-braiding machine which is a second generation version based on our awarded patent for a micro-braiding apparatus. We rebuilt the 2nd machine in order to produce more precise tubular braids, in a robust and fully automated manufacture and provide more advanced complex braiding options. We redesigned mechanical components for safer wire handling, and smoother, but faster processing and developed our own electronic devices and a PC control program to control the 28 stepper motors used in braid plate actuation independently and automatically. This new machine can handle 12 single or bundle

groups of strands (Vs. 6 single strands by the 1st prototype machine) independently so that it can produce both complex tubular microbraid structures with 12 single strands, and also complex varying tubular braid structures. By controlling braiding directions the machine supports combinatorics applications, which can increase yields of neural recording via combinations of multiple recording sites on a single wire and precise juxtapositions of multiple recording sites on multiple wires. The automation of Z axis with 0.4µm resolution for the max 400mm travel stroke along the braid axis allows a realized braid pitch that is specific and consistent along the length of a braid and the maximum length of braid structures can now be up to 40cm for larger animal applications. With the 2nd micro-braiding machine, we not only manufacture more precise and complex braided microelectrodes in easier, more reliable and faster ways, but also potentially extend the use of braided microelectrodes to various different electrophysiological applications including combinatorics, peripheral nerve interface as intrafascicular electrodes, deep brain research in a larger animal, spinal implants, etc. We plan for the braided microelectrodes distribution for test applications in cooperation with other neuroscientists and neural engineers in various fields, and with potential for future clinical application.

Disclosures: T. Kim: None. **S.F. Giszter:** None.

Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

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Topic: I.04. Physiological Methods

Support: NIH Grant R01-MH109289
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NIH Grant U01-NS098976

Title: Circuit based dissection of neuronal oscillations

Authors: *P. LAKATOS[1,3], S. A. NEYMOTIN[4], M. N. O'CONNELL[5], A. BARCZAK[6], T. M. MCGINNIS[2], S. BICKEL[7]
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Abstract: Despite decades of research into the function of neuronal oscillations, we still lack an understanding of their mechanistic functions. One possible explanation is that most studies aimed at uncovering the function of a given oscillation still use arbitrarily defined and sometimes

overlapping frequency bands. To remedy this, we set out to revise the taxonomy of brain oscillations based on their thalamocortical circuit mechanisms. We examined rhythmic neuronal activity patterns in multi-scale neuroelectric activity of several cortical regions (auditory and motor), thalamic structures (relay nuclei, thalamic reticular nucleus and pulvinar), and the dorsal striatum of non-human primates. We analyzed frequency specific effects on cell type specific neuronal activity as well as functional and effective connectivity in the dataset without any prior assumptions to classify neuronal oscillations solely based on their spatiotemporal fingerprints, and to infer the underlying circuitry generating each distinct oscillatory class. We then incorporated a computational modeling approach to refine the rudimentary circuits defined by electrophysiological recordings. The analyses of concurrent electrocorticographic (ECoG) or electroencephalographic (EEG) recordings of neuronal activity enabled us to identify macroscale markers of the distinct thalamocortical oscillatory patterns and compare these to motifs of human ECoG and EEG recordings.

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Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

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Topic: I.04. Physiological Methods

Support: NIH U01 NS094375
NIH R01 MH101697
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NIH R01 NS078435

Title: Cellular-scale probes for high-density, precisely-localized neurophysiology

Authors: D. EGERT[1], J. R. PETTIBONE[1], I. BATMUTSKY[2], C. M. CALDWELL[3], D. H. ROOSSIEN, JR[4], P. R. PATEL[5], S. LEMKE[2], K. GANGULY[2], D. CAI[6], C. A. CHESTEK[5], *J. D. BERKE[2]
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Abstract: Understanding the information processing achieved by neural microcircuits will require recordings from large numbers of interconnected neurons. Although neural devices with large numbers of electrodes have become an important neuroscience tool, they are typically not suitable for this goal. To achieve the stiffness needed to penetrate into the brain, each element

typically has a width of at least 25-100µm (with cross-section in the thousands of μm^2). This large foreign body causes substantial direct mechanical damage and is also detected and rejected by the brain's immune system. The immune reaction leads to loss of neurons in the vicinity of the electrodes, one factor that frequently curtails the duration of chronic neural recordings. Furthermore the spacing between elements must be large to avoid total destruction of the tissue (for example, in a standard Blackrock array contacts are separated by 400µm). Finally, large devices must generally be removed from the brain before processing the tissue for histological analysis, impeding the ability to visualize recording sites within intact circuits. Here we report next-generation silicon-based neural probes at cellular-scale (5x10µm cross-section), with ultra-high-density packing (as little as 66µm between shanks) and 64 or 256 closely-spaced recording sites per probe, distributed across 32 shanks. The relatively short (500µm) distal portion with cellular-scale cross-section is coupled to a more robust upper portion (far from the recording sites). The fabrication process is based on the Michigan probes process with extensions to integrate stiffeners and e-beam lithography, allowing for high density interconnects. We show that these probes can achieve stable, chronic, *in vivo* single-unit recordings from superficial or deep brain structures in freely-moving rats. Gliosis and neuronal loss were greatly reduced compared to prior silicon probes. In a preliminary recording from primary motor cortex, 149 units were simultaneously detected and large single-units were still observed after 143 days of implantation. For each unit, an average of 37 other units were recorded within a 300µm radius, close enough to be directly interacting partners. Furthermore, we demonstrate a slice-in-place approach for the precise registration of the recording sites relative to adjacent neurons and to anatomical features. This scalable technology provides a valuable tool for the examination of information processing within neural circuits, and potentially for human brain-machine interfaces.

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Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

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Program #/Poster #: 431.09/LLL44

Topic: I.04. Physiological Methods

Support: The University of Texas at Dallas

Title: Design and development of ultrathin microelectrode arrays

Authors: *J. O. USORO, F. DEKU, J. J. PANCRAZIO, S. F. COGAN
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Abstract: Current state-of-the-art intracortical electrode arrays can record useful signals for cortical mapping as well as for control of brain-machine interfaces (BMI). However, reported mechanical failure modes suggest that these devices lack the mechanical/electrical stability needed for chronic applications. Amorphous silicon carbide (a-SiC) intracortical microelectrode arrays (MEA) have shown promise in being used as chronically implantable devices for recording extracellular neural activity. A-SiC is well tolerated in the cortex, resistant to corrosion, and exhibits flexibility as a thin film, which may help mitigate insertion and micromotion-induced foreign body response. Here, we report the design and fabrication of ultrathin microelectrode arrays tailored for long-term neurobehavioral studies in rats. The a-SiC MEAs were fabricated using standard thin-film fabrication techniques. Films of a-SiC were deposited by plasma enhanced chemical vapor deposition and metal traces were formed through sputter deposition and patterned through lift-off photolithography. Electrodes are 200 μm^2 and coated with a sputtered iridium oxide film to reduce impedance. Electrical connection to the MEAs was achieved by mounting an Omnetics connector directly on the array. The shanks are 2 mm in length with 8 electrodes per shank. Shank pitch is 200 µm in an effort to enable recordings from individual cortical columns. The shanks are 6 µm thick and 53 µm wide so that at least one dimension falls within the ultra-thin range for probes, which have been shown to reduce glial scar formation. Initial experiments to test the utility of this novel technology in a behaving rat model are underway. MEAs will be assessed by recording single unit activity (SUA) and performing electrochemical impedance spectroscopy over 16 weeks from 4 devices implanted in the motor cortex of rats which have been previously trained to perform a knob supination task. From previous studies, we expect to resolve SUA on the order of 50-200 µV with an array yield of active channels similar to, or greater than the 60% seen in our acute experiments. A decoding algorithm correlating the neural activity and supination task will consist of linear filters and is expected to perform with accuracy similar to that seen in literature. Preliminary data from the pilot study will be presented at the conference. The results from this pilot study will demonstrate the feasibility of a-SiC MEAs for chronic recording and decoding applications in a behaving animal model. Moreover, they will inform future studies of neural decoding using a-Sic MEAs in terms of device geometry, performance, and single unit stability.

Disclosures: J.O. Usoro: None. **F. Deku:** None. **J.J. Pancrazio:** None. **S.F. Cogan:** None.

Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

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Program #/Poster #: 431.10/LLL45

Topic: I.04. Physiological Methods

Support: NSF INSPIRE (CBET-134193)

Title: Long-term evaluation of a parylene-based multi-electrode array for recordings from the hippocampus of behaving rats

Authors: *H. XU, W. JIANG, A. HIRSCHBERG, K. SCHOLTEN, E. MENG, D. SONG
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Abstract: Obtaining single-unit activities from multiple neurons in neural networks from behaving animals is crucial for the understanding of cognitive functions of brain circuits and is also the foundation to develop effective cortical prosthetic devices. However, severe immune response caused by micromotion between stiff implants, such as microwire electrode array, and surrounding brain tissue often limits the signal quality and lifetime of penetrating devices. To reduce the stiffness mismatch between recording devices and brain tissue, we developed a flexible, polymer based multi-electrode array for recording single neuron activities from multiple sub-regions of the rat hippocampus, a major subcortical structure that closely associates to the formation of new long-term memory. Parylene C, a biocompatible polymer, was used as the structural and insulation material of the multi-electrode array. 64 Platinum (Pt) recording electrodes were placed in groups along eight individual shanks to conform to the distribution of hippocampal neurons. By supporting the top half of the probe array with dissolvable polyethylene glycol (PEG), the effective length of the Parylene array was temporarily reduced and bucking threshold of the array was increased and enabled the straight insertion of the flexible array into the desired hippocampal region without introducing extra shuttle devices into the brain tissue. Over forty units were recorded with Parylene arrays from acute implantations. Signal to noise ratio (SNR) of spike activities recorded with the Parylene array was comparable to that of the commonly used microwire electrode array. Next step, we will evaluate the performance of the Parylene array over long term. The Parylene array will be chronically implanted and neural recordings will be collected from free moving rats. Signal qualities, including units yield, SNR and signal stability over time will be examined and compared with recordings collected with microwire electrode arrays. Long-term tissue respond to the implants will also be tested through immunohistochemistry stains.

Disclosures: H. Xu: None. **W. Jiang:** None. **A. Hirschberg:** None. **K. Scholten:** None. **E. Meng:** None. **D. Song:** None.

Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

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Program #/Poster #: 431.11/LLL46

Topic: I.04. Physiological Methods

Support: NIH Grant U01NS099687

Title: Recordings of extracellular action potentials from freely moving rats by perforated polyimide multielectrode platforms

Authors: *S.-H. HUANG[1,2], M. M. JANKOWSKI[1,3], H. EREZ[1,4], N. SHMOEL[1,2], I. NELKEN[1,3], M. E. SPIRA[1,4,2]
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Abstract: Long-term recordings of extracellular spikes by chronically implanted electrodes in vertebrate brain remain an unsolved challenge. To extend the recording time and improve the quality of the recorded signals we prepared scalable single shank polyimide (PI) platforms (280 µm wide and 16 µm thick). The platform is subdivided into a distal perforated part and a solid proximal part. The perforated part (opening area = 45 - 65 µm by 8 µm) of the PI platform tapers to form a sharp tip. The PI platforms were composed of 2 or 16 planar electrodes (Ø = 25 µm) located along the edges of the platforms tip. Electrodes were implanted into the rat primary somatosensory cortex (S1) or to frontal associative area (FrA). Immunohistological cryosections of the PI platform together with the surrounding tissue revealed that 2 weeks after implantation, microglia, astrocytes and neurites extended into the perforated platform, leading to apparent local "anchoring" of the brain tissue to the platform. To test the durability and recording quality of the system we conducted weekly telemetric recordings from freely moving rats. The longest recording that we have obtained lasted 8 months (the recordings were terminated because of skin infection). Recordings of the electrophysiological signals for weeks revealed large extracellular action potentials. The recorded amplitudes varied over time without a dominant trend of amplitude decline. This suggests that either the current source was not stable or that the impedance between the current source and the sensing pads was not constant. The immunohistological cryosections of the PI platform together with the surrounding tissue revealed minimal foreign body response. These observations are consistent with the view that the use of perforated PI platform functionalized by polyethylenimine and laminin facilitates mechanical integration of the platform with cortical tissue and thereby supports long-term stable recordings from freely moving rats.

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Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

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Program #/Poster #: 431.12/LLL47

Topic: I.04. Physiological Methods

Support: BrainLinks-BrainTools, DFG ExC1086

Title: How flexibility and probe size influence chronic reliability: A study on batch processed polyimide-based intracortical neural arrays

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Abstract: For short and long term neural applications, it is desirable to reduce or ideally eliminate the foreign body response to the implanted devices. One way to do so is to minimize the size of the device and thus the bending stiffness of the implant affecting the soft brain tissue. Recent findings in the field of neural engineering have led to the development of ultra-thin and ultra-flexible intracortical electrode arrays, presumably “invisible” for the host tissue and unable to trigger a noticeable immune response in long-term applications. However, reduced probe size comes at the cost of fewer individual electrodes, more challenging handling and fabrication, as well as mechanical properties less suited for chronic in vivo experiments. It is therefore important to better understand which design parameters offer the best trade-off.
In this study, we compare two kinds of polyimide-based electrode arrays which differ only in their width: one kind is only 30 µm wide while the other has a width of 100 µm. Both device types are 12 µm thick and display electrodes 20 µm in diameter, spaced so that they target each one of the six cortical layers. The devices were chronically implanted into the brain of adult rat models and different time points were selected for perfusion and subsequent evaluation and quantification of the differences in the elicited immune response of the brain (via immunohistochemistry). Some of the probes were also used for long term recording of electrophysiological activity. Control devices (i.e. identical but without connector) were used to study the influence of the connector and cement on the short and long term tissue reaction to the implants.
The wider probes (100 µm width) featured a pair of identical electrodes per cortical layer (instead of a single one) with the aim to achieve a better reconstruction of the neuronal configuration and status around the probe. The study’s goal is to systematically investigate the influence of selected geometrical parameters on the performance of thin-film polyimide based neural prostheses in vivo.
Key concept: This study is part of a bigger project which aims to find a clear answer to the question “how small does a neural probe have to be to become invisible for the host tissue and yet to record long term high quality signals?”

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Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

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Andreas Schaefer is a Wellcome Trust Investigator (110174/Z/15/Z)

Title: jULIEs: Microwire based neural probes for localized extracellular recordings and stimulation in the mouse brain

Authors: *R. R. RÁCZ[1], M. KÖLLő[2], T. ACKELS[1], G. RACZ[1], C. BULZ[1], T. WARNER[1], A. SCHAEFER[1]
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Abstract: Research into brain dynamics at biophysical, circuit and systems level is of fundamental importance for the advancement of our understanding on how the central nervous systems works and drives individual behaviour. Micro-fabricated charge sensing extracellular probes are considered one of the most powerful techniques capable of addressing cell populations in different brain regions at relevant read-out frequencies and resolution. Although promising, microfabricated devices have limitations in terms of shank size and overall geometry, recording fidelity and available modalities or lateral site density and channel count. To overcome these challenges, here we introduce a nanomaterials modified microwire platform with superior flexibility capable of extracellular recordings and stimulation of neural activity. These modalities are enabled by functionalization of the inert high specific surface recording site with a high charge storage capacity material packaged to fit industry standard read-out and stimulation electronics. At the core of the jULIEs probes are atomically-flat-shank microwires with diameters <25µm and sites ranging between 1 and 10 µm ± 0.2µm, resulting in minimal stray capacitances (<0.1pF/mm length) and superior coupling with the extracellular space. Microwire tips are angle-polished to <30° to facilitate insertion and bi-directional movement in tissue

during unit localization. Charge capacity and interfacial impedances are controlled and adjusted by modifying recording sites with nanosized IrO_x to achieve coupling capacitances above 1pF/μm^2 at ~1kHz. Cyclic voltammetry and electrochemical impedance spectroscopy between 1Hz and 200kHz around the open circuit potential in 150mM saline has been used to characterize the electrode-tissue interface. jULIEs neural probes containing 16, 32, 64, 128 channels have been packaged with connectors compatible with Intan RHD2000 amplifiers and tested in the olfactory bulb (OB) of anaesthetized mice (4-6 weeks old, Ketamine/Xylazine anesthesia). Under 2p imaging guidance we performed localized stimulation in the OB of Tbet-GCaMP transgenic mice using a NLA8000A stimulation isolator. Action potentials were reliably recorded with amplitudes up to 1.6mV and activity has been reproducibly evoked by electrical stimulation at current levels between 20 and 50µA. Thus, the combination of nanostructured high specific surface material with high charge storage capacity IrOx provides a versatile platform for minimally invasive, highly localized neural recordings and stimulation.

Disclosures: **R.R. Rácz:** A. Employment/Salary (full or part-time):; Cyberdeal S.r.L. **M. Köllő:** None. **T. Ackels:** None. **G. Racz:** None. **C. Bulz:** None. **T. Warner:** None. **A. Schaefer:** A. Employment/Salary (full or part-time):; Paradromics Inc..

Poster

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Topic: I.04. Physiological Methods

Support: NIH Grant U01NS099687

Title: Effective structural integration of rat cortical brain tissue with perforated polyimide microelectrode platform improves long term recording stability

Authors: ***N. SHMOEL**[1,2], H. EREZ[1,3], S.-H. HUANG[1,2], M. M. JANKOWSKI[1,4], B. M. IGNATOWSKA-JANKOWSKA[3], I. NELKEN[1,4], M. E. SPIRA[1,2,3]
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Abstract: Long-term recording of neuronal activity from freely behaving rodents over extended periods is critical for basic and applied brain research. The recording and stimulating qualities of the majority of the currently used in vivo multielectrode array (MEA) platforms deteriorate over time due to chronic immune response of the brain to foreign implants, the so called brain foreign body response (FBR). Earlier studies suggested that the use of perforated MEA platforms may help improve the duration and recording quality of implanted devices. Nevertheless, the impact

of MEA-platform perforation on the duration of recording in freely behaving rat and the underlying structural relationships formed between the brain tissue and the perforated platform were not studied. To study these parameters we prepared perforated polyimide (PI) platforms (280 µm wide, 16 µm thick and pore sizes of 8x45-65µm) with 2 to 16 planar microelectrodes (Ø 25 µm) located along the edges of the platforms tip. Weekly telemetric recordings from freely moving rats revealed large field potentials for months (currently the longest recording period is 8 months) without deterioration. Immunohistochemical confocal imaging of cross sections through the brain tissue together with the PI-MEA platform (two weeks and 8 months after the platform implantation) revealed that the overall density of microglia and astrocytes was only slightly elevated adjacent to the PI-MEA perimeters. No large-diameter neuronal "kill zone" was observed. Occasionally neuronal somata and neurites appeared to form direct contact with the MEA surface. Importantly, neurons branches along with astrocytes and few microglia extended into the platforms perforations leading to local “anchoring” of the brain tissue to the MEA. The long-term electrophysiological recordings using the perforated PI-MEA platforms together with the observed ingrowth of neurites, astrocytes and microglia into the perforated platform suggest that beside for the reduced FBR the ingrowth of brain tissue into the perforation significantly improve the long-term recording stability.

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Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

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Topic: I.04. Physiological Methods

Support: Hungarian Brain Research Program - Grant No. KTIA_13_NAP-A-IV/1,2,3,4,6;the
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KAP-1.1-14/032
OTKA-K119443

Title: Simultaneous intra- and linear extracellular recordings with corresponding morphology: Towards a ground-truth data for multichannel electrodes

Authors: *D. MESZENA[1,2], I. PAL[2], B. P. KEREKES[1], G. MARTON[2], K. TOTH[2], L. WITTNER[2], Z. SOMOGYVARI[3], I. ULBERT[2,1]
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Abstract: An integrative experimental method is presented for simultaneous recording of extra- and intracellular activity. In spite of the widespread use of multi-channel extracellular electrodes, very limited knowledge is available about the intracellular validation of these signals. Whole-cell patch clamp recordings were used to detect intracellular single cell activity, in rat hippocampal slices, *in vitro*. Simultaneous extracellular signal was detected from the vicinity of the same neuron with a novel, 32-channel laminar edge-probe. Electrophysiological measurements were completed with subsequent histological analysis. Dendritic and axonal morphology of the intracellularly recorded and filled cell was revealed by three dimensional reconstruction performed with the aid of the Neurolucida system. The presented method allows the investigation of single cell contribution on the extracellularly recorded signals. Furthermore, our experimental system let us determine the exact Euclidean cell-electrode distances. The knowledge about the spatial location of the cell compartments and the electrode contacts can help to determine the impact of single cell activity on extracellular recordings. The simultaneous, multimodal signals recorded with our system can yield additional information for various model-based calculations of neuronal dynamics.

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Poster

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Program #/Poster #: 431.16/LLL51

Topic: I.04. Physiological Methods

Support: NIH BRAIN program (USA), project number 2015/2018-NIH 1U01NS094190

Title: A scalable active pixel electrode array CMOS-probe for large-scale intracortical recordings

Authors: *F. BOI[1], G. ANGOTZI[1], A. LECOMTE[1], E. MIELE[3], G. MANDELBAUM[4], S. ZUCCA[2], T. FELLIN[2], J. ASSAD[4], B. SABATINI[4], L. BERDONDINI[2]
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Abstract: Monitoring neural signals at cellular scale, within and among brain circuits is of the utmost importance to understand how the brain processes information. However, this goal remains challenging and requires the development of neurotechnologies capable of high spatial and temporal recording resolutions over large brain areas. Over the last decade significant advancements in CMOS electrode array devices permit large-scale electrophysiological recordings (in vitro and ex vivo) at sub-millisecond temporal resolution from large

microelectrode arrays (*doi: 10.1039/b907394a*), and this technology has recently started to be transferred to high-density CMOS neural probes. For instance, a CMOS probe with 384 recording channels that can address 960 recording sites was successfully developed (*doi: 10.1038/nature24636*). However, this choice of architecture implies that only a configurable sub-set of electrodes is accessible for recording thus effectively decreasing the density of available channels. In this work, we propose an 'active pixel' solution to achieve true high-density (>1000 electrodes/mm^2) full-frame recordings. A first generation of our probes was designed in a standard 180nm CMOS technology to provide low-power (< 3 mW, well below the limit of 40 mW reported in the literature) circuits for 512 active sensing electrodes (size of 15 µm × 15 µm, centre-to-centre pitch of 29 µm). The design includes in-pixel circuits for amplification underneath each electrode site and on-chip circuits for reading out the whole-array at 30 kHz/electrode using time division multiplexing. For implantation, silicon micromachining techniques were used to shape the CMOS devices (110 µm W × 9 mm L) and to reduce the thickness down to 40-50 µm. Electrodes sites were finally modified by electrodepositing platinum and PEDOT, resulting in pixel impedance below 1 MΩ at 1 kHz. Experimental performances of a first generation of these CMOS probes demonstrate the capability of recording both LFPs and spiking signals in head-fixed anesthetized or behaving mice and the capability to avoid light-stimulation artefacts due to the continuous recalibration of the in-pixel amplifiers. Low-noise performances down to 12 µV rms (full bandwidth up to 10 kHz) can be achieved with optimized in-pixel circuit designs. Furthermore, this technology is eligible for a recently-developed real-time closed-loop setup that is able to manage recording/stimulating procedures. In perspective, our approach allows to investigate modular probe geometry and the integration of these CMOS-probes with ultra-low power wireless circuits that we have recently reported (*doi: 10.1109/TCSI.2017.2762159*).

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Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 431.17/LLL52

Topic: I.04. Physiological Methods

Title: Simplifying stereotaxic insertion of cannula and electrodes using 3D printing of surgical guides designed with freely available software

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Abstract: Stereotaxic guided procedures are regularly used in preclinical neuroscience to make injections and record neuronal activity in the cortex and deep nuclei. This involves anesthetizing and mounting animals stereotaxic frame using ear bars and a nose clamp and positioned carefully. After exposing the skull, precise micromanipulators are used to mark the skull at locations relative to the skull sutures, providing access to the target structure. Small hole(s) are carefully drilled through the skull without damaging underlying tissues. Then manipulators are used to insert a guide cannula through the hole to the depth of the target structure, and the cannula(e) are affixed to the skull using dental acrylic secure the cannula(e) to small screws inserted nearby. In skilled hands, stereotaxic insertion of a single cannula can be made with good precision in a short time. But as the number of target structures increase, it becomes increasingly difficult and time consuming to precisely position and anchor the cannula to the skull manually. Many excellent commercial devices have been created to simplify precise insertion of multiple cannulae and electrodes using standard production methods. With the emergence of 3D printing, methods have been published showing how to produce remarkably complex implants and surgical guides that can be customized and printed economically. Customization of these 3D printed models, however, requires skill in manipulating and editing 3D surfaces.
Here, we present a method that simplifies the creation and customization of 3D printed surgical implants designed for insertion of guide cannula. Some 3D printed implants are custom fitted to the contours of the skull with MRI or other image and sophisticated 3D printing design software, requiring a depth of skill and knowledge to design and edit the implants. For the relatively flat skulls of rodents, we simplify the design process by modeling the stereotaxic atlas coordinate system for a standard weight animal into the dimensions of a single flat implant that is stereotaxically attached to the skull. This simulates the process of stereotaxic placement but removes the need to microposition each cannula independently. Furthermore, 3D design requires only XYZ plane movement and scaling. Our inexpensive, autoclavable design can be easily edited using free or inexpensive 3D design software and has a wide range of applications in preclinical research.

Disclosures: R.W. Sikes: None. **A.F. Paquette:** None.

Poster

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Topic: I.04. Physiological Methods

Support: NIH Grant R01MH111359
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NIH Grant S10RR029050

Title: Towards reconstruction of neuronal circuit activity from electrophysiological signals obtained from the cortical surface

Authors: *N. ROGERS[1], L. HOSSAIN[2], M. THUNEMANN[3], K. KILIÇ[3], J. HERMIZ[2], M. GANJI[2], P. SAISAN[3], Q. CHENG[3], K. L. WELDY[3], A. M. DALE[3,4], V. GILJA[2], S. DAYEH[2], A. DEVOR[3,4,5]
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Abstract: A challenge of using minimally invasive surface electrode arrays is that mapping of neuronal circuit activity onto electrophysiological signals detectable from the cortical surface is poorly understood. To address this challenge, we combined surface and laminar electrophysiological recordings to achieve sufficient spatiotemporal information for reconstruction of regional variations in 3D current flow between the intra- and extracellular compartments. This information can then be used for prediction of the most plausible circuit activity. In addition, we combined these electrophysiological recordings with calcium imaging in layer 5 pyramidal cells to evaluate the contribution of active dendritic calcium conductances in these cells to the extracellular potential. All experiments were done in the mouse Barrel cortex in vivo under general anesthesia. Our surface electrode array was composed of 32 channel microelectrodes (pitch=200 υμ, diameter=20 um) and made of a semiconducting polymer, PEDOT:PSS. The substrate consisted of a biocompatible and transparent parylene C layer with a total thickness of 5-10 um. These PEDOT:PSS/Parylene surface electrode arrays provided outstanding optical transparency allowing 2-photon imaging throughout the cortical thickness (using excitation wavelengths longer than 1100 nm). The laminar electrode array, consisting of 23 channels spaced by 100 um, was inserted along the cortical depth axis, immediately adjacent to the surface array. To generate well controlled localized neuronal activity, we stimulated single whiskers on the mouse snout using a piezoelectric stimulator. Laminar (depth) profiles of extracellular potentials at different distances from the principle barrel column were estimated by keeping the laminar electrode in place, and stimulating single whiskers 1-4 columns away. In this way, we obtained an average 3D volume estimate of the extracellular potential induced by a single-whisker stimulus. In addition, single trial analysis in response to repeated single-whisker stimuli revealed variability not only in the amplitude but also in the spatial location of spiking and local field potentials. Our study illustrates the power of novel neuroscience tools allowing 3D electrophysiological data acquisition and integration with optical imaging modalities, opening the door for mechanistic studies of neuronal circuit dynamics during stimulus-induced and ongoing (spontaneous) neuronal activity that until now have been out of reach.

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Poster

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Program #/Poster #: 431.19/LLL54

Topic: I.04. Physiological Methods

Support: LDRD award 17-SI-002

Title: Extracellular matrix enhances neural network formation and activity on multi-electrode array

Authors: *D. LAM[1], H. A. ENRIGHT[1], S. K. G. PETERS[1], J. OSBURN[1], A. P. DE OLIVEIRA SALES[2], J. E. CADENA PICO[2], D. SOSCIA[2], K. KULP[1], E. K. WHEELER[2], N. O. FISCHER[1]
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Abstract: The brain's extracellular matrix (ECM) is a highly organized structure that has a heterogenous molecular composition (e.g. collagen, proteoglycans, glycoproteins and glycosaminoglycans) and accounts for approximately 20% of the total volume in the adult brain. ECM acts as a physical barrier to reduce the diffusion of soluble and membrane-associated molecules and cell migration. Additionally, it has important functions during development, in synaptic plasticity, and following injury. While studies have investigated the effects of individual ECM molecules in 2D and 3D in pure neuronal cultures or co-cultured with glia, it is important to recognize the complexity of ECM in the brain. In this study, we examined the effects of decellularized rat brain ECM and a commercial human ECM cocktail, MaxGel, on a co-culture of primary neuron and astrocytes *in vitro*. We compared electrical activity of the neuronal networks grown with or without ECM interfaced to a PDL-coated Multi-Electrode Array (MEA) device. The MEA devices present a noninvasive experimental approach for long-term interrogation of neuronal networks. We have recently developed a complex culture system on a MEA device using primary rat neurons and glial cells (i.e. astrocytes, oligodendrocyte precursor cells). Increasing the cellular complexity in culture has shown firing responses (e.g. firing rate, burst events) and synchronized activity at earlier stages in culture compared to neuronal cultures alone. Here, we also observed a similar response (i.e. early firing responses, increased active electrodes) by increasing the molecular complexity of the extracellular matrix. Flow cytometry and immunocytochemistry were used to identify the cell-type specific populations, cell morphology, and the phenotypic state of astrocytes. We report that the 2D construct represents a

reproducible and reliable method to study the complex neuronal-astrocyte network and can be further enhanced to recreate a 3D tissue model relevant to the brain.

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Poster

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Program #/Poster #: 431.20/LLL55

Topic: I.04. Physiological Methods

Support: NSF Grant 1743694
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Title: Platinum nanorod (ptnr) microelectrodes record action potentials from the cortical surface

Authors: *M. GANJI[1,2], L. HOSSAIN[3], E. ARNEODO[4], J. HERMIZ[2], A. PAULK[6], V. GILJA[2], S. CASH[6], E. HALGREN[5], T. GENTNER[4], S. DAYEH[2]
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Abstract: Microelectrode arrays on flexible substrates are promising for mechanically compliant neural interfaces and for large-scale electrophysiological recording from intact brains. Recently, there has been a great interest in devising flexible microelectrode arrays that can achieve cellular resolution. Here, we introduce a new biocompatible microelectrode array composed of platinum nanorods (PtNRs) on micrometer thin parylene C layers fabricated using scalable microfabrication processes. We show by extensive electrochemical testing that PtNRs exhibit 16 times larger charge injection capacities (CIC) and 10 times lower impedances compared to planar Pt. The devices are compatible with standard in-hospital sterilization techniques with minimal degradation in their performance. We carried out in-vivo characterization in starling birds and measured action potentials from the cortical surface with electrode diameters of 20, 40, and 100μm. Overall, we believe that the new PtNR microelectrodes with facile integration with standard microfabrication can advance neural probe technology by offering outstanding features with regard to next generation chronic neural stimulation and recording from the central and peripheral nervous systems.

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Poster

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Topic: I.04. Physiological Methods

Support: ARO grant W911NF-14-1-0173
NIDCD grant

Title: A modular high-density 294 channels μECoG system on macaque vlPFC for auditory cognitive decoding

Authors: *C. CHIANG[1], J. LEE[2], C. WANG[1], A. J. WILLIAMS[1], Y. E. COHEN[2,3], J. VIVENTI[1]
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Abstract: Background: Discerning sound is a complex neurocomputational problem because the stimuli of interest in the real world change simultaneously along multiple dimensions and are often mixed together with other environmental sounds. It is not well understood how the brain transforms a mixture of acoustic stimuli into distinct perceptual representations. There is broad consensus that auditory perceptual decisions are mediated by the ventral auditory pathway. At the later stages of this pathway, including the ventrolateral prefrontal cortex (vlPFC), neurons can encode decision outcomes. However, the spatiotemporal resolution of the neural code that underlies perception and cognition over this region is still unclear; and until recently, it was not possible to record from large numbers of brain sites simultaneously. We, therefore, developed a modular high-resolution electrode array system with long-term viability and used it to study the information that could be decoded from vlPFC micro-electrocorticographic (μECoG) signals during an auditory detection task.
Method: We molded three separate μECoG arrays into one with silicone and implanted this high-density modular system in one adult male rhesus macaque. A custom 3D-printed titanium chamber was mounted on left hemisphere using MRI-based targeting (BrainSight software, Rogue Research). The molded 294-contact polyimide μECoG array (30 μm total thickness; 229 μm diameter gold contacts; 610 μm contact spacing; 10.4 by 11 mm^2 sensing area) was implanted subdurally over vlPFC. Intan headstages and an OpenEphys recording system were used to record μECoG activity while the monkey participated in a “hearing-in-noise” task in which they reported hearing a “target” vocalization from a background “chorus” of

vocalizations. We titrated task difficulty by varying sound level of the target vocalization, relative to the chorus.
Result: We present a decoding analysis of the μECoG signal relationship to behavior with respect to spatial resolution, neural frequency bands, time, and cognitive/behavioral state. We found that individual channels varied significantly in their ability to decode various behavioral parameters (e.g., hits versus misses): some channels were at chance, whereas others performed significantly better than chance. When we considered those channels that performed better than chance, we found that, as we increased the number of channels, decoding performance improved. This improvement was seen across a variety of neural frequency bands.
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Poster

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Topic: I.04. Physiological Methods

Support: DARPA VAPR HR0011-14-2-0001
NIH R21 EY027570
DARPA ELM DE-AC02-05CH11231

Title: A high-density carbon fiber intracortical electrophysiological recording array technology

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Abstract: The ideal intracortical electrophysiological recording array would have a breadth of characteristics, including minimal adverse biological response, full-volume sampling of single-unit activity, and scalability to a large number (>thousands) of recording electrodes. No device yet meets all of these criteria. This work presents significant steps toward such a device, demonstrating a high-density 32-channel carbon fiber microwire neural recording array capable of acute in-vivo recording of single-unit extracellular potential. Departing from the in-plane architectural paradigm of conventional microwire-style neural recording arrays, a two dimensional array substrate is microfabricated in silicon and 5 μm diameter carbon fiber monofilaments are threaded through holes in that silicon substrate to create an array of carbon

fiber extracellular recording electrodes; the method can be scaled to an arbitrary number of recording electrodes. In addition to scalability, this device architecture enables electrode pitch four times finer than the state of the art among microwire arrays for extracellular electrophysiology. The fine diameter of the carbon fibers affords both minimal cross-section and nearly three orders of magnitude greater lateral compliance compared to traditional 25 μm tungsten microwires, with these features serving to minimize the adverse biological response of the implanted electrodes.
The substrate microfabrication and array assembly processes are robust and repeatable, and with the introduction of a robotic system to automate the insertion of carbon fibers into the through-silicon vias with submicron precision, the processes are fundamentally scalable to an array with a large number of electrodes. A specially formulated isotropically conductive adhesive mechanically and electrically bonds the carbon fiber recording electrodes to the silicon substrate, and post-processing of both the adhesive and the recording sites serves to further lower the impedance for superior electrophysiological characteristics. Acute extracellular electrophysiological recording is demonstrated in M1 in a rat, with single-unit action potentials being recorded on many channels. This carbon fiber microwire intracortical electrophysiological recording array is a promising technology for increasing information density while minimizing the adverse biological response, particularly in applications where microwire arrays are already commonplace.

Disclosures: T.L. Massey: None. **S.R. Santacruz:** None. **J.F. Hou:** None. **K.S.J. Pister:** None. **J.M. Carmena:** None. **M.M. Maharbiz:** None.

Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 431.24/LLL59

Topic: I.04. Physiological Methods

Support: NIH R21-1R21EY028381-01
Simons Center for the Social Brain
NEC Corporation Fund for Research in Computers and Communications
NIH 1FMH108086-01

Title: Open ephys++: High performance open-source firmware, apis, and hardware for closed-loop neuroscience experiments

Authors: *J. P. NEWMAN[1], J. ZHANG[1], J. VOIGTS[2], M. T. HARNETT[3], M. A. WILSON[4]
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Abstract: Testing certain hypotheses about neural coding requires experiments that can execute complex closed-loop algorithms on fast timescales. Existing acquisition systems can cope with high data rates or low latencies. However, recording form many channels while maintaining low closed-loop latencies usually requires highly specialized hardware. We present open-source hardware designs, protocols, and programming interfaces that enable sub-millisecond bidirectional communication with arbitrary arrangements of head borne sensors and actuators. This interface allows scientists to implement closed-loop control algorithms entirely in software on a commodity PC. Our architecture is generic and can be used to serialize and control any mixture of sensors over a tiny, easily commutated 2-conductor microcoxial cable. We provide concrete examples of this architecture in the form of next generation multifunction headstages used for rodent tetrode electrophysiology. First are 256 or 64-channel headstage (using neural amplifier chips from Intan Technologies) for use with behaving rats or mice, with integrated FPGA, high-bandwidth data serializer, 9-axis IMU, and 2 or 32-channel LED drivers. Second is an galvanically-isolated PC-housed acquisition board containing headstage deserialization circuitry, digital I/O, and FMC interfacing circuitry. Additionally, we demonstrate seamless integration with the popular UCLA microendoscope. Due to the simplicity, modular nature, and performance of these designs they may prove an appealing generic acquisition backend to support the development of next-generation head-borne sensors.

Disclosures: J.P. Newman: None. **J. Zhang:** None. **J. Voigts:** None. **M.T. Harnett:** None. **M.A. Wilson:** None.

Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 431.25/LLL60

Topic: I.04. Physiological Methods

Support: NIH BRAIN Initiative U01 NS099697-01
NIH BRAIN Initiative Research Supplements to Promote Diversity U01 NS099697-02S1
Steven W. Smith Fellowship (Duke University)

Title: The effect of contact size on the µECoG signal acquired from the surface of the rat auditory cortex

Authors: *A. J. WILLIAMS, M. TRUMPIS, B. BENT, C. CHIANG, J. VIVENTI
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Abstract: Micro-electrocorticography (µECoG) is a method of reliably recording brain activity at high-resolution from the surface of the cortex without damaging brain tissue (Viventi et al.,

2011). Currently, µECoG electrodes are designed using a wide range of contact sizes to measure brain activity; however, the influence of size of the recording contact on the signal acquired *in vivo* is still undetermined (Chang, 2015; Khodagholy et al., 2015; Slutzky et al., 2010). This work will show a thorough analysis of the effect of contact size within a µECoG array on signal metrics from acute recordings taken from rat auditory cortex.

A 60-channel gold µECoG electrode array was designed with four different contact sizes of 20, 50, 100, and 150 µm diameter arranged in a grid pattern as shown in Figure 1. The array was fabricated on a polyimide substrate with standard micro-fabrication methods. Subdural *in vivo* recordings were taken from the surgically exposed auditory cortex of anesthetized female Sprague Dawley rats aged 4-6 months. Recordings were carried out in a sound-attenuated chamber with responses to tone pips of 13 frequencies at eight sound pressure levels recorded to reconstruct frequency intensity response areas. Responses to brief broadband click stimuli and *in vivo* impedance for each electrode were also recorded. Acquired neural signals were analyzed using previously established methods evaluating evoked-signal-to-noise ratio (ESNR), spatial resolution, auditory response tuning and decoding accuracy (Insanally et al., 2016; Trumpis et al., 2017).

Preliminary data from epidural recordings has shown no difference between signals acquired using contact sizes, most likely due to the presence of the dura attenuating signals from the brain. In future work, we will repeat the experiment using a subdural preparation and investigate action potentials recorded from the µECoG devices. We anticipate that the ability for the array to record action potentials from the surface of the brain will more strongly depend on the size of the electrode contacts.

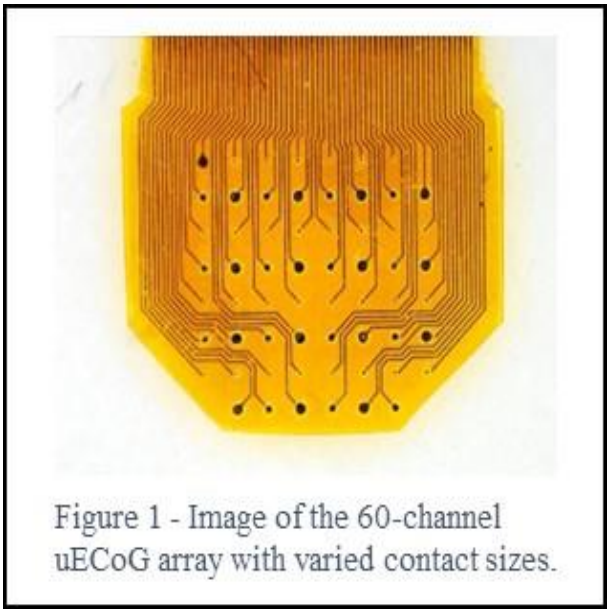

Figure 1 - Image of the 60-channel uECoG array with varied contact sizes.

Disclosures: A.J. Williams: None. **M. Trumpis:** None. **B. Bent:** None. **C. Chiang:** None. **J. Viventi:** None.

Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 431.26/LLL61

Topic: I.04. Physiological Methods

Support: Canadian Institutes of Health Research, CIHR
National Science and Engineering Research Council of Canada

Title: Quick and consistent method for stability assessment of microelectrodes

Authors: M. MA[1], T. P. ZANOS[3], M. R. KRAUSE[1], C. C. PACK[2], *T. E. KENNEDY[4]
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Abstract: Cortical multielectrode arrays (MEA) offer some of the highest resolution technology for detecting clinical user intent for controlling prostheses, such as robotics and functional electrical stimulation. The long term performance of these neuron-machine interfaces (NMI) depends on signal recording stability. This project analyses over seven months of Utah Array recordings from inferotemporal and prefrontal cortices of adult macaque monkeys, to quantify both the gradual decline of individual channels and implant level failures. Stability assessment was first performed by counting stable units, via regular spike sorting; and subsequently by counting stable channels, via a novel rule-based method applied to the firing rate distribution. The latter relies only on the timing of above-threshold events - befitting newer NMI decoding techniques, and hence is both quick (fully automated) and consistent (not requiring subjective steps involved in spike sorting and matching between single-units). The method is implemented in Matlab and enables efficient and objective evaluation of future microelectrode technologies.

Disclosures: M. Ma: None. **T.P. Zanos:** None. **M.R. Krause:** None. **C.C. Pack:** None. **T.E. Kennedy:** None.

Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 431.27/MMM1

Topic: I.04. Physiological Methods

Support: NIH NINDS 2R44NS065545-03A1

Title: Electrode array platform for transcutaneous neural recording and stimulation

Authors: *I. P. CLEMENTS, A. B. HARRISON, A. C. WILLSIE, A. J. PREYER, E. A. BROWN, J. D. ROSS
Axion Biosystems, Atlanta, GA

Abstract: Improved capabilities for non-invasive sensing and manipulation of neuromuscular activity would advance areas of clinical diagnostics, therapeutic stimulation, and neural prosthetics. Here we describe a non-invasive, automated platform for targeted monitoring and manipulation of human nerves and muscles. Flexible arrays of transcutaneous electrodes were coupled to a custom-designed IC offering up to 64 channels of simultaneous stimulation and recording. Signals were further conditioned with custom electronics and array-based processing techniques. Neural signals were manipulated and recorded with high precision and sensitivity through conformable, transcutaneous electrode arrays. Array-based stimulation enabled stimulation selectivity without requiring precise anatomical placement. Array-based recordings enabled 2D mapping of neural propagation and longitudinal tracking of nerve signals between sessions. EMG signals were also mapped, with H-reflex and M-wave recruitment curves automatically generated. The developed platform increases efficiency of typical diagnostics, in which bipolar electrodes are repeatedly repositioned by a highly skilled operator. Novel capabilities are enabled, such as automatic anatomical registration, longitudinal monitoring, and 2D spatial mapping of nerve signals to reveal branching or focal slowing. The system is designed for scalable and cost-effective manufacturing. Ultimately, this platform will enable rapid advancements in both neuromuscular research and medical device development, with applications in neuropathological diagnostics, advanced therapeutics, and neural prosthetics.

Disclosures: I.P. Clements: A. Employment/Salary (full or part-time):; Axion Biosystems. **A.B. Harrison:** A. Employment/Salary (full or part-time):; Axion Biosystems. **A.C. Willsie:** A. Employment/Salary (full or part-time):; Axion Biosystems. **A.J. Preyer:** A. Employment/Salary (full or part-time):; Axion Biosystems. **E.A. Brown:** A. Employment/Salary (full or part-time):; Axion Biosystems. **J.D. Ross:** A. Employment/Salary (full or part-time):; Axion Biosystems.

Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 431.28/MMM2

Topic: I.04. Physiological Methods

Support: Wellcome Trust 205093 102264
Human Frontier Sciences Program

Gatsby Charitable Foundation GAT3531
EU Horizon 2020
Marie Curie No 656528
European Union's Seventh Framework Programme 600925

Title: On the shape and extent of extracellular action potential waveforms across the rodent brain

Authors: *S. CHEN[1,2], J. P. NETO[3], M. PACHITARIU[1], A. R. KAMPFF[3], N. A. STEINMETZ[2]
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Abstract: Extracellular recording of action potentials is a gold-standard technique widely used to measure neuronal activity with high temporal and spatial precision. However, little is known about the detailed shapes of the extracellular fields associated with the action potentials of various types of neurons, and how these characteristics influence the experimenter's ability to successfully record neurons in diverse brain regions.
Here we used a custom "ultra-dense" electrode array with 255 small (5x5 μm), densely packed (6 μm center-to-center spacing) TiN recording sites, spanning 89x101 μm, to measure these shapes at higher resolution than previously possible. We performed recordings with this probe in urethane-anesthetized rats from five brain regions: isocortex, hippocampus, striatum, thalamus, and cerebellum. We analyzed the data using Kilosort (Pachitariu et al. 2016), a spike sorting algorithm designed to optimally incorporate information from many recording sites that each observe the same neuron, as is the case with these probes.
We observed in all regions that some neurons had broad field extents, detectable at high amplitude across all or most of the recording sites, as expected from recordings with less dense arrays. However, we furthermore observed that in all regions some neurons had extracellular potentials confined to extremely small areas, such that only one or a few sites had detectable action potential amplitudes (>50 μV), implying a total detectable extracellular field extent of ~5-10 μm radius.
We suggest that such small-field neurons may be typically un-detectable by other electrophysiological tools. Arrays with larger sites will not resolve these small fields with detectable amplitude, and the classic technique of isolating neurons by moving a sharp-tipped electrode to stay near the neuron will have difficulty because of the adjustment precision required to keep within a few microns of these neurons. Moreover, these neurons will be particularly susceptible to any movement of the brain relative to the probe (as in electrode "drift"), as small-field neurons will effectively disappear into the gaps between sites and become un-trackable. The existence of neurons with such small fields across the brain may provide a partial answer to the classic "dark matter" problem in neuroscience - i.e. that fewer neurons are observed than expected with extracellular techniques (Shoham et al. 2006).

Disclosures: S. Chen: None. **J.P. Neto:** None. **M. Pachitariu:** None. **A.R. Kampff:** None. **N.A. Steinmetz:** None.

Poster

431. Physiological Methods: Electrophysiology: Electrode Arrays I

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 431.29/MMM3

Topic: I.04. Physiological Methods

Support: Center for Brain Activity Mapping (CBAM) at UC San Diego
NSF No. ECCS-1351980
NSF No. ECCS-1743694
NSF No. CMMI-1728497

Title: Translation of PEDOT/Parylene C ECoG microelectrode arrays for recording stimulus driven action potentials in songbirds

Authors: *L. A. HOSSAIN[1], J. HERMIZ[2], Z. ARNEODO[3], M. GANJI[2], N. ROGERS[4], T. GENTNER[3], V. GILJA[2], S. A. DAYEH[1]
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Abstract: Understanding cognitive processing in intact brains is the subject of intense research efforts to resolve stimulus-evoked activity of individual and networks of neurons across different cortical layers. Traditionally, single unit activity is conveniently recorded with penetrating depth electrodes. But these electrodes cause damage to natural structure and connection in the brain tissue. Recording single unit activity from the cortical surface has been demonstrated recently with low impedance PEDOT:PSS microelectrodes built on thin flexible substrates. Here, we build similar devices with 'through' vias in between the microelectrode contacts and insert depth electrodes to validate and correlate surface recorded activity with activity at underlying cortical layers and demonstrate stimulus-evoked single unit activity at the cortical surface in songbird experiments.
The PEDOT devices were fabricated using a conventional surface micromachining procedure in arrays of 5x6 microelectrodes with 20μm diameter and 200μm center-to-center spacing. The metal lines were embedded in ~2.9μm parylene C on both sides, and the PEDOT:PSS was spun-cast from solution and cured in ambient at 140°C for 1 hour. Electrochemical impedance spectroscopy was used to evaluate the properties of the electrochemical junction and yield of our process. The microelectrode impedance magnitudes were 67.6kΩ±17kΩ at 1kHz, with a 96% yield on the best devices. We implanted the microarrays acutely into the HVC region of the cortex on starlings (n=6) and optimized the procedure for conformal contact to the brain surface. Recordings were on both male and female starlings with Testosterone administered 2-5 weeks before recording. Upon playing a pre-recording of the bird's own song, stimulus-evoked high gamma activity was observed from the HVC. By altering the auditory stimuli, distinct activity

was recorded that is highly correlated to the stimuli when the song was known to the bird and less correlated for the unknown stimuli. The enhanced electrochemical properties of our microelectrodes also enabled the detection of single units from the surface. Like the LFP, these surface spikes were evoked by the auditory stimuli of the bird's own song. These characteristics match the spiking patterns previously recorded from depth electrodes in the same birds. Therefore, we believe that validation of these microelectrode arrays in decoding stimulus-evoked activity from the surface may pave the way for better understanding of information processing in superficial layers in intact brains and without penetrating the brain to record from deeper layers.

Disclosures: L.A. Hossain: None. **J. Hermiz:** None. **Z. Arneodo:** None. **M. Ganji:** None. **N. Rogers:** None. **T. Gentner:** None. **V. Gilja:** A. Employment/Salary (full or part-time):; Neuralink Corp. **S.A. Dayeh:** None.

Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 432.01/MMM4

Topic: I.05. Biomarker and Drug Discovery

Support: BK21 PLUS
NRF-2016R1C1B2010206

Title: Exploring pathogenesis-based biomarkers for Parkinson's disease using various components of peripheral blood

Authors: *J. KANG[1], H. HEO[1], Y. KIM[1], J. CHANG[1,2]
[1]Dept. of Biomed. Sci., Ajou Univ. Grad. Sch. of Med., Suwon, Korea, Republic of; [2]Dept. of Brain Sci., Ajou Univ. Sch. of Med., Suwon, Korea, Republic of

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder which appears motor symptoms including shaking, rigidity and bradykinesia. Despite its rapid increase in prevalence, there is currently no effective treatment for PD. In addition, since symptoms of PD begins to appear only after a loss of significant numbers of nerve cells at the affected brain region, it is critical to start the appropriate medical intervention at the early stage of the disease progression. To diagnose the early stage of PD before the development of Lewy bodies inside nerve cells, which is a major pathologic feature of PD progression, it is necessary to find biomarkers that are specific for early PD patients. Though efforts to find biomarkers for PD have been done in many ways, no clinically-applicable biomarkers have yet been identified. Here, we aimed to investigate whether groups of selected proteins based on previous studies on the pathogenesis of PD could be candidates of

useful biomarkers for PD. Considering the clinical use of biomarkers, peripheral blood is a good resource because it can be obtained through relatively non-invasive ways as well as the advantage of containing components that are distinct from cerebrospinal fluid. For this study, a total of 20 patients with idiopathic PD and age-matched 20 patients with essential tremor (ET) which were diagnosed according to the UK Brain Bank Criteria were enrolled consecutively at Ajou University Hospital. There were no significant differences in age, sex, general cognition or medical history between the PD and the ET groups. We first screened which selected pathogenesis based-proteins in the peripheral blood of patients could be quantitatively analyzed by western blotting. As a result, it was found that 4 proteins in the whole blood, 14 proteins in the plasma, and 37 proteins in peripheral blood mononuclear cells can be analyzed. Through additional quantitative analysis of these targets, we finally have found multiple biomarker candidates that significantly increased or decreased in the blood of PD patients.

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Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 432.02/MMM5

Topic: I.05. Biomarker and Drug Discovery

Support: BAEF Post-doctoral Fellowship

Title: Discovering Miro inhibitors as a novel approach to treat Parkinson’s disease

Authors: *R. VANHAUWAERT, X. WANG
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Abstract: Cells balance their energy homeostasis and minimize oxidative stress by regulating mitochondrial function and transport and by eliminating dysfunctional mitochondria. It is not surprising that defects in this regulation will be detrimental to all cells; this is especially true for neurons because of their remarkable length and complexity of axons as well as the variable and specialized energetic demands of these highly-polarized cells.
Our lab recently implicated misregulated mitochondrial transport and clearance in the pathogenesis of Parkinson’s disease (PD). Interestingly, we found that PD-causing genes PINK1, Parkin and LRRK2 target Miro for removal from damaged mitochondria and their pathogenic mutations impair Miro removal and delay mitophagy. This defect renders vulnerable neurons to accumulate damaged mitochondria, causing energy shortage and oxidative stress, and consequently leading to neurodegeneration (Hsieh et al., 2016). Miro is a motor/adaptor on the mitochondrial surface that mediates mitochondrial motility and is removed from damaged

mitochondria to enable mitochondrial clearance via mitophagy. Remarkably, partial reduction of Miro arrests damaged mitochondria and restores mitophagy, both in LRRK2 mutant iPCS-derived neurons from PD patients and flies (Hsieh et al., 2016).
Since partial reduction of Miro rescues functional neuronal defects in both *in vitro*and *in vivo*models for PD (Hsieh et al., 2016), a small molecule that partially inhibits Miro could lead to new therapeutic treatments for this devastating disease. In order to adapt our cell-based and animal models to a high throughput drug screen, we have developed three Miro detection methods. Besides the traditional Western blot detection, we established a Miro ELISA and an immunohistochemistry approach using fibroblasts from PD patients and healthy controls. In collaboration with industrial partners we are conducting a targeted Miro inhibitor screen. Preliminary results reveal an initial 72 compounds that specifically target Miro. Now, we are testing their efficiency to reduce Miro levels in PD-patient fibroblasts using our assays. Our approaches could facilitate the discovery of powerful Miro-inhibitors with the hope to translate those to more effective treatments for Parkinson's patients.
Hsieh, C.H., et al. Functional Impairment in Miro Degradation and Mitophagy Is a Shared Feature in Familial and Sporadic Parkinson's Disease. Cell stem cell (2016).

Disclosures: R. Vanhauwaert: None. **X. Wang:** None.

Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 432.03/MMM6

Topic: I.05. Biomarker and Drug Discovery

Support: NSERC Grant: RA4981A01
CRC Grant: 950-230372
CERC Grant: 215063
Lawson IRF

Title: Structural and functional biomarkers of Parkinson's disease: Using structural and functional neuroimaging to identify the presence and severity of Parkinson's disease

Authors: *P. MACDONALD[1], N. M. HIEBERT[2], A. R. KHAN[3], L. NACI[6], A. VO[4], B. T. WANG[3], A. M. OWEN[4], K. N. SEERGOBIN[5]
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Abstract: Parkinson's disease (PD) is a progressive disorder for which there are no reliable biomarkers. The aim of this study was to explore the potential of structural and functional

analyses to assess the presence and track the progression of PD patients. Parkinson's disease (PD) is a common, complex, heterogeneous neurodegenerative illness with a broad range of possible motor and non-motor symptoms. The neural changes underlying most PD symptoms and signaling disease progression, however, remain poorly understood. Using a 3T MRI scanner, patients with PD and healthy controls were tested on two separate occasions, with one completed on and the other off dopaminergic medication. On both days, functional data were acquired while participants watched a short film, as well as diffusion tensor (DTI) and T1-weighted scans. While watching these films, distinct neural networks are activated in a specific and reproducible manner among healthy controls. Deviations in the synchronicity within each of these neural networks provides and innovative means to capture deficits in sensory and cognitive processing which may be used as functional biomarkers for disease. Frontal-parietal control network (FCPN), default mode network (DMN), dorsal attentional network (DAN), and primary visual sensory networks were investigated. DTI data were used to first parcellate the striatum into seven distinct sub-regions, guided by cortical regions to which they are reciprocally connected. The segmentation resulted in caudal-motor, rostral-motor, executive, limbic, parietal, occipital, and temporal striatal sub-regions. Volume of each sub-region was calculated for each striatal sub-region. Functional data revealed that synchronicity within FCPN was significantly greater in healthy controls, compared to PD patients when both were tested in the OFF state. Additionally, synchronicity within the DMN network was reduced in patients in the early compared to late-stages of PD. Structurally, the caudal-motor striatum was significantly atrophied in PD patients compared to controls. Finally, volume of limbic striatum, the only striatal sub-region innervated by the later-degenerating ventral tegmental area, was sensitive to disease progression using both motor and cognitive indices. Structural measures of striatal sub-regions, as well as cortex-wide functional connectivity measures, provided a sensitive means for distinguishing PD patients from controls and for tracking disease progression.

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Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 432.04/MMM7

Topic: I.05. Biomarker and Drug Discovery

Support: UK MRC
Wellcome Trust

Title: Normative data on the hippocampus in 12,247 participants of the UK Biobank

Authors: *L. NOBIS[1], S. MANOHAR[2], S. M. SMITH[3], F. ALFARO-ALMAGRO[3], M. JENKINSON[3], C. E. MACKAY[1], M. HUSAIN[2]
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Abstract: In Alzheimer's Disease (AD), pathophysiological processes affecting the structure of the brain are estimated to begin up to 20 years before clinically significant cognitive symptoms become evident. Thus, structural neuroimaging markers, such as hippocampal volume, may facilitate detection of AD-related pathology in the pre-clinical stage. Challenges in the processing of structural MRI scans, as well as a lack of normative data, have so far prevented this method from being used in a quantitative manner in clinical practice. However, with the imaging data from UK Biobank, and recently developed automated MRI processing tools, the utilisation of this method in a clinical setting becomes more feasible.
Here we report detailed normative information on the hippocampus in relation to age, sex, whole brain volume, and symmetry in 12,245 healthy participants of the UK Biobank. Brain images were processed, with imaging-derived phenotypes made available for general access. Hippocampal volume was estimated with FIRST. Information on hippocampal volume was then further analysed to provide normative volume percentiles across age.
We present percentiles, or nomograms, for hippocampal volume to serve as comparison values in both research and clinical settings in the largest cohort of healthy participants currently available. Examination of hippocampal volume across age also revealed an acceleration of hippocampal atrophy around ages 62-68 years. In addition, the analysis revealed larger mean hippocampal volumes in males than in females, but larger hippocampal volume in relation to total brain size in females. Finally, the data indicate asymmetry of the hippocampus, as the right hippocampus was slightly, but significantly, larger than the left hippocampus.
The reported nomograms will allow the clinician to assess a patient's hippocampal volume in relation to their age-group and thereby provide a potential indication of their risk for dementia. Openly available standardised and automated MRI processing pipelines will simplify the process of obtaining a measure of hippocampal volume from standard MRI scans in clinical practice. The results also suggest that the hippocampus may be more vulnerable to pathological processes than other brain tissue in later life, possibly linked to an increasing risk of dementia. While the current findings are based on cross-sectional data, the longitudinal health outcomes that will become available in UK Biobank over the next years will add invaluable information on the significance of hippocampal atrophy in healthy and pathological ageing.

Disclosures: L. Nobis: None. **S. Manohar:** None. **S.M. Smith:** None. **F. Alfaro-Almagro:** None. **M. Jenkinson:** None. **C.E. Mackay:** None. **M. Husain:** None.

Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

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Program #/Poster #: 432.05/MMM8

Topic: I.05. Biomarker and Drug Discovery

Support: NIG Grant NS087070
P30 CA030199
The Tanz Family Funds

Title: Oligomerization can convert a cyclic peptide antagonist into a potent and selective activator of the EphA4 receptor

Authors: *M. GÓMEZ-SOLER, S. LOMBARDI, C. ZHAO, B. C. LECHTENBERG, S. J. RIEDL, E. B. PASQUALE
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Abstract: Eph receptor tyrosine kinases and their ephrin ligands are key players in many processes occurring during neural development and in the adult nervous system. For example, aberrant EphA4 activity has been associated with inhibition of nerve regeneration after injury and exacerbation of neurodegenerative diseases. We previously identified peptide antagonists selectively targeting the extracellular ligand-binding domain of EphA4. These peptides have been useful not only as research tools but also for demonstrating the potential therapeutic value of pharmacologically inhibiting EphA4 in *in vivo* animal models of spinal cord injury, amyotrophic lateral sclerosis (ALS) and Alzheimer's disease. Modifications of one of these peptides have yielded a potent and stable cyclic peptide antagonist (designated APY-d3) that selectively inhibits ephrin-induced EphA4 activation. In contrast, selective activation of EphA4 remains a challenge. Soluble forms of the ephrin ligands have been used to activate EphA4 in *in vitro* and *in vivo* studies. However, the promiscuous nature of the ephrin ligands prohibits their use for selective activation of individual Eph receptors. Because ephrin binding leads to Eph receptor oligomerization and subsequent activation, we hypothesized that oligomerization of the APY-d3 peptide antagonist might transform it into a potent and selective EphA4 agonist. Thus, we designed an APY-d3 peptide linked to biotin (APY-d3-bio), which can be oligomerized by complexation with the tetrameric avidin. By measuring inhibition of ephrin-A5 binding to EphA4 in ELISAs, we determined that APY-d3 biotinylation does not affect peptide potency, while oligomerization through avidin binding increases potency without compromising selectivity. In addition, treatment of HEK293 cells stably expressing EphA4 with oligomeric APY-d3-bio increased phosphorylation of Y779 in the EphA4 activation loop, which is indicative of receptor activation. We used BT549 breast cancer cells as a model for cells with

endogenous co-expression of EphA4 and other EphA receptors that can be activated by the same ephrins. Treatment of these cells with oligomeric APY-d3-bio allowed us to identify cellular signaling networks specifically activated by EphA4. To ensure scientific rigor, ELISAs were performed at least 3 times, with each experiment including triplicate measurements and controls. Experiments with cells were repeated at least 3 times with similar results. This work illustrates the value of APY-d3 oligomerization as a strategy to selectively activate EphA4 in cells expressing multiple Eph receptors and to elucidate EphA4-specific downstream signaling pathways.

Disclosures: M. Gómez-Soler: None. **S. Lombardi:** None. **C. Zhao:** None. **B.C. Lechtenberg:** None. **S.J. Riedl:** Other; Inventor on patent applications covering EphA4-targeting peptides. **E.B. Pasquale:** Other; Inventor on patent applications covering EphA4-targeting peptides.

Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 432.06/MMM9

Topic: I.05. Biomarker and Drug Discovery

Title: Functional ultrasound - Novel in-vivo imaging technique for pre-clinical CNS drug discovery

Authors: *A. SHATILLO, T. MIETTINEN, J. KOPONEN, A.-M. KÄRKKÄINEN, D. MISZCZUK, A. J. NURMI
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Abstract: Functional ultrasound (fUS) is a novel imaging technique that was recently introduced to the field of preclinical CNS research. Method utilizes latest technological advancements for ultrafast plane-wave acquisition of doppler ultrasound signal with real-time data processing. Applied to neuroimaging in laboratory animals, this approach enables high sensitivity imaging of relative cerebral blood volume (rCBV) with unrivaled temporal and spatial resolution. High penetration depth allows whole brain coverage in both rats and mice. Moreover, non-invasive signal recording and small footprint of the fUS sensor creates a perfect opportunity for awake imaging in non-anesthetized animals with minimal stress and habituation period. Physiologically, fUS readout is based on the same neurovascular coupling mechanisms as commonly used functional MRI (MRI). Taken together, fUS imaging will likely soon become an invaluable method for basic research and pre-clinical drug discovery tool, allowing fast screening of the novel compounds for modulating neuronal metabolism and hemodynamic response in the brain. Similarly to fMRI, fUS can be used to study sensory processing, mapping of pharmacological response and resting-state functional connectivity analyses. Multiple experimental paradigms

(e.g. acute dosing vs. pretreatment-challenge) combined with different approaches to data analysis gives flexibility to look into specific aspects of the test compound-induced brain effects. Deliverables include multiple quantitative temporal (time to peak, full width at half-maximum, area under the curve, amplitude etc.) and spatial (statistical maps of activity) characteristics.
As early adopters of the technology, Charles River Discovery presents here our wide validation of fUS imaging for pre-clinical drug testing. This work is focusing on brain responses to commonly used psychoactive compounds as well as fUS applications in several CNS disease models.
Provided data and methodology established within this study, demonstrates extensive research and drug testing capabilities of functional ultrasound platform in pre-clinical setting.

Disclosures: T. Miettinen: None. **J. Koponen:** None. **A. Kärkkäinen:** None. **D. Miszczuk:** None. **A.J. Nurmi:** None.

Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 432.07/MMM10

Topic: I.05. Biomarker and Drug Discovery

Support: NIH R01 NS052318

Title: Reproducibility of free water imaging in Parkinson's disease

Authors: *W. T. CHU[1,2], D. B. ARCHER[2], R. G. BURCIU[2], S. LAI[3], S. WU[4], M. S. OKUN[5,6], N. R. MCFARLAND[5,6], D. E. VAILLANCOURT[2,1,5]
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Abstract: Quantitative and reproducible Parkinson’s disease (PD) progression markers are essential for the development of therapeutics. However, reproducible imaging biomarkers have been elusive. Free water is a measure that can be calculated from a diffusion tensor imaging scan of the brain and has recently been shown to predict the progression of motor symptoms in PD patients. The objective of this study was to evaluate the reproducibility of free water measurements in the substantia nigra, putamen, caudate, globus pallidus, subthalamic nucleus, thalamus, cerebellar peduncles, cerebellar vermis and lobules V and VI, and corpus callosum of the brain in PD patients. The study examined 19 PD patients who individually received two identical diffusion scans (test and retest). After the test scan, subjects were pulled out of the scanner and briefly repositioned before being placed back in the scanner for the retest scan. Two methods for analyzing regions of interest (ROIs) were evaluated: (i) a hand-drawn ROI method

and (ii) a standard ROI method using the same ROIs for all subjects. Paired t-tests were used to assess test-retest differences in free water. Test-retest variability was defined as the absolute difference of test minus retest divided by the mean of test and retest and expressed as a percent. The results demonstrated that there was no significant difference ($p > 0.05$) between test and retest free water using either ROI method. Test-retest variability of free water in the posterior substantia nigra using hand-drawn ROIs = 9.1 ± 8.7% and using standard ROIs = 8.0 ± 6.4%. Other ROIs had test-retest variability ranging from 3.6 ± 2.2% to 11.1 ± 9.2%. These data suggest that free water is a highly reproducible measure in PD patients and supports the feasibility of using free water as a PD progression biomarker.

Disclosures: W.T. Chu: None. **D.B. Archer:** None. **R.G. Burciu:** None. **S. Lai:** None. **S. Wu:** None. **M.S. Okun:** None. **N.R. McFarland:** None. **D.E. Vaillancourt:** None.

Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 432.08/MMM11

Topic: I.05. Biomarker and Drug Discovery

Support: NMRC/STaR/0009/2012

Title: Proteomic profiling of human neural cell line derived exosomes to identify cell type specific surface protein markers

Authors: *G. H. D. POPLAWSKI[1], A. SIDDIQUEE[1], R. SOBOTA[5], H. CHOI[2], R. CHIA[1], J. CHONG[3], M. LAI[3], E. KOO[1,4,6]
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Abstract: Due to constant increase in life expectancy, dementias such as Alzheimer's Disease (AD) are becoming one of the leading health issues of our time. One of the major challenges in dementia research is the ability to provide accurate diagnosis in the premortem setting, especially before clinical symptoms are evident. Indeed, one often cited reason for repeated failures in AD clinical trials is that treatment is begun too late in the disease course. While highly desirable, non-invasive, early diagnostic tools are not yet available. "Liquid biopsies" utilizing the exosomal content of patient-derived plasma have been proposed as a promising approach to the identification of dementia-related biomarkers. Indeed, recent studies have demonstrated that neurally-derived exosomes from AD patients carry AD biomarkers such as amyloid-β and

phospho-Tau, as well as pre- and post-synaptic markers, which can potentially be used to differentiate between preclinical and clinical disease progression. Neurally-derived exosomes can be enriched via immunoprecipitation utilizing antibodies targeting the neuronal cell adhesion molecule, L1CAM. As AD pathology does not only involve the degeneration of neurons but also encompasses neuroinflammation and gliosis, new biomarkers are likely to be found in other neural cell types, such as glia and microglia. In order to isolate exosomes from different neural cell types from patient plasma, we sought to identify cell-type specific exosomal surface markers that can be used for immunoaffinity enrichment. As a proof of principle, we isolated exosomes by ultra-centrifugation methodology from the human glioblastoma cell line U251 and performed mass-spectrometric analysis, which identified 1053 unique proteins. Gene ontology analysis indicated that 65% of these proteins are associated with extracellular exosomes (string-database) and the NCI-60 cancer cell line database indicated a high probability that proteins are derived from the U251 cancer cell line and are of astrocytic origin (Enrichr-database), thus validating our approach. Additional multiplex proteomic characterization of exosomes is currently underway analyzing human cells of astrocytic, oligodendrocytic, neuronal, microglial and neuro-endothelial origin. We will further evaluate if cell specific surface proteins can be utilized to successfully enrich exosomes originating from distinct cell types of the human brain, which may lead to the development of biomarkers for diverse disorders of the brain including dementia, stroke and aging.

Disclosures: G.H.D. Poplawski: None. **A. Siddiquee:** None. **R. Sobota:** None. **H. Choi:** None. **R. Chia:** None. **J. Chong:** None. **M. Lai:** None. **E. Koo:** None.

Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 432.09/MMM12

Topic: I.05. Biomarker and Drug Discovery

Support: H2020-MSCA-COFUND-2014-665919
IJCI-2015-24576
ERC-2014-StG-638106

Title: Cell-specific mitochondrial proteomic analysis in a mouse model of mitochondrial disease

Authors: *A. GELLA, P. PRADA, I. BOLEA, E. SANZ, A. QUINTANA
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Abstract: Leigh Syndrome (OMIM: #256000) is the most common pediatric presentation of a defined mitochondrial disease (MD). This progressive neurodegenerative disorder is

characterized by a rapid deterioration of cognitive and motor functions, in most cases resulting in death due to respiratory failure. To date, no general curative treatment is available for this devastating disorder. Strikingly, MD neuropathology presents a remarkable selectivity for certain neuronal populations, which may likely be driven by differential mitochondrial protein content in neurons. Using a mouse model of MD that is a correlate of the human Leigh Syndrome, we isolated cell-type specific intact mitochondria and protein alterations were estimated by using protein- and peptide-based approaches and LC-MS/MS methods. These results widen the spectrum of candidate proteins driving susceptibility to MD and open new targets to develop effective treatments for MD.

Disclosures: A. Gella: None. **P. Prada:** None. **I. Bolea:** None. **E. Sanz:** None. **A. Quintana:** None.

Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 432.10/MMM13

Topic: I.05. Biomarker and Drug Discovery

Title: SUVN-I6107: A novel true muscarinic M1 receptor positive allosteric modulator (M1-PAM) devoid of cholinergic side effects

Authors: J. TADIPARTHI, G. RAMALINGAYYA, N. GANUGA, S. YATHAVAKILLA, R. MEDAPATI, A. VUYYURU, R. KALLEPALLI, N. MUDDANNA, R. PALACHARLA, G. BHYRAPUNENI, T. THATIPARTHI, T. NARASIMHULA, S. JARUGUMALLI, *K. MUDIGONDA, R. NIROGI
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Abstract: Targeting the cholinergic system is a viable option for developing new treatments for Alzheimer's disease (AD). Compounds that target the muscarinic (M1) receptor such as M1 agonist and M1-PAM though demonstrated efficacy in animal models, also cause cholinergic side effects restricting their clinical use. SUVN-I6107, a M1-PAM was evaluated for its binding potential at the orthosteric and allosteric site of muscarinic receptors, its ability to potentiate the effect of acetylcholine in the reporter gene assay was evaluated. The pharmacokinetics and brain penetration was evaluated in rodents. The efficacy of SUVN-I6107 was evaluated in animal models of cognition. The effect on the modulation of soluble amyloid precursor protein-α (sAPP-α) was studied in rat cortex. Effect on neuronal oscillations was evaluated in combination with donepezil. Cardiovascular safety was assessed using the patch clamp technique. Cholinergic side effects were evaluated in mice, rat and dog. SUVN-I6107 is a M1-PAM with no agonistic activity. It has good selectivity over closely related muscarinic receptor subtypes. SUVN-I6107

modulated acetylcholine potency when tested in in-vitro IP1 assay and produced about 3-4 fold increase in striatal levels of IP1 at doses several fold higher than therapeutically active dose. It has adequate water solubility and found to be orally bioavailable (67%) with good brain penetration and free fraction. SUVN-I6107 demonstrated efficacy in the object recognition task and enhanced cerebral blood flow in rats. At therapeutically effective doses, SUVN-I6107 potentiated the effects of donepezil on elicited hippocampal theta levels and promoted non-amyloidogenic APP processing in rats. SUVN-I6107 did not show cholinergic side effects in rat and dogs up to dose several fold higher than the pharmacological active dose in animal models. SUVN-I6107 was found to be safe when tested in hERG patch clamp assay and in early stage animal toxicity studies. SUVN-I6107 did not induce convulsion (mice and rat) and found to non-mutagenic in Ames assay. SUVN-I6107 demonstrated robust efficacy in animal models without inducing cholinergic side effects. SUVN-I6107 is a true M1-PAM demonstrating robust efficacy in animal models and is devoid of side effects associated with M1-PAM.

Disclosures: J. Tadiparthi: A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **G. Ramalingayya:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **N. Ganuga:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **S. Yathavakilla:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **R. Medapati:** A. Employment/Salary (full or part-time):; rajeshbabum@suven.com. **A. Vuyyuru:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **R. Kallepalli:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **N. Muddanna:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **R. Palacharla:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **G. Bhyrapuneni:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **T. Thatiparthi:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **T. Narasimhula:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **S. Jarugumalli:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **K. Mudigonda:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **R. Nirogi:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.

Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 432.11/MMM14

Topic: I.05. Biomarker and Drug Discovery

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Parkinson's Foundation Grant PF-FBS-1778

Title: Automated non-invasive imaging procedure for distinguishing parkinsonism: A multisite cohort across MRI vendors

Authors: *D. B. ARCHER, A. ROY[1], W. CHU[1], R. BURCIU[1], S. COOMBES[1], H. LI[2], J. MCCRACKEN[1], N. BOHNEN[3], M. MULLER[3], R. ALBIN[3], F. KRISMER[4], G. DU[6], M. M. LEWIS[7], K. SEPPI[5], X. HUANG[6], O. PASTERNAK[8], N. MCFARLAND[1], M. OKUN[1], D. VAILLANCOURT[1]
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Abstract: Parkinson's disease (PD), multiple system atrophy (MSA), and progressive supranuclear palsy (PSP) are neurodegenerative disorders that are difficult to distinguish clinically as they share similar motor and non-motor features. Whereas dopamine transporter imaging can help identify Parkinsonism, it cannot distinguish between different forms of Parkinsonism. Diffusion MRI is a promising technique that allows for the *in vivo* quantification of brain microstructure, and recent small sample studies at one site have shown it can distinguish between different forms of Parkinsonism. A large scale diffusion MRI study using automated methods that is effective across sites with different pulse sequences would be advantageous given the heterogeneity in scanners across the world. The purpose of this study was two-fold: (1) determine which of several possible normalization pipelines provides the best inter-subject alignment in 17 Parkinson's-related regions of interest by comparing free-water within hand-drawn regions to template-derived regions in 104 subjects (31 controls, 40 PD, 17 MSA, 16 PSP) from one site, and (2) evaluate the clinical diagnostic capability of free-water using 5-fold cross validation artificial neural network modelling in the largest PD/MSA/PSP cohort to date, collated across 14 different MRI scanners and including 746 subjects (240 controls, 399 PD, 52 MSA, 55 PSP). We found that normalizing the fractional anisotropy image to a high-resolution fractional anisotropy template using symmetric diffeomorphic mapping in the Advanced Registration Tools software provided the best alignment of Parkinson's-related regions. Our neural network modelling displayed high test accuracy for all models (Control vs. PD/MSA/PSP: 94.3 ± 1.5%, PD vs. MSA/PSP: 86.1 ± 2.5%; MSA vs. PD/PSP: 86.3 ± 2.1%; PSP vs. PD/MSA: 88.7 ± 2.3%). This study provides an automated normalization pipeline and region of interest template that accurately distinguishes between different forms of Parkinsonism using clinically-relevant regions and variable pulse sequences.

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Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 432.12/MMM15

Topic: I.05. Biomarker and Drug Discovery

Title: Multi-plex digital spatial profiling validation of neurodegenerative and neuroinflammation protein targets in human ffpe brain sections

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Abstract: Neurodegenerative diseases, including Alzheimer's, Parkinson's, and amytrophic lateral sclerosis (ALS), affect millions of people worldwide and represent an increasing burden in terms of healthcare economics and societal impact. The investigation and characterization of the abundance, distribution and co-localization of neurodegenerative and neuroinflammatory targets within the human brain are critical for the advancement of our understanding of progressive degenerative disease. Nanostring's Digital Spatial Profiling (DSP) technology allows for simultaneous analysis of >40 proteins from discrete regions of interest (ROI) in FFPE tissue sections, providing morphological context to high plex molecular analysis. The assay relies upon antibody probes coupled to photocleavable oligonucleotide tags. After hybridization of probes to slide-mounted formalin fixed paraffin-embedded (FFPE) tissue sections, the oligonucleotide tags are released from discrete regions of the tissue via UV exposure. Released tags are quantitated in a standard nCounter® assay, and counts are mapped back to tissue location, yielding a spatially-resolved digital profile of analyte abundance. In this study, we validate an antibody-based panel designed to characterize key proteins of interest on the DSP platform. To validate antibodies for this technology, the specificity and sensitivity of each antibody was assesses for predicted immunohistochemistry (IHC) staining pattern on appropriate tissues as well as signal-to-noise ratios of positive counts above background. Each target was also evaluated in single versus multiplex to ensure there are no effects of multiplexing antibodies. Immunohistochemical analysis of antibodies chosen for this panel display indistinguishable staining patterns on control tissues for unconjugated primary antibodies and oligo-conjugated primary antibodies. Key targets validated include Tau, Map-2, NeuN, Tdp-43, GFAP, MBP, CD11b, CD68, HLA-DR, Vimentin, Iba1, S100B, VCAM1, Olig2, APP, CNPase, Park5, Park7, Synaptophysin, β3-tubulin etc. Ongoing efforts will expand the antibodies validated for with DSP specific for brain tissue.

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Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

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Program #/Poster #: 432.13/MMM16

Topic: I.05. Biomarker and Drug Discovery

Support: NIH grant R01AG048108
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Title: Brain correlates of fractal regulation in motor activity—Results from the Rush Memory and Aging Project

Authors: *P. LI[1,2], L. YU[3], K. ARFANAKIS[3,4], A. S. P. LIM[5], A. S. BUCHMAN[3], J. A. SCHNEIDER[3], D. A. BENNETT[3], K. HU[1,2]
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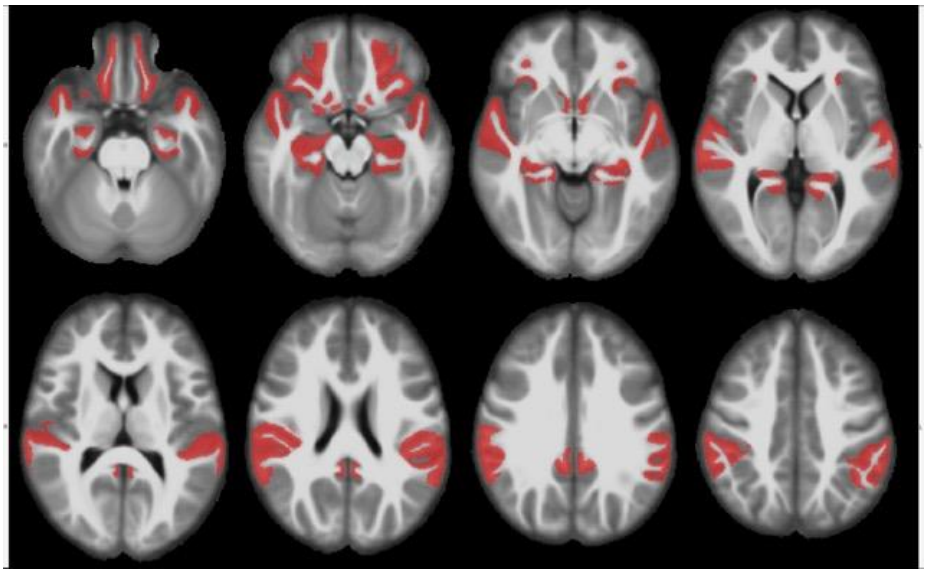
Abstract: Human motor activity possesses fractal structures as characterized by similar fluctuation patterns across multiple time scales from seconds to hours. Such fractal regulation (FR) is robust in healthy young subjects and is degraded with aging. Our recent study further showed that FR degradation is predictive of increased risk of developing incident Alzheimer's dementia. However, the underlying brain correlates of FR are still unclear. Here we tested whether FR degradation is associated with specific changes in brain structure. We studied 338 older non-demented adults (age: 81.5±7.1 [SD]) in the Rush Memory and Aging Project who had motor activity continuously recorded for up to 10 days and underwent antemortem magnetic resonance imaging (MRI) scan. The temporal correlations in activity fluctuations at time scales ~0.1-1.5h were examined to assess FR. Based on the structural MRI scans, gray matter volumes of 34 cortical and 10 subcortical regions were obtained. FR in activity were positively associated with gray matter volumes of 14 cortical regions and 2 subcortical regions (Bonferroni corrected $p<0.05$). After adjustment for age, sex, and education, the associations remained in 13 cortical and 1 subcortical regions, including 7 regions that are known to be linked to Alzheimer's disease (AD) pathology (as highlighted in red in Figure): lateral orbitofrontal, supra marginal, isthmus cingulate, superior temporal, fusiform, and accumbens area. To conclude, degradation of FR in motor activity is associated with lower cortical gray matter volumes in various brain regions/sub-

regions which possibly are the brain correlates responsible for the maintenance of FR. The overlap with AD-related regions suggests that FR degradation may share or contribute to the etiology of AD pathology.



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Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

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Program #/Poster #: 432.14/MMM17

Topic: I.05. Biomarker and Drug Discovery

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Title: How early can fractal regulation predict the risk for Alzheimer’s dementia?

Authors: C. HU[1], L. YU[2], J. YANG[2], D. A. BENNETT[2], *K. HU[1], P. LI[1]
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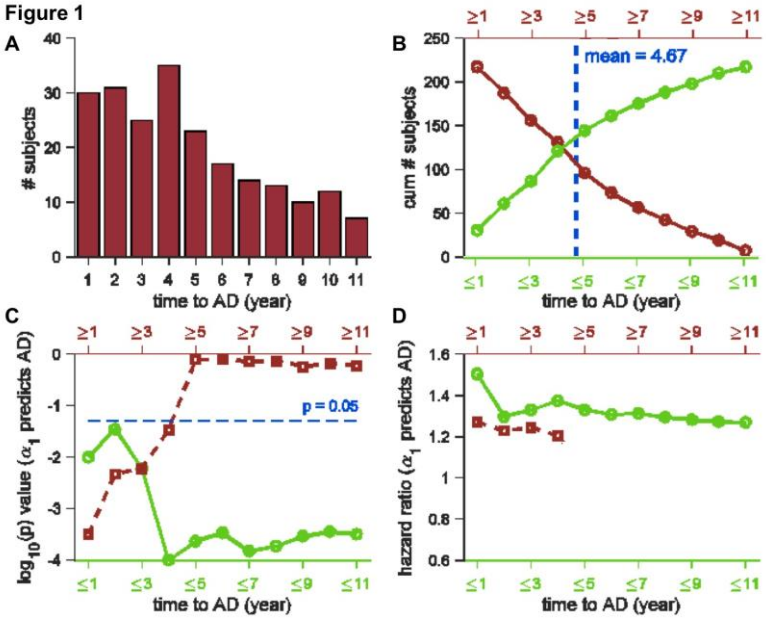
Abstract: Many neurophysiological systems exhibit fractal regulation (FR) as indicated by similar fluctuation patterns in their outputs at different time scales. Our recent study of 1,097 subjects in the Rush Memory and Aging Project showed that degraded FR in motor activity is associated with increased future risk of developing Alzheimer's dementia (AD) in older individuals. Here we tested whether the association depends on time lag between the assessment of FR and diagnosis of AD. To this end, we re-examined the same datasets in the previous study, in which 220 (out of 1,097) non-demented subjects at baseline developed AD later (AD subjects) and the other 877 subjects did not (non-AD subjects). The time lag from baseline to AD onset ranges between 1-11 years with an average of 4.7 years (Figure 1A). Actigraphy was monitored continuously for up to 10 days at baseline and was used to assess FR at time scales of ~1-90 minutes. Using a Cox proportional hazards model, we examined the association of baseline FR with incident AD repeatedly in multiple subsets of these 1,097 subjects. Two types of subsets were considered for each selected threshold of time lag (i.e., 1, 2, ⋯ , 11 years): (Type 1) AD subjects who developed AD before the threshold (green line in Figure 1B); and (Type 2) AD subjects who developed AD after the threshold (red line in Figure 1B). All non-AD subjects were included in each subset. For Type 1 subsets, we found that degraded FR, as indicated by smaller FR metric, was associated with increased risk for incident AD for all time lag threshold values with the smallest p value at time lag ≤4 years (green line in Figure 1C) and the largest hazard ratio at time lag of ≤1 year (green line in Figure 1D). For Type 2 subsets, the association was only significant when the threshold value of time lag was not greater than 4 years (red lines in Figure 1C and D). Our results indicate that FR can better predict the risk of incident AD when the assessment of FR is within or equal to 4 years before the clinical diagnosis of AD.

Figure 1



Disclosures: C. Hu: None. **L. Yu:** None. **J. Yang:** None. **D.A. Bennett:** None. **K. Hu:** None. **P. Li:** None.

Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 432.15/MMM18

Topic: I.05. Biomarker and Drug Discovery

Title: Brain delivery and central effects of peptide drug by intranasal administration in mice

Authors: *J.-I. OKA[1], S. SASAKI-HAMADA[1,2], R. NAKAMURA[1], T. FUNANE[1], Y. NAKAO[3], C. YAMASHITA[3]
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Abstract: We previously reported that the intracerebroventricular (i.c.v.) administration of glucagon-like peptide-2 (GLP-2) exerted antidepressant-like effects both in naïve mice and adrenocorticotropic hormone (ACTH)-treated mice, and that the i.c.v. administration of neuromedin U (NMU) inhibited lipopolysaccharide (LPS)-induced memory impairment and neuronal cell-death. However, the i.c.v. administration is invasive, costly, and impractical for the delivery of drugs into human brains, and thus we need to develop a non-invasive and effective way to deliver GLP-2 or NMU into the brain. In order to utilize GLP-2 or NMU as a clinical treatment tool for depression or amnesia, we prepared three peptide derivatives containing cell-penetrating peptides (CPPs) and a penetration-accelerating sequence (PAS) for the intranasal (i.n.) administration. CPPs including arginine-rich peptides have been shown to deliver various bioactive molecules with low membrane permeability into cells, and led to the regulation of cell functions. Macropinocytosis is transient, actin-driven fluid-phase endocytosis that involves membrane ruffling and the formation of large vacuoles called macropinosomes, which plays an important role in the cellular uptake of arginine-rich CPPs, resulting in highly efficient intracellular delivery. The addition of a PAS to CPPs has been reported to enhance the efficiency of the intracellular delivery of bioactive peptides by promoting endosomal escape *in vitro*, but no *in vivo* study was done to demonstrate brain delivery of peptides with a PAS and CPPs. In the present study, we examined i.n. delivery to the brain and central effects of peptide derivatives containing a PAS and CPPs. PAS-CPPs-GLP-2 (i.n.) was effectively delivered into the brain, and exerted antidepressant-like effects on naïve and ACTH-treated mice. Moreover, we prepared two NMU derivatives containing CPPs, octaarginine (R8), and each penetration-accelerating sequence, namely FFLIPKG (PASR8-NMU) and FFFFG (F4R8-NMU), for the i.n. administration. In the Y-maze test in mice, the i.c.v. administration of LPS significantly decreased spontaneous alternation behavior, which was prevented by the prior i.n. administration of PASR8-NMU or F4R8-NMU. The i.n. administration of PASR8-NMU or F4R8-NMU just

before the Y-maze test improved LPS-induced memory impairment in mice. We herein provided for the first time the efficiency of the addition of a PAS to CPPs *in vivo*. These results suggest that these peptide derivatives are useful in the clinical treatment of psychiatric and neurological disorders by the intranasal administration.

Disclosures: J. Oka: None. **S. Sasaki-Hamada:** None. **R. Nakamura:** None. **T. Funane:** None. **Y. Nakao:** None. **C. Yamashita:** None.

Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 432.16/MMM19

Topic: I.05. Biomarker and Drug Discovery

Support: MGH ECOR Formulaic Award
NIH grant R01NS092838
NIH grant R21NS090049

Title: Intracranial pressure monitoring for the safety optimization of intrathecal drug delivery to the CNS

Authors: *V. BELOV[1,2,3], J. APPLETON[1], P. GIFFENIG[1], M. PAPISOV[1,2,3]
[1]Massachusetts Gen. Hosp., Boston, MA; [2]Harvard Med. Sch., Boston, MA; [3]Shriners Hosp. for Children, Boston, MA

Abstract: Objectives. High volume intrathecal (IT) administration is a promising method for barrier-free delivery of macromolecular therapeutics to the CNS. Estimates of its safety thresholds are currently based on limited empirical data thus demanding more comprehensive mechanistic investigation. The goal of this exploratory study is to characterize the dynamics of pressure disbalances in the cerebrospinal fluid (CSF) caused by various modes of IT administration. **Methods**. A 1F piezoresistive diffused semiconductor pressure sensor mounted at the tip of a 20 cm long 0.8 F polyimide catheter (Millar, Inc) was used in all intracranial pressure (ICP) measurements. In rats, the sensor was inserted through a cannula into the cisterna magna. In monkeys, the configuration of the subcutaneous injection ports prevented the sensor placement in the subarachnoid compartment; therefore, ICP was measured within a catheter connected to the port. The recorded values were corrected for the hydrodynamic resistance in the catheter line, based on in vitro measurements. Signal processing was carried out using a FE221 Bridge Amplifier coupled with a PowerLab 4/35 (ADInstruments). Control of both modules and waveform analysis was performed using LabChart 8 software (ADInstruments). **Results.** In rats, a safely tolerable linear elevation of the steady state ICP values at 0.4 mmHg•μl^{-1}•min was

characteristic for the slow infusion rates in the range of a good compensatory reserve (<37 µl/min, ICP stabilization under 18±3 mm Hg) and for the higher rates in the range of a poor compensatory reserve (37-62 µl/min, ICP stabilization between 18±3 and 28±4 mm Hg). Physiological compensation was impaired above 62 µl/min, leading to the exponential ICP elevation found intolerable for > 10 min. However, the acute ICP elevations followed by rapid relaxations, characteristic for bolus injections, were not lethal up to 200 mm Hg (1.5 ml at 4.4 ml/min) and were sigmoidally related to injected volumes. In monkeys, ICP elevation was a linear function of the infusion rate and appeared to be impacted by the pressure resistance in the external catheter line. **Conclusions.** Accelerated CSF drainage and factors of IT compliance enable safe physiological accommodation of the added volume in the subarachnoid space in a rate- and ICP-dependent manner. Monitoring of ICP during the IT injection/infusion procedure can enable safe IT delivery of even large volumes.

Disclosures: V. Belov: None. **J. Appleton:** None. **P. Giffenig:** None. **M. Papisov:** None.

Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 432.17/MMM20

Topic: I.05. Biomarker and Drug Discovery

Title: Effects of silver- and gold nanoparticles on the nervous system- *In vitro* and *in vivo*

Authors: *F. JOHANSSON[1], N. ABDULLA[2], E. SÖDERSTJERNA[1], U. ENGLUND JOHANSSON[3]
[2]Clin. Sci., [3]Div. of Ophthalmology, [1]Lund Univ., Lund, Sweden

Abstract: Much attention is given to nanoparticles (NPs) as carriers in drug delivery of therapeutical agents to central nervous system, including the retina. Nanomaterials are increasingly used in diagnostics, imaging and targeted drug delivery. AuNPs are employed as *e.g.* anti-cancer agents and AgNPs are commonly used due to their antibacterial effects. Despite widespread use, the documentation is limited on the direct effect of Ag- and AuNPs on eukaryotic cells, including neural cells and tissue. Hence, we investigate the uptake and distribution of especially Ag- and AuNPs, as well as their possible toxic effect in a battery of assays ranging from human neural stem cells (HNSC) to the mouse eye *in vivo*. Low concentrations of Ag- and AuNPs (0.022- 0.4 µg/ml) are studied at a cellular-, tissue and organ level, using a HNSC lines and administration to the mouse eye *in vivo*. Uptake and distribution of the NPs are analyzed using TEM. HNSC viability, (MTT and TUNEL assays), cell proliferation (Ki67 marker) and cell cycle analysis and phenotypic differentiation (GFAP (glial/neural) and DCX (neuronal) markers) were studied, including function using

electrophysiology. Adverse effects after intravitreal inj. eere studied using the parameters: gross morphology, glial- and microglial response, cytotoxic effects (apoptosis (TUNEL assay) and oxidative stress (AvidinD staining)). $AgNO_3$ is used as a positive control for the reported toxic effect of Ag ions. Ag- and AuNPs of two different sizes are included, 20 and 80 nm, respectively. Initial data show that in the HNSC assay 20 and 80 nm Ag- and AuNPs are taken up into the cytoplasm, nucleus and mitochondria. Moreover, NP exposure may facilitate HNSC differentiation and neurite outgrowth, and affect the electrophysiological characteristics of human neurons. In *in vivo* studies using the eye as a model, neuronal toxicity (apoptosis and oxidative stress) was primarily seen in the outer nuclear layer, harboring the photoreceptors. Our results strongly suggest careful investigation of the eventual adverse effects of Ag- and AuNPs if these are considered for usage in both daily consumer products and medicine, since models of our nervous systems are clearly affected.

Disclosures: F. Johansson: None. **N. Abdulla:** None. **E. Söderstjerna:** None. **U. Englund Johansson:** None.

Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 432.18/MMM21

Topic: I.05. Biomarker and Drug Discovery

Support: Lundbeck Foundation

Title: Targeted delivery to brain endothelial cells and transport across the blod-brain barrier of shark antibodies (vNAR) following intravenous administration

Authors: *C. L. JACOBSEN[1], K. B. WICHER[2], L. RUTKOWSKI[2], F. S. WALSH[2], T. MOOS[1]
[1]Aalborg Univ., Aalborg Ost, Denmark; [2]Ossianix, Inc, Philadelphia, PA

Abstract: Large molecules in the circulation do not pass through the BBB in sufficient amount to elicit a pharmacological response in the central nervous system (CNS), which is a huge challenge for the treatment of neurological diseases. However, a novel class of small single chain antibodies adapted from the shark immune system (a.k.a. vNAR antibodies) may overcome the obstacle of the BBB when targeted to brain capillary endothelial cells (BCECs). The present study aimed to exploit the cerebral distribution after intravenous injection in female BALB/c mice (n=16) of two vNAR antibodies targeting either the transferrin receptor (TfR) (K4_B2-hFc) expressed by BCECs or both the TfR and CD20 (DUV-B2(HC2N). Using immunohistochemistry, vNAR immunoreactivity was observed in BCECs throughout the entire

CNS. Brain slides of non-immune vNAR-injected animals were devoid of immunoreactivity, except for choroid plexus epithelial cells. The latter were however also labeled irrespective of the injected compound suggesting non-specific uptake at the blood-CSF barrier. K4_B2-hFc and DUV-B2(HC2N) immunoreactivity were observed intraneuronally in several brain regions of the CNS, including forebrain and midbrain regions, but immunoreactivity was mainly in the lower brain stem where the pontine, trigeminal motor, facial motor, and vestibular nuclei, as well as the Purkinje cells of the cerebellum exhibited prominent labeling. Our results show that systemically administered vNAR antibodies accumulate in BCECs in the entire brain as well as neurons primarily in the lower brain stem. These findings indicate that the vNAR antibodies targeting the TfR carry the potential of delivery of therapeutics across the BBB.

Disclosures: C.L. Jacobsen: None. **K.B. Wicher:** None. **L. Rutkowski:** None. **F.S. Walsh:** None. **T. Moos:** None.

Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 432.19/MMM22

Topic: I.05. Biomarker and Drug Discovery

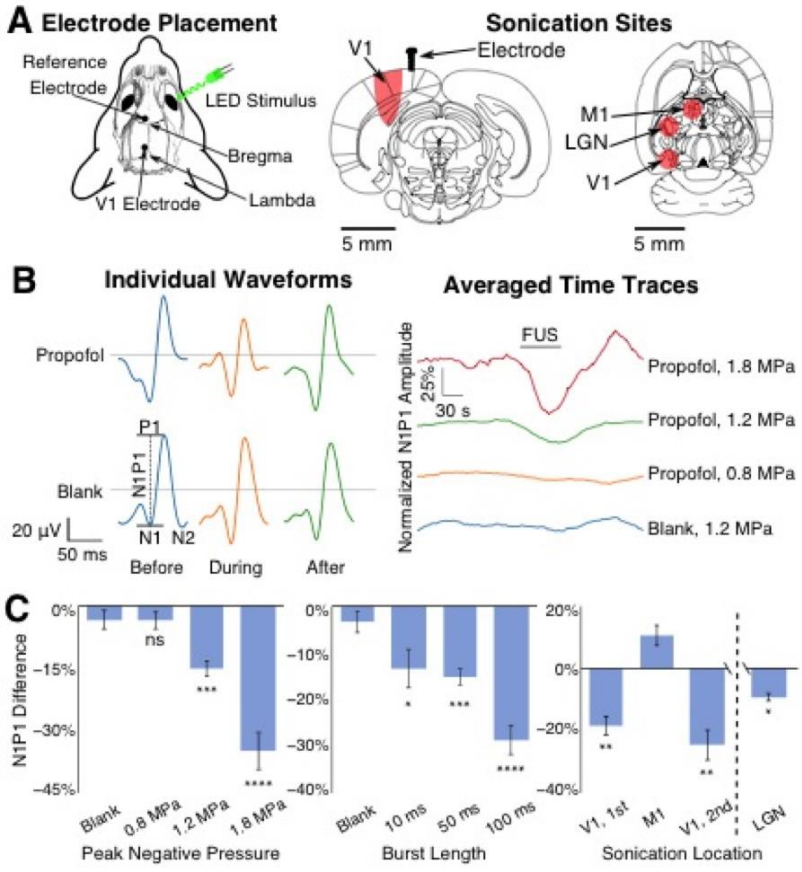
Support: BRAIN Initiative RF1 MH114252
Stanford Center for Cancer Nanotechnology Excellence
Foundation of the American Society for Neuroradiology
Wallace H. Coulter Foundation
William T. Newsome, PhD and the Stanford Neurosciences Institute

Title: Noninvasive neuromodulation with ultrasonic drug uncaging: Electrophysiological assessment

Authors: *M. ARYAL, J. B. WANG, Q. ZHONG, R. D. AIRAN
Radiology, Stanford Univ., Palo Alto, CA

Abstract: Current neuromodulation techniques suffer from invasiveness or limited tissue penetration or spatiotemporal resolution. We have proposed that polymeric perfluorocarbon nanoemulsions can be used to uncage neuromodulatory drugs with focused ultrasound (FUS), enabling noninvasive pharmacological neuromodulation. Here, we accomplish noninvasive neuromodulation with ultrasonic uncaging of the anesthetic propofol. We use visual evoked potentials (VEPs) to determine the temporal kinetics and dose-response relationship of the neuromodulatory effect. Visual evoked potentials were recorded from subdural electrodes implanted on male Long-Evans rats (~200gm). FUS (650 kHz, 1 Hz burst frequency) was used

while varying in-situ pressure, burst length, and brain target. Percentage change of the N1P1 VEP amplitude was measured before, during, and after ultrasonic drug uncaging after administration of propofol (1 mg/kg) versus blank nanoparticles. With visual cortex (V1) sonication, VEPs were attenuated by 15±5%, 34±10% with 1.2, 1.8 MPa sonication pressure, respectively (Fig.1). VEPs were attenuated by 13±8%, 15±5%, 27±8%, with 10, 50, 100ms ultrasound bursts, respectively (Fig.B). While sonicating lateral geniculate nucleus (LGN), VEPs were attenuation by 9±3%, but were not attenuated at motor cortex (M1, Fig.B). The temporal kinetics of these effects had half-lives of 8.8-14.8 s. This work establishes that noninvasive pharmacological neuromodulation with ultrasonic drug uncaging is temporally precise with respect to sonication, with dose-response relationships with sonication pressure and burst length, and with spatial specificity to the sonication site. Presented as mean +/- S.E.M., N=5-10. ns: not significant; *:p<0.05, **:p<0.01, ***:p<0.001; ****:p<0.0001 by two-tailed t-tests.
Fig.1 (A) Schematic of recording electrode placement (*left*) and sonication sites (*right*), represented by the expected full-width half-maximum of the ultrasound field in each location. (B-C) Dose-response relationship, spatial specificity of ultrasonic propofol uncaging.



Disclosures: J.B. Wang: None. **Q. Zhong:** None. **R.D. Airan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 17-163 – Provisional application with Stanford University; QZ and RDA, PCT/US2017/033226 with Johns Hopkins University; RDA.

Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 432.20/MMM23

Topic: I.05. Biomarker and Drug Discovery

Title: Imaging the pharmacokinetics and pharmacodynamics of intrathecally administered antisense oligonucleotides in the rat

Authors: *C. MAZUR[1], K. ZASADNY[2], J. SULLIVAN[2], D. A. WOLF[3], B. POWERS[1], J. HESTERMAN[2], M. SEAMAN[2], R. HOLT[2], I. POLYAK[2], R. COELHO[2], V. GOTTUMUKKALA[2], C. GAUT[2], J. HOPPIN[2], E. SWAYZE[1], A. VERMA[4]
[1]Neurosci. Drug Discovery, Ionis Pharmaceuticals, Inc., Carlsbad, CA; [2]inviCRO, LLC, Boston, MA; [3]Biogen, Inc., Cambridge, MA; [4]United Neurosci., Dublin, Ireland

Abstract: The development of therapeutics for CNS disorders has been impeded by the inability of many potentially therapeutic molecules to cross the blood brain barrier (BBB) and engage their targets. Antisense oligonucleotides (ASOs) are promising therapeutics for treating CNS disorders due to their specific targeting and extended pharmacological effect, however their size and charge impedes their ability to cross the BBB. The intrathecal (IT) dosing route offers a solution for bypassing the BBB and delivering drugs directly to the CNS. Determining the pharmacokinetics (PK) and pharmacodynamics (PD) of ASOs presents unique challenges imposed by anatomical and functional properties of the IT space and reliance upon ex vivo histological molecular techniques. We developed an imaging approach using radio and fluorophore-labeled ASOs tracking PK and employed neuroreceptor targeting ASOs to enable tracking of PD using receptor-targeting radiotracers.
We demonstrate these PK/PD principles using two ASOs which target the MALAT1 non-coding RNA and the GABA-A receptor subunit GABRA1 mRNA. Dynamic SPECT/CT imaging with the ^{125}I-MALAT1 ASO showed widespread time and dose dependent exposure of the neuroaxis tissues following lumbar IT injections, with exposure in cortical structures. A dosing study using either unlabeled GABRA1 or MALAT1 ASO (n=4 per cohort) demonstrated progressive decline in ^{18}F-flumazenil uptake specific to the GABRA1 ASO, with the effect in cortical structures. We confirmed with ex vivo studies that the reduction of ^{18}F-flumazenil uptake corresponded to GABRA1 mRNA and protein reduction produced by the ASO.
We also imaged a Cy7-labeled GABRA1 ASO using 3D cryofluorescence imaging to demonstrate the correlation between the distributions of the IT administered ASO with the regional receptor knockdown demonstrated by the ^{18}F-flumazenil. This 3D cryofluorescence imaging technique offers a bridge between in vivo molecular imaging and ex vivo histology enabling the 3D visualization of the distribution of the fluorescently labeled ASO.

Disclosures: C. Mazur: A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals, Inc. **K. Zasadny:** A. Employment/Salary (full or part-time):; inviCRO, LLC. **J. Sullivan:** A. Employment/Salary (full or part-time):; inv. **D.A. Wolf:** A. Employment/Salary (full or part-time):; Biogen, Inc. **B. Powers:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals, Inc. **J. Hesterman:** A. Employment/Salary (full or part-time):; inviCRO, LLC. **M. Seaman:** A. Employment/Salary (full or part-time):; inviCRO, LLC. **R. Holt:** A. Employment/Salary (full or part-time):; inviCRO, LLC. **I. Polyak:** A. Employment/Salary (full or part-time):; inviCRO, LLC. **R. Coelho:** A. Employment/Salary (full or part-time):; inviCRO, LLC. **V. Gottumukkala:** A. Employment/Salary (full or part-time):; inviCRO, LLC. **C. Gaut:** A. Employment/Salary (full or part-time):; inviCRO, LLC. **J. Hoppin:** A. Employment/Salary (full or part-time):; inviCRO, LLC. **E. Swayze:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals, Inc. **A. Verma:** A. Employment/Salary (full or part-time):; United Neuroscience.

Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 432.21/MMM24

Topic: I.05. Biomarker and Drug Discovery

Title: Blood to brain delivery mechanisms of cholesterol conjugated antisense oligonucleotides

Authors: *C. WRIGHT DWYER[1], B. POWERS[1], M. JACKSON[1], T. P. PRAKASH[2], E. E. SWAYZE[2], P. P. SETH[2], F. RIGO[1]
[1]Neurosci. Drug Discovery, [2]Medicinal Chem., Ionis Pharmaceuticals, Carlsbad, CA

Abstract: Antisense oligonucleotides (ASOs) are a versatile drug platform for the treatment of many neurological diseases. Currently ASO therapies must be administered directly into the cerebral spinal fluid to gain access to the central nervous system. Drug delivery approaches to achieve blood to brain transfer of systemically administered ASO therapies represent a promising future avenue with many clinical benefits. We have demonstrated that a cholesterol conjugated RNase H gapmer ASO achieves blood to brain delivery after intravenous administration. Through a series of imaging studies using fluorescently modified ASOs, we determine the mechanisms leading to blood-to-brain transfer. Chemical structure-activity relationship studies uncover important principles in the design of cholesterol conjugated ASOs for achieving blood to brain transfer. The results from our studies document the feasibility of achieving blood to brain delivery of ASOs.

Disclosures: C. Wright Dwyer: A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual

property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals. **B. Powers:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals. **M. Jackson:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals. **T.P. Prakash:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals. **E.E. Swayze:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals. **P.P. Seth:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals. **F. Rigo:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals.

Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 432.22/MMM25

Topic: I.05. Biomarker and Drug Discovery

Title: Development and characterization of an *in vitro* human iPSC-derived blood-brain barrier model

Authors: *P. HAYDEN, M. KLAUSNER, J. MOHN
Mattek Corp., Ashland, MA

Abstract: Objective and Rationale: Solute distribution between blood and brain is strictly regulated by the blood-brain barrier (BBB). The BBB poses a challenge for delivering therapeutics, including anticancer, antibiotic or antipsychotic drugs into the brain. Preventing potentially damaging molecules from overcoming the BBB is also an increasing problem, especially when combinations of therapeutics are encountered. Medical and pharmaceutical scientists therefore have a growing need for rapid, reliable in vitro models of the BBB for preclinical screening of pharmaceutical BBB transport properties. Differentiation of human iPSC into blood-brain barrier endothelial cells was recently reported. The goal of the current exploratory work is to build upon this breakthrough to produce scalable and reproducible human

iPSC-derived BBB models for use in toxicology and drug development applications.
Methods: iPSCs (male, 68 years old) were differentiated to endothelial progenitors by activating canonical Wnt signaling. Subsequent treatment with retinoic acid led to further endothelial differentiation and acquisition of a BBB-phenotype. The differentiated cells were seeded onto microporous membrane inserts and development of BBB specific properties were assessed at various times and culture conditions. Development of barrier function was assessed by measurement of transendothelial electrical resistance (TEER). Immunocytochemical (ICC) staining was performed to evaluate the expression of tight junction protein ZO-1, and BBB-specific markers including glucose transporter GLUT1 and various drug transporter proteins.
Results: Optimization of seeding density and culture conditions resulted in uniform endothelial cell monolayers with evidence of robust tight junction and barrier formation. TEER > 1000 ohms X cm^2 was maintained for up to 6 days. ICC staining demonstrated uniform expression of the tight junction protein ZO-1 localized along the endothelial cell borders. Permeation of Lucifer yellow across the BBB culture was low, further demonstrating development of barrier function. GLUT1 showed diffuse staining.
Conclusions: These results show significant progress in development of a reliable human BBB model that will be useful for preclincal screening of candidate pharmaceutical compounds. The model is easily scalable and can be adapted to 96-well microporous membrane plate formats, allowing use in high-throughput applications. Further efforts will continue to characterize expression and activity of BBB drug transporters, and the inventory of iPSC-derived BBB cells will be expanded to include an array of ages, gender and disease conditions.

Disclosures: P. Hayden: None. **M. Klausner:** None. **J. Mohn:** None.

Poster

432. Biomarker and Drug Discovery: Drug Delivery and Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Monday, November 5, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 432.23/MMM26

Topic: I.05. Biomarker and Drug Discovery

Support: NSF Grant 1632881
Civitan International Research Center Emerging Scholars Award

Title: Focused ultrasound blood brain barrier opening mediated delivery of MRI-visible nanoclusters for noninvasive neuromodulation with spatiotemporal precision

Authors: *M. C. RICH[1], J. SHERWOOD[3], Y. BAO[3], F. LUBIN[1], M. BOLDING[2]
[1]Neurobio., [2]Radiology, Univ. of Alabama Birmingham, Birmingham, AL; [3]Dept. of Chem. and Biol. Engin., Univ. of Alabama at Tuscaloosa, Tuscaloosa, AL

Abstract: Systemic drug delivery produces off target effects which limits the ability to pharmacologically treat or investigate region specific brain function. Furthermore, side effects can often outweigh clinical treatment potential and confound preclinical results. Additionally, many potentially useful drugs cannot penetrate the blood brain barrier (BBB) requiring drug modifications that jeopardize or completely abolish its therapeutic action. Local drug delivery currently requires invasive methods such as injections, cannulae, and pumps that damage tissues confounding results and limiting translational efficacy. Agents that are not BBB permeable can now be delivered to specific brain regions via focused ultrasound (FUS) mediated BBB opening. FUS can be targeted anywhere in the brain with high spatial resolution to open the BBB, allowing location specific passage of systemic agents into target brain regions. However, FUS BBB opening by itself requires the use of BBB impermeable agents, lacks temporal control and circulating drugs can still cause systemic effects. Here we employ an MRI visible albumin based nanoclusters to encapsulate neuromodulators for delivery to target brain regions noninvasively via FUS facilitated BBB opening. We show that nanoclusters can encapsulate neuromodulators such as glutamate and NBQX with a very low baseline release rate. In addition, we show that IV injected nanoclusters can locally diffuse into the brain with FUS facilitated BBB opening (BBBO) and provide enhanced MRI contrast at the site of delivery. Drug release into brain tissue is triggered by a second FUS treatment (FUS-release) using different parameters from the FUS used to open the BBB. Furthermore, FUS-release causes a change in MRI contrast providing in-vivo confirmation of drug release. Importantly, we show that the drug loading capacity of the nanoclusters is sufficient for inducing localized changes in neural activity in response to glutamate release from nanoclusters in vivo. This new platform will provide noninvasive activation and silencing of spatially precise brain locations to test for effects on behavior while providing independent validation of the site of neuromodulation. This avoids the circularity of using the neuromodulatory effect to verify location of delivery.

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