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Excitatory to inhibitory transition in GABAergic currents guides circuit formation of cortical interneurons

Authors: *K. ZAVALIN*, A. HASSAN, Z. KHERA, E. DELPIRE, A. H. LAGRANGE;  

Abstract: Introduction: Excitatory GABA is a crucial developmental cue that guides perinatal neuronal migration, synaptogenesis, and circuit formation. The switch from embryonic depolarizing GABA to mature hyperpolarizing responses is mainly determined by the onset of KCC2 expression. Previous work by other groups has found that prematurely upregulated KCC2 function produces dramatic abnormalities in brain development, especially in GABAergic cortical interneurons (INs). However, few studies address the repercussions of delayed onset of hyperpolarizing GABA. Here, we investigate how indefinitely-prolonged depolarizing GABA responses in INs adversely affect cortical circuit development using an IN-specific knockout (KO) mouse -DLX5:cre-IRESEGFP; KCC2flox.

Hypothesis: We hypothesize that GABAergic interneuron migration and inhibitory circuit formation is critically dependent on the timing of hyperpolarizing GABA onset. We expect an anachronistic excitable physiology and altered distribution/circuit integration in KCC2KO INs.

Results/Discussion: KO mice exhibit abnormal neurological development, including: spontaneous seizures, a 45% faster onset of fluorothyl-induced seizures, reduced body weight, and late postnatal mortality, but normal gross and histological anatomy of organs, including feeding structures and the brain. We observe the earliest cortical KCC2 expression in layer 5 (L5) INs, as early as E16-18 in wild-type mice. We thus expected these cells to be the most affected by loss of KCC2, but instead found comparable densities of cells in both maturing (Dlx5:GFP+) and all INs (pan-IN marker) in L5 of KO and sibling. In addition, inhibitory synapses are roughly similar in quantity and GABA receptor subtype composition.

We then postulated that the effects of KCC2 loss might be specific to IN subtypes, which in L5 consist mainly of somatostatin (SST) and parvalbumin (PV) INs with distinctly different circuit
functions and developmental sequences. We found a 24% increase in L5 density of SST INs in KO, but no change in PV IN density. Accordingly, we found no change in frequency of L5 principal cell sIPSCs, which reflect somatic and proximal dendrite input of predominantly PV IN projections. Hence, while a timely KCC2 expression in INs is vital for cortical circuit functionality, our preliminary data suggest that only the L5 SST INs, but not the L5 PV INs, are affected in the KO. We are now investigating (1) the circuit-level repercussions of abnormal SST IN development in the KO, and (2) if an earlier IN developmental trajectory is particularly sensitive to KCC2 loss.


Poster

276. Nervous System Patterning and Transplantation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 276.02/A2

Topic: A.01. Neurogenesis and Gliogenesis

Title: Effect of body weight and breeding position within the uterine horn on the hippocampus of the Wistar rat

Authors: *B. RUIZ VELÁSQUEZ1, R. HUDSON2, I. JIMÉNEZ3, P. PACHECO1, M. ALVARADO1;
1Neurobiología del Desarrollo, Inst. De Neuroetología, Univ. Veracruzana, Xalapa, Veracruz, Mexico; 2Inst. de Investigaciones Biomédicas, UNAM, Mexico, Mexico; 3Dept. de Fisiología, Biofísica y Neurociencias CINVESTAV-IPN, Mexico, Mexico

Abstract: Introduction: In the rat, the body weight of the individual is important during the first postnatal ages (P), in the obtaining of resources and in the development of the systems. The pups have been classified as heavy, intermediate and light. It has been shown that before birth, the position of the offspring within the uterine horn (UH) and the neighboring brothers affect the hormonal levels of the breeding. They influence spatial memory capacity in adult ages, where mainly the hippocampus is one of the structures involved. Objective: To determine the number of hippocampal cells in the fetuses that presented differences in body weight (heavy/light) and their position in the UH on embryonic day 21 (E21). Methods: Seven litters were obtained from pregnant females, who underwent cesarean section in E21 to obtain the fetuses. The following biological parameters were taken into account: body weight, sex and position of the offspring within the UH (O, close to the ovary, M middle region and C near the cervix). By litter the heaviest and lightest brood of each sex was selected (n=7 in each group). Heavy male: HM; light male: LM; heavy female: HF; light female: LF. The histological process and Nissl staining were performed. The hippocampus was analyzed with the ImageJ program to obtain cell density. For
the statistical analysis, correlations were made (r=≥0.50) and a hierarchical analysis was carried out where Fisher's post hoc test was carried out (P=0.05). Results: the heavy offspring; HM and HF, were located in 90% in C, being the first to be born. The HF of the left uterine horn obtained values between 13000 μm² to 15000 μm², those of the right UH show values between 6000 μm² to 8000 μm². The HM, present values around 15000 μm². Regarding the correlations, it was observed that the pupils located in O, when increasing the body weight, the total number of cells in the hippocampus was higher (r=0.51). Significant differences were found between HM and HF in the total number of hippocampal cells; HM x̅=5878.0 ± 753.1 and HF x̅=4780.9 ± 846.1 (P=0.04). Conclusion: The heavy offspring are the first to be born. The body weight and sex of the individual, affect the development of the hippocampus from prenatal ages.


Poster

276. Nervous System Patterning and Transplantation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: A3

Program #/Poster #: 276.03/A3

Topic: A.01. Neurogenesis and Gliogenesis

Title: Effect of the intake of blue tortilla additioned with organic acids and processed with microwave, on the development of hippocampus in Wistar rat

Authors: *P. GONZÁLEZ NIETO1, M. ALVARADO2, J. CHÁVEZ SERVIA3, R. GUZMÁN GERÓNIMO4;
1Inst. De Neuroetologia, Xalapa Veracruz, Mexico; 2Inst. de Neuroetologia, Univ. Veracruzana, Xalapa Veracruz, Mexico; 3CIIDIR-Unidad Oaxaca, Inst. Politécnico Nacional, Oaxaca, Mexico; 4Inst. de Ciencias Básicas, Univ. Veracruzana, Xalapa Veracruz, Mexico

Abstract: During pregnancy, the adequate supply of nutrients is essential for the proper development of the fetus. Basic foods such as corn (Zea mays) and specifically blue corn contain anthocyanins (antioxidants) that are bioactive compounds that help stimulate neurogenesis. However, the process of nixtamalization degrades anthocyanins. This work explored the effects of nixtamalization and alternative processes to maintain their biological properties. The nixtamalization by microwaves added with gallic acid was selected to continue with the biological test, obtaining 479.79 ± 0.06 mg total polyphenols Eq of gallic acid/100g and of anthocyanins 61.02 ± 1.80 mg C3G/100g with an increase of 43% and 69% respectively compared to traditional nixtamalization. The effect on the dentate gyrus of the hippocampus was evaluated, which participates in learning processes, memory, spatial perception and especially neurogenesis; process that takes place in the prenatal stage and continues in adulthood in this area. It was developed in 5 groups with Wistar female rats (control, traditional nixtamalization...
with and without acid and nixtamalization by microwaves with and without acid) the fetuses were extracted on day 20 of gestation. The fetuses used were obtained from 6 average litters of each one a male was selected. The histological process was performed on the brains of the fetuses, afterwards the count of the number of cells per unit area (cells / mm²) of the dentate gyrus was carried out. The group of tortilla nixtamalizada by microwaves added with gallic acid predominated with values of 7305.09 ± 515.23 cells/mm² having an increase of 33% compared with the control which obtained 5465.02 ± 267.27 cells / mm² and an increase of 23% compared to the traditional nixtamalization without acid having values of 5900.28 ± 202.65 cells / mm². Further, the use of microwaves favors the cells size, the range < 30 μm² the use of the microwave and the favorable ranges ≥ 30 μm² microwaves doubled the cells size, while in the ranges greater than 60, without the addition of acid stimulated the increase. These data suggest that the process of nixtamalization by microwaves and added with gallic acid retains a greater quantity of anthocyanins and improves its biological activity.


Poster

276. Nervous System Patterning and Transplantation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 276.04/A4

Topic: A.01. Neurogenesis and Gliogenesis

Title: Generation of vascularized brain organoid and a model of brain angioenesis

Authors: *X. SUN;
Inst. of Neurosci., Shanghai, China

Abstract: Brain organoids derived from human pluripotent stem cells (PSCs), including induced PSCs (iPSCs) and embryonic stem cells (ESCs), can self-organize to form distinct brain regions and have been used to recapitulate developmental programs of human fetal brain, model developmental brain diseases. However, the lack of vasculatures composed mainly of endothelial cells (ECs), which have been shown to regulate embryonic neurogenesis, brain disorders, and aging process, limits its applications. During embryonic development, endothelial progenitor cells arise from migrating vascular progenitors (VPs) derived from mesodermal cells, while the ectodermic neural fate is triggered by the inhibition of BMP signaling, which makes it difficult to generate brain and vascular simultaneously. In this study, we develop the sequential induction approaches for vascular and brain organoids respectively, which could be fused together to generate the vascularized brain structures. Meanwhile, the blood-brain-barrier(BBB) structures could be detected in cultured brain organoids.
Disclosures:  R. Beattie: None.  S. Laukoter: None.  F.M. Pauler: None.  K.I. Nakayama: None.  S. Hippenmeyer: None.
**Poster**

**276. Nervous System Patterning and Transplantation**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 276.06/A6

**Topic:** A.01. Neurogenesis and Gliogenesis

**Support:** Fondecyt 1150444
Conicyt 21160410

**Title:** AP-4 modulates ApoER2 secretory trafficking and reelin induces dendritic development

**Authors:** *M. O. CARACCI, V. MACIAS, M.-P. MARZOLO*
Biologia Celular y Mol., Pontificia Univ. Catolica De Chile, Santiago, Chile

**Abstract:** Adaptor Protein Complex 4 (AP-4) is a heterotetrameric complex (β4, σ4, µ4 and ε) which regulates protein trafficking from the TGN to endosomes and the plasma membrane. Each subunit has been associated to hereditary spastic paraplegia (HSP). The µ4 subunit directly recognizes tyrosine-based motif through structurally distinct canonical and non-canonical binding pockets. Few transmembrane protein cargos have been described, among them the Amyloid Precursor Protein (APP) and the Autophagy-related protein 9A (ATG9A) have received considerable attention. In the present work we show that ApoER2 secretory trafficking is disturbed by AP-4 deficiency. First, we followed the secretory pathway of ApoER2 using FM4-ApoER2-GFP co expressed with µ4 WT or binding defective mutant for the canonical and non-canonical binding pockets of µ4 in hippocampal neurons and HeLa cells. We found that expression of defective canonical binding pocket lead to a delayed exit from the TGN in HeLa cells and to differential ApoER2 distribution in hippocampal neurons. We further confirmed these observations using previously described HeLa ε KO cells where we found again a delayed release from the TGN. Surface expression of tagged ApoER2 was also reduced in HeLa KO cells compared to WT, further suggesting AP-4 complex regulates ApoER2 secretory pathway. Since ApoER2 is a bonafide Reelin receptor we tested if expression of WT or binding defective mutants affected Reelin mediated enhancement or primary dendrites. Surprisingly we observe that the effect of Reelin is greatly reduced in defective canonical binding pocket expressing neurons while expression of µ4 WT and defective non-canonical binding pocket expressing neurons showed enhanced Reelin induction of primary dendrites. All together our results show that ApoER2 is a potential novel protein cargo of the AP-4 complex, ascribing a role for this receptor and for Reelin signalling pathway in the development AP-4 associated neurodevelopmental disorders such as HSP.

**Disclosures:** M.O. Caracci: None. V. Macias: None. M. Marzolo: None.
Title: A role of autism related Chd8 in normal cerebellar development and motor functions

Authors: *C. Xiang*¹, C. Dong², Y. Lin¹, W. Zhou¹, R. Q. Lu²;¹Children's Hosp. of Fudan Univ., Shanghai, China;²Cancer and Blood Dis. Institute, EHCB, Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract: Autism spectrum disorder (ASD) is a complex neurodevelopmental disease defined by deficits in social interaction, repetitive and restrictive behaviors. Motor impairment and developmental co-ordination disorder, the cardinal feature in ASD, are among the earliest signs of an autistic phenotype. Cerebellum is essential for the spatial accuracy and temporal coordination of movement. Abnormal cerebellar pathology has been observed in autistic individuals. Recent studies indicate that CHD8 represents one of the most high-risk susceptibility genes in ASD. A cohort of ASD patients exhibit cerebellar hypoplasia and develop ataxia, however, the underlying mechanisms of Chd8 in regulation of cerebellum development remain poorly understood. Here, we find that Chd8 is broadly expressed in the developing cerebellum. The mice with Chd8 deletion in cerebellar neural progenitor cells exhibit severe malformation in cerebellar cytoarchitecture and lamination, as well as develop locomotion defects. In addition, genetic inactivation of Chd8 in cerebellar granule neuron progenitors (GNP) leads to cerebellar hypoplasia and GNP dysgenesis in mice. The Chd8 mutant mice develop ataxia detected by rotarod test and beam walking test. Transcriptome profiling further indicate that Chd8 regulates a set of GNP-associated genes and signaling pathways. Our studies reveal that Chd8 is necessary for GNP development and cerebellar formation.

Single-cell RNA sequencing of cell populations in the forebrain of embryonic chickens

**Authors:** *L. L. BRUCE*¹, Y. ZHANG¹, S. KURAPATI², N. J. CHOI¹, J. ZUO¹;
¹Biomed. Sci., ²Pharmacol., Creighton Univ., Omaha, NE

**Abstract:** The neural plate of all vertebrates is believed to be composed of homologous cell populations of pluripotent progenitor cells. During embryonic development these progenitor cells gradually become specialized and acquire distinct functions and morphologies. The chicken forebrain is generally subdivided into pallium (cortex- and hippocampus-like) and subpallium (striatum- and pallidal-like) regions. The goal of this project is to investigate the dynamics and timing of molecular specification of chicken neuronal populations within pallium and subpallium regions at early developmental time periods. We will test the hypothesis that each forebrain region is born from a distinct progenitor region rather than from continuous gradients. Forebrain tissue was removed from euthanized chicks at embryonic day 5 and cells were dissociated using papain. Single cell RNA sequencing was performed using a 10X Genomics Chromium single cell 3’ reaction, followed by DNA sequencing using an Illumina HiSeq X platform. A tSNE plot and heat map for 2 samples of 3000 cells each (3 forebrains per sample) was used to visualize cell clusters. Combinatorial gene expression patterns identified major clusters as red blood cells, collagen producing cells, neural crest cells, and brain-related cells. Within the brain-related cell cluster, cells were further clustered according to their developmental state, based on genes strongly expressed in (1) mitotic progenitor, neuroepithelial, and glial cells and (2) in young, maturing neurons. Forebrain cells were identified based on FoxG1+ expression. Of these, 23% of the FoxG1+ forebrain cell progenitor population expressed one or more pallium-specific genes (Emx1, Emx2, Tbr1, Eomes; 9%) or subpallium-specific genes (Dlx1, Dlx2, Dlx5, Isil, Lhx6, Lhx8, Shh; 11%), or both (3%), whereas 94% of the maturing neuron population expressed pallial (18%), subpallial (71%) specific genes, or both (3%). Thus, during chick development, the forebrain regional specification begins at progenitor stages and is well established in young
maturing neurons. Combinatorial expression of transcription factors will be used to identify further clustering within these populations and to reconstruct the transcriptional trajectories of cell populations.


Poster

276. Nervous System Patterning and Transplantation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 276.09/A9

Topic: A.01. Neurogenesis and Gliogenesis

Support: Ruth K. Broad Foundation
NIH EY024694
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Title: Large scale death of retinal astrocytes during normal development mediated by microglia

Authors: V. M. PUNAL¹, C. E. PAISLEY¹, F. BRECHA¹, M. A. LEE¹, R. PERELLI², E. G. O’KOREN¹, C. R. ACKLEY³, D. R. SABAN¹, B. E. REESE⁴, * J. N. KAY¹;
²Neurobio., ¹Duke Univ., Durham, NC; ³Univ. of California, Santa Barbara, Santa Barbara, CA; ⁴Neurosci. Res. Inst., Univ. of California, Santa Barbara, CA

Abstract: Naturally-occurring cell death is a fundamental developmental mechanism for regulating cell numbers and sculpting developing organs. This is particularly true in the central nervous system, where large numbers of neurons and oligodendrocytes are eliminated via apoptosis during normal development. Given the profound impact of death upon these two major cell populations, it is surprising that developmental death of another major cell type - the astrocyte - has rarely been studied. It is presently unclear whether astrocytes are subject to significant amounts of developmental death, or how it occurs. Here we address these questions using mouse retinal astrocytes as our model system. We show that the total number of retinal astrocytes declines by over 3-fold during a death period spanning postnatal days 5-14. Surprisingly, these astrocytes do not die by apoptosis, the canonical mechanism underlying the vast majority of developmental cell death. Instead, we find that microglia kill and engulf astrocytes to mediate their developmental removal. Genetic ablation of microglia inhibits astrocyte death, leading to a larger astrocyte population size at the end of the death period. However, astrocyte death is not completely blocked in the absence of microglia, apparently due to the ability of astrocytes to engulf each other. Nevertheless, mice lacking microglia showed
significant anatomical changes to the retinal astrocyte network, with functional consequences for the astrocyte-associated vasculature leading to retinal hemorrhage. These results establish a novel modality for naturally-occurring cell death, and demonstrate its importance for formation and integrity of the retinal gliovascular network.


Poster

276. Nervous System Patterning and Transplantation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 276.10/A10

Topic: A.01. Neurogenesis and Gliogenesis

Title: Timing and specificity of Emx2 in area patterning


Abstract: All developmental processes are tightly regulated with respect to onset, cessation, as well as the specificity of actions undertaken. Transcription factors (TFs) are often utilized in the coordination and regulation of these processes, and homeobox TFs in particular regulate patterning of tissues and organs during development. Emx2 is a homeobox TF that has been previously demonstrated to regulate the patterning of functional areas in the developing telencephalon (area patterning), in particular the primary visual area (V1). Recent data and the development of novel approaches allowed for better exploration of the timing of Emx2’s role in area patterning. Most early cortex-specific deletions have been performed utilizing the Emx1-IRES-Cre mouse line to mediate the enzymatic removal of floxed target genes. However a Cre-driver mouse line utilizing the Foxg1 promoter (i.e. Foxg1-IRES-Cre) has been generated which allowed a target gene to be deleted one day earlier than the Emx1 Cre-driver line. Through this comparison we determined that Emx2 function in area patterning occurred at the onset of expression as earlier deletion of Emx2 caused a larger reduction in V1 size in comparison to deletion at the later time point. In addition to timing we also explored the specificity of Emx2 function. Recent research has demonstrated that deletion of Emx1 yields an area patterning phenotype similar to the cortex-specific deletion of Emx2. The similarities in phenotype paired with the similarities in evolutionary origin, expression pattern, and timing of expression suggested that some functional redundancy might exist. We employed a multiple allele deletion approach to assess whether there is a gene dosage effect of the Emx genes on area patterning. Our results indicated that the actions of Emx1 and Emx2 were distinct, as double heterozygous
mutants did not exhibit a significant change in V1 size, while single gene homozygous mutants did. Additional Emx allelic deletion combinations were also generated and the area patterning phenotypes observed were consistent with the initial finding that the actions of Emx1 and Emx2 are distinct.


Poster

276. Nervous System Patterning and Transplantation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 276.11/A11

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01NS099099

Title: Transcriptional regulation of cortical development by CoupTF1, Emx2 and Pax6

Authors: *A. R. YPSILANTI*¹, K. PATTABIRAMAN¹, R. CATTO-PRETTA³, O. GOLONZHKA¹, S. LINDTNER¹, K. TANG⁴, I. JONES², Y. SHEN², A. ABNOUSI⁵, I. JURIC⁵, M. HU⁵, M. J. HAWRYLYCZ⁶, C. L. THOMPSON⁷, H. ZENG⁷, I. BAROZZI⁸, A. S. NORD³, J. L. RUBENSTEIN⁰;


Abstract: This study aims to uncover the transcription network that regulates cortical regional patterning. Using in situ hybridization, we screened the expression of around 700 transcription factors (TFs) at E11.5 in the mouse, and identified around 30 that are expressed in rostrocaudal and/or dorsoventral gradients in the ventricular zone. Next, we tested whether their cortical expression was altered in CoupTF1, Emx2 and Pax6 mutant mice, and classified their phenotypes according to their change of expression in the context of abnormal regional architecture. Next, to assess whether their expression changes were due to direct regulation by CoupTF1, Emx2 and Pax6, we performed TF ChIP-seq, in conjunction with assays for epigenomic marks, DNA accessibility and chromatin-looping conformation in wild-types and mutants. This enabled us to map potential regulatory enhancers around the genetic loci of the 30
TFs of interest. We provide evidence that these TFs are combinatorially regulated by CoupTF1, Emx2 and Pax6.


Poster

276. Nervous System Patterning and Transplantation

Location:  Hall A  

Time:  Monday, October 21, 2019, 8:00 AM - 12:00 PM  

Program #/Poster #:  276.12/A12  

Topic:  A.01. Neurogenesis and Gliogenesis  

Support:  NIH Grant K08-NS099502  

ProjectALS  

Title:  Genetic mapping of diversity among developing brainstem motor neuron subtypes at single cell resolution  

Authors:  *M. F. ROSE*¹, A. P. TENNEY³, D. CREIGHTON³, A. GELBER³, M. A. TISCHFIELD³, T. COLLINS³, A. A. NUGENT², P. ANG³, S. IZEN³, M. R. BAUER³, W. HUANG³, R. SATIJA⁴, O. ROZENBLATT-ROSEN⁵, A. REGEV³, E. C. ENGLE²;  

¹Pathology, ²Boston Children's Hosp., Boston, MA; ³Boston Children’s Hosp., Boston, MA; ⁴New York Genome Ctr., New York, NY; ⁵The Broad Inst., Cambridge, MA  

Abstract:  The brainstem ocular motor neurons (OMNs) mediate eye movements and are differentially affected compared with other motor neuron (MN) groups in some disorders. Specific subsets of OMNs show disrupted or aberrant innervation in congenital cranial dysinnervation disorders (CCDDs). In contrast, OMNs continue to function in Amyotrophic Lateral Sclerosis (ALS), while spinal and other brainstem MNs degenerate. Here we define unique developmental gene expression patterns among OMNs, and generate a toolbox of genetic markers to help study these disorders. We isolated and compared seven distinct motor neuron populations: the three ocular motor nuclei (CN3, CN4, CN6) and the other primary MN types (CN5, CN7, CN9/10/12 in the brainstem, and spinal cord MNs). We used microdissection and fluorescence-activated cell sorting (FACS) from multiple mouse lines to follow their developmental trajectories: embryonic days E9.5-E12.5 (*Islet-1:GFP, Hb9:GFP*), E13.5-E14.5 (*IsIlCre;Rosa26tdTomato*), and E14.5-E18.5 (*ChatCre;Rosa26tdT-2A-H2B-GFP*). Pooled RNA-seq analysis at E10.5-E11.5 (Genesifter, DESeq2) was combined with single cell and single nuclei
RNA-seq at E9.5-E18.5 (plate- and droplet-based; Cell Ranger, Seurat, SPRING). Gene expression was validated with database analysis and in situ hybridization. We co-labeled for BrdU/EdU after injection on different developmental days (E8.5-E11.5) to correlate the gene expression differences with cell age. Each MN population showed unique gene expression patterns, including novel markers of OMNs, providing a genetic fingerprint of developing MNs for future studies. Spatially- and temporally-distinct subpopulations were identified within the ocular motor nuclei, corresponding to specific divisions of the oculomotor nerve, selectively affected in some CCDDs. The single nuclei FACS RNAseq protocol offers a new tool to enrich for small populations with unique gene expression at late embryonic/postnatal times when it is difficult to collect intact neurons. These data uncover distinct developmental gene expression patterns and markers of the various cranial motor neurons and provide new tools to study their selective vulnerability in the CCDDs.


Poster

276. Nervous System Patterning and Transplantation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program#/Poster#: 276.13/A13

Topic: A.01. Neurogenesis and Gliogenesis

Title: Pou3f1 identifies a novel subtype of glutamatergic cerebellar nuclear neurons in the mouse cerebellum

Authors: *J. T. YEUNG¹, J. P.-H. WU³, M. RAMIREZ², R. ROBERT⁴, F. CONSORTIUM⁵, D. GOLDOWITZ⁶;
¹Med. Genet., ²Genome Sci. and Technol., Univ. of British Columbia, Vancouver, BC, Canada; ³Mol. and Cell Biol., Univ. of California, Berkeley, Berkeley, CA; ⁴Univ. de Rennes 1, Brittany, France; ⁵RIKEN Japan, Yokohama, Japan; ⁶Med. Genetics; U of BC, Ctr. for Mol. Med. & Ther., Vancouver, BC, Canada

Abstract: The generation of diverse cell types in the brain is governed by sequential expression of genes. To elucidate the underlying gene regulatory cascades that produce cerebellar cell types, our lab and FANTOM5 consortium collected a dynamic transcriptomic dataset of cerebellum across embryonic (E) and neonatal (P) timepoints (E11-P9). Gene regulatory network analysis of this dataset has identified novel candidate transcription factors that have not previously been associated with cerebellar development. One of these genes, Pou3f1, is highly expressed during gastrulation and in developing mouse brain, and has been suggested to play a role in neural
differentiation, but its role in cerebellar development is unclear. Our FANTOM5 data indicates that in mouse cerebellum, Pou3f1 transcript expression peaks at E12. *In-situ* hybridization demonstrated that Pou3f1 transcript is localized to the newly generated progenitors that arise from the rhombic lip and during their migration along the subpial surface of the cerebellum at E11-12. The expression pattern of Pou3f1 suggested that these cells may be glutamatergic cerebellar nuclear (CN) neurons. To test this hypothesis, we examined the Pou3f1-expressing cells in a mutant that lacks all glutamatergic lineages in the cerebellum, i.e. the *Atoh1*-null cerebellum. Pou3f1+ cells are missing in the *Atoh1*-null cerebellum indicating that these are glutamatergic neurons. We previously found that Pax6 is another key molecule expressed during the development of glutamatergic CN neurons and regulates the survival of these cells. Examination of Pou3f1 and Pax6 in wildtype E13 cerebellum shows that the two molecules are not co-expressed. To further examine the relationship between Pax6 and Pou3f1, we studied Pou3f1 expression in the *Pax6*-null *Small Eye (Sey)* mutant. Surprisingly, Pou3f1 expression is observed in the *Sey* mutant cerebellum. The lack of co-staining between Pou3f1 and Pax6 in the CN neuron progenitors, as well as the presence of Pou3f1-expressing cells in the *Sey* mutant suggests that Pou3f1+ nuclear neurons are a novel subtype of glutamatergic CN neuron. To map the position of Pou3f1+ CN neurons in the cerebellum, we examined the expression pattern of Pou3f1 with other CN neuron markers such as Tbr1, Brn2 and Irx3 in the E18 cerebellum. While Tbr1+ glutamatergic CN neurons reside in the fastigial nuclei, the GABA-ergic CN neurons (Brn2+ and Irx3+) are found in the interposed and lateral nuclei. Pou3f1 largely co-labeled with Brn2 and Irx3. Our findings reveal that Pou3f1 marks a novel subtype of glutamatergic CN neurons and revises the lineage map of CN neurons distribution.


**Poster**

**276. Nervous System Patterning and Transplantation**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 276.14/A14

**Topic:** A.01. Neurogenesis and Gliogenesis

**Title:** Characterizing Msx genes in mouse cerebellar development

**Authors:** *I. GUPTA*, F. CONSORTIUM*, J. YEUNG*, *D. GOLDOWITZ*;  
*Med. Genet., Univ. of British Columbia, Vancouver, BC, Canada; RIKEN, Yokohama, Japan

**Abstract:** The cerebellum accounts for 80% of the neurons in the brain. Developmental defects of the cerebellum impair fine motor functions (Ataxia) and cognitive functions (ASD, Schizophrenia, ADHD). During embryogenesis, the cerebellar anlage is influenced by multiple signaling centers including the adjacent fourth ventricle roof plate, which secretes Bmp, Wnt,
and retinoic acid. Cerebellar neurons are then produced from two distinct progenitor zones: the *rhombic lip* (RL) and *ventricular zone* (VZ). Bmp signaling activates the Msx (muscle segment homeobox) family of genes that has conserved homeodomains. They have been studied in fruit fly development and are implicated in mammalian CNS patterning, but their exact function is unknown. Different functional studies inactivating BMP signaling components in cerebellum have resulted in the loss of, or defects in, RL stem cell specification, and reduced number of Purkinje cells and interneurons. In mice and humans, the Msx family has 3 members - Msx1, Msx2 and Msx3. Our aim is to characterize these 3 genes in cerebellar development in the mouse. We participated in the FANTOM5 consortium to create a transcriptomic expression dataset, which was collected from the developing cerebellum tissue at 12 timepoints - embryonic days (E) 11.5 to E18.5 and postnatal days 0, 3, 6 and 9 - through cap analysis of gene expression (CAGE) sequencing. Temporal expression patterns for the 3 Msx genes obtained from this dataset show that they are expressed highest in early embryonic ages, and *in situ* hybridization (ISH) on these early age tissue shows their expression being limited to the progenitor zones. This suggests their involvement in early developmental processes like proliferation, specification and migration. We found that Msx1 and Msx2 are co-expressed with Atoh1 in the RL, which marks all glutamatergic lineages that arise from the RL. While Msx1 is more limited to RL, Msx2 expression is also seen in the VZ albeit more weakly. Msx3 is very strongly expressed in the VZ at E11.5 and E12.5. At these ages, Msx3 is expressed beyond the Ptf1a positive domain, while still being limited to the VZ. Interestingly, at E13.5 and E14.5, Msx3 shows compartmentalized expression within the VZ along with lateral-medial differences. This pattern imitates that of Olig2, a marker for Purkinje cell progenitors. Olig2 and Gsx1 are known to control the temporal identity transition of VZ progenitors by virtue of their compartmentalized expression pattern, and Msx3 may also be involved. To test this, we will study the loss-of-function effect of Msx3 on Olig2 and Gsx1 expression by knocking down the gene in an organotypic cerebellum tissue culture system at E11.5.

**Disclosures:** I. Gupta: None. F. Consortium: None. J. Yeung: None. D. Goldowitz: None.

**Poster**

**276. Nervous System Patterning and Transplantation**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 276.15/A15

**Topic:** A.01. Neurogenesis and Gliogenesis

**Title:** Understanding the development of spinal sensory interneurons through *in vitro* differentiation of mouse embryonic stem cells

**Authors:** *S. GUPTA;*  
UCLA, Los Angeles, CA
Abstract: Spinal cord injury (SCI) patients can lose somatosensation, the ability to sense the environment when sensory interneurons (dI1-dI6) are damaged. These six classes of interneurons arise in the developing dorsal spinal cord from distinct progenitor pools (dP1-dP6), that are marked by the expression of specific transcription factors. The identities of these neurons are dependent on roof plate derived-Bone Morphogenetic Protein (BMP) family members and somite derived-Retinoic Acid (RA). However, it remains unresolved how RA and BMP signaling direct a variety of dI fates in the spinal cord. To resolve this question, we have differentiated mouse embryonic stem cells (mESCs) in the presence of either RA or RA+BMP4. We have observed that RA alone is sufficient to direct ESCs towards the Lhx1/5+/Pax2-dI2 (unknown function) and Lhx1/5+/Pax2+ dI4 (pain-sensing) neural fates, while treatment with RA+BMP4 suppresses dI2/dI4 fates and concomitantly induces Lhx2+ dI1 ( proprioception) and Isl1+ dI3 (mechanosensing) neurons. The simultaneous occurrence of either dI2/dI4 or dI1/dI3 neurons in our protocol suggest these neurons have a shared developmental origin. We hypothesize that RA and BMP4 activate different transcriptional networks to drive ESCs towards an as yet unidentified bipotential progenitor (dP2/dP4 and dP1/dP3), which then differentiates into either the dI2/dI4 or dI1/dI3 fates. We are evaluating this model using RNA-Seq analysis of mESCs undergoing dI differentiation to identify the RA and RA+BMP4 specific transcriptional networks. We are characterizing the progenitor types induced by RA and RA+BMP4 in mESCs by single-cell RNA-Seq to identify the novel bipotential progenitors of dI neurons. This analysis aims to provide fundamental insights into how specific classes of sensory interneurons are generated in the dorsal spinal cord. This information will shed light on our ability to design clinically relevant ESC protocols to derive specific dI neurons that can be used as cell replacement therapies to treat SCI.

Key words: Mouse, differentiation, embryonic stem cells, spinal cord, spinal cord injury.

Disclosures: S. Gupta: None.

Poster

276. Nervous System Patterning and Transplantation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 276.16/A16

Topic: A.01. Neurogenesis and Gliogenesis

Support: National Natural Science Foundation of China (31771133 and 31811530148, L. Zhou; 31671067, Y. Qu) Guangdong Natural Science Funds for Distinguished Young Scholars (2016A030306001, Y. Qu)

Title: Early forebrain neurons and scaffold fibers in human embryos
Authors: J. QIN\textsuperscript{1}, M. WANG\textsuperscript{1}, T. ZHAO\textsuperscript{2}, A. GOFFINET\textsuperscript{1}, *Y. QU\textsuperscript{1}, L. ZHOU\textsuperscript{1};
\textsuperscript{1}GHM Inst. of CNS Regeneration, Jinan Univ., Guangzhou, China; \textsuperscript{2}Dept. of Anesthesiol., Guangzhou Women and Children’S Med. Ctr., Guangzhou, China

Abstract: Neural progenitor proliferation, neuronal migration, areal organization and pioneer axon wiring are critical events during early forebrain development, yet remain incompletely understood, especially in human. Here, we studied forebrain development in human embryos aged 5 to 8 post conceptional weeks (WPC5-8), stages that correspond to the neuroepithelium/early marginal zone (WPC5), telencephalic preplate (WPC6 & 7) and incipient cortical plate (WPC8). We show that early forebrain neurons are formed at the neuroepithelial stage and originate from dorsal radial progenitors and possibly from the olfactory placode. At the preplate stage, forebrain organization is quite similar in human and mouse in terms of areal organization and of differentiation of Cajal-Retzius cells, pioneer neurons and axons. Like in the mouse, axons from pioneer neurons in prethalamus, ventral telencephalon and cortical preplate cross the diencephalon-telencephalon junction and the pallial-subpallial boundary, forming scaffolds that could guide thalamic and cortical axons at later stages. In accord with this scaffold guidance model, pioneer neurons express CELSR3 and FZD3, two molecules with key roles during mouse forebrain wiring. Furthermore, at the early cortical plate stage (WPC8), corticofugal axons cross the pallial-subpallial boundary and run in ventral telencephalon in close contact with scaffold neurons.


Poster

276. Nervous System Patterning and Transplantation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 276.17/A17

Topic: A.04. Transplantation and Regeneration

Support: SENS 342335

Title: Engineering cortical layers to enhance engraftment of neurons

Authors: *A. QUEZADA, N. IFEDIORA, J. M. HEBERT; Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: Several neurodegenerative disorders result in the loss of cortical neurons and ultimately the impairment of essential neural functions such as sensory processing or controlling movement. Neural stem cell (NSC) transplantation is a promising therapeutic strategy to replace neurons and repair damaged neural circuits. In this study, we plan to devise novel approaches to
replace lost or dying neurons in the cortex using NSC transplantation. First, to test if human vascular cells and neurons can integrate with the mouse host vascular and neural networks, we transplanted undissociated human fetal cortical tissue (gestational week 19) into a lesioned area of the cortex of immunocompromised mice (NSG, age 6-8 weeks). At one month post engraftment, we performed immunohistochemistry to assess graft survival and development. We observed both human and mouse blood vessels within the transplant as well as fusion of mouse and human blood vessels. This indicates that the mouse vasculature infiltrated the human graft and anastomosed with the human fetal derived blood vessels. Future experiments will include assays to demonstrate functionality of the blood vessels located within the graft. In addition, electrophysiological recordings will be performed to determine neural development of transplanted neural precursors and synaptic integration with the host. Since we now validated human cortical transplantation in mice, we will continue optimization using a more practical source of cells - human embryonic stem cells (hESC). We plan to do this using two complementary methods. First, to increase long-term survival of transplanted cells, the transplants will contain human vascular endothelial cells that spontaneously develop an integrated blood vessel network, as a method of pre-vascularization. Furthermore, to encourage repair of damaged neural circuits, cortical pyramidal neurons derived from hESC will be organized by cortical layers prior to transplantation in order to promote local circuit formation. Thus, we will compare transplants that are unvascularized and contain a disorganized mix of cortical neurons to transplants that are pre-vascularized and are organized by layers. Following transplantation into the motor cortex of immunocompromised mice, immunohistochemistry will be used to assess cell survival and structure of the transplant. Viral tracings will be performed to map out the axonal projections of transplanted neurons, while two-photon calcium imaging will be used to determine neurophysiological maturation of the graft.

Disclosures:  A. Quezada: None. J.M. Hebert: None. N. Ifediora: None.

Poster

276. Nervous System Patterning and Transplantation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 276.18/A18

Topic: A.08. Development of Motor/ Sensory/ and Limbic Systems

Title: Reassembling neural circuits in rat-mouse interspecies chimeras

Authors: *B. T. THROESCH*¹², J. WU³⁴, R. MUNOZ CASTANEDA⁵, M. SAKURAÍ³⁴, P. OSTEN⁵, J. IZPISUA BELMONTE⁴, K. K. BALDWIN¹;
¹Neurosci., The Scripps Res. Inst., La Jolla, CA; ²Neurosci., Univ. of California San Diego, La Jolla, CA; ³Mol. Biol., Univ. of Texas Southwestern, Dallas, TX; ⁴Salk Inst., La Jolla, CA; ⁵Cold Spring Harbor Lab., Cold Spring Harbor, NY
Abstract: Interspecies chimeras are an emerging method that can be used to disentangle the roles cell autonomous programs versus environmental factors play in neural circuit development. Furthermore, this tool may provide a means to test the function of cells from less accessible species, such as humans, in a genetically-modifiable, whole-brain system. Despite recent advances in producing chimeras amongst rodents and other species, the extent to which different neuronal subtypes can develop, integrate, and contribute to circuit function and behavior remains poorly understood. Here, we have successfully produced rat-mouse chimeric animals (mRats) by injecting fluorescently-labeled rat pluripotent stem cells into mouse blastocysts. Using whole-brain imaging, we identify the contribution of rat cells to diverse cellular subtypes and circuits throughout the mouse brain. To assess the capacity of these rat neurons to function within the mouse, we have taken advantage of transgenic models in which specific neurons in the mouse olfactory system are ablated or silenced. These experiments demonstrate that rat olfactory sensory neurons can be produced during embryogenesis and are sufficiently specified to form glomeruli in the mouse olfactory bulb. However, when mouse olfactory sensory neurons are ablated, the rat neurons’ ability to form glomeruli is compromised. In contrast, silencing mouse sensory neurons while maintaining their structural viability permits rat sensory neurons to form robust glomeruli and elicit activity in downstream neurons, suggesting the possibility of functionally replacing the mouse’s olfactory sensory capacity with that of the rat. By quantifying the neural circuits to which neurons of other species can and cannot contribute and establishing the rules by which they integrate, these mRat chimeric studies will inform our understanding of evolutionarily conserved mechanisms of neural circuit development and plasticity applicable to species and circuits beyond the rodent olfactory system.


Poster

276. Nervous System Patterning and Transplantation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 276.19/A19

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIMH Grant RC2MH089921

Title: An anatomical reference atlas of developing human brain at middle gestation: Segmentation based on a combinatorial expression of marker genes

1Allen Inst. For Brain Sci., Seattle, WA; 2Univ. of Washington, Seattle, WA
Abstract: Prenatal development of the human brain is a dynamic and complex process that includes cell proliferation, differentiation, migration, target finding, synaptogenesis and maturation. Unlike adult brain, very few anatomical references are available to accurately describe the complex developmental anatomy in human brain. To provide a helpful and accurate reference tool that reflects structural organization in early development, we present, as a first step, a digital anatomical reference atlas of the developing human brain at post-conceptional week 15. In contrast to typical anatomical atlases based mainly on conventional Nissl and myelin stains, this atlas combinatorically incorporates in situ expression data from 46 marker genes and Nissl-stain histology from the same whole brain. A total of 236 structures were annotated in this atlas, including major cortical areas, developing cortical lamina, many transient structures (e.g. ganglionic eminences, rostral migratory stream and callosal sling), permanent subcortical nuclei, and major white mater tracts. This atlas represents the first detailed anatomical reference atlas for whole human brain at middle gestation, and can serve as a baseline reference for studies of abnormal development in neurological and psychiatric diseases as well as a guide for new techniques for cellular-level analysis of human brain development.

Poster

277. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 277.01/A20
Topic: A.02. Postnatal Neurogenesis

Title: Normal brain development and impaired exploratory behavior in transgenic mice overexpressing the polysialyltransferase ST8SiaIV in neuron

Authors: *S. NGAMLI FEWOU¹, I. RÖCKLE², H. HILDEBRANDT², M. ECKHARDT³; ¹Fac. of Hlth. Sciences, Univ. Des Montagnes, Bangangte, Cameroon; ²Inst. für Klinische Biochemie, Medizinische Hochschule Hannover, Hannover, Germany; ³Inst. für Biochemie und Molekularbiologie, Univ. Bonn, Bonn, Germany

Abstract: A large body of literature has demonstrated that the polysialic acid (polySia) modification of the neural cell adhesion molecule (NCAM) is a key regulator of cellular interactions during brain development, maintenance, and plasticity. To properly fulfill these functions, polySia concentration has to be carefully controlled. This is done by the regulation of the expression of the two polySia synthesizing enzymes ST8SiaII and ST8SiaIV. From this point of view we and others have demonstrated that downregulation of ST8SiaIV during oligodendrocyte differentiation is a prerequisite for efficient myelin formation and maintenance. Here, we addressed the question whether the prevention of polySia downregulation in neurons affects brain and particularly myelin development and functioning. For this purpose, we developed transgenic mouse lines overexpressing the polysialyltransferase ST8SiaIV in neurons. Transgenic expression of ST8SiaIV prevented the postnatal downregulation of polySia and most of the polySia in the forebrain and brain stem of adult transgenic mice was associated with NCAM-140 and NCAM-180 isoforms. Structural examination of the brain revealed no overt abnormalities of axons and myelin. In addition, ultrastructural and Western blot analyses indicated normal myelin development. However, behavioral studies revealed reduced rearing activity, a measure for exploratory behavior, while parameters of motor activity were not affected in transgenic mice. Taken together, these results suggest that a persisting presence of polySia in neurons has no major effect on brain structure, myelination and myelin maintenance, but causes mild behavioral changes.

Disclosures: S. Ngamli Fewou: None. I. Röckle: None. H. Hildebrandt: None. M. Eckhardt: None.

Poster

277. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 277.02/A21

Topic: A.02. Postnatal Neurogenesis

Support: New Investigator Program - Wisconsin Partnership Program

NIH Grant - 1DP2OD025783-0
Title: Characterization of nesprin-3 in mammalian neural stem cells

1Neurosci., Univ. of Wisconsin - Madison, Madison, WI; 2Cell Biol., The Netherlands Cancer Inst., Amsterdam, Netherlands

Abstract: Throughout life, hippocampal neural stem cells (NSCs) generate new neurons in a process referred to as neurogenesis, contributing to cognitive flexibility. The mechanisms regulating adult neurogenesis are still being established. Recently, we have shown that dividing NSCs asymmetrically segregate protein cargoes, including the intermediate filament vimentin, between the two resulting daughter cells. The daughter cell inheriting these cargoes has a reduced proliferation, while the other “clean” daughter remains unaffected. Disruption of the nuclear envelope (NE) leads to symmetric cargo segregation, suggesting NE proteins may be involved in the molecular mechanism underlying cargo segregation. Interestingly, vimentin can interact with nesprin-3, an outer NE protein, whose function in the brain is unknown. Using multiple in vitro and in vivo techniques, we have characterized nesprin-3 and its interaction with vimentin in NSCs, within the developing and adult mammalian brain.


Poster

277. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 277.03/A22

Topic: A.02. Postnatal Neurogenesis

Title: Early generated interneurons regulate neuronal circuit formation during early postnatal development

Authors: C.-Z. WANG, J. MA, Y.-Q. XU, S.-N. JIANG, *Y.-C. YU;
Fudan Univ., Shanghai, China

Abstract: A small subset of interneurons that are generated earliest as pioneer neurons are the first cohort of neurons that enter the neocortex. However, it remains largely unclear whether these early-generated interneurons (EGIns) predominantly regulate neocortical circuit formation. Using inducible genetic fate mapping to selectively label EGIns and pseudo-random interneurons (pRIns), we found that EGIns exhibited more mature electrophysiological and morphological properties and higher synaptic connectivity than pRIns at early postnatal stages of the
somatosensory cortex. In addition, when stimulating one cell, the proportion of EGIns that influence spontaneous network synchronization is significantly higher than that of pRIns. Importantly, toxin-mediated ablation of EGIns after birth significantly reduce spontaneous network synchronization and decrease inhibitory synaptic formation during the first postnatal week. These results suggest that EGIns can shape developing networks and may contribute to the refinement of neuronal connectivity before the establishment of the adult neuronal circuit.


Poster

277. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 277.04/A23

Topic: A.02. Postnatal Neurogenesis

Title: Effects of neonatal exposure to an anesthetic midazolam on neural stem / progenitor cell behavior in the adult mouse hippocampus

Authors: *H. DOI, T. MATSUDA, K. YAMAURA, S. HOKA, K. NAKASHIMA;

1Stem Cell Biol. of Med., Grad. Sch. of Med. Sciences, Kyushu Univ., Fukuoka, Japan;

2Anesthesiol. and Critical Care Med., Grad. Sch. of Med. Sciences, Kyushu Univ., Fukuoka, Japan

Abstract: Adult neural stem/progenitor cells (aNS/PCs) in the subgranular zone (SGZ) of the adult hippocampal dentate gyrus (DG) proliferate and give rise to new neurons continuously throughout life to maintain hippocampus-dependent cognitive functions. Recent studies have demonstrated that gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter in the adult brain, suppresses aNS/PC proliferation in the SGZ. Midazolam, a GABA<sub>A</sub> receptor agonist, is widely used in clinical and surgical procedures, such as induction and maintenance of anesthesia, and sedation. Although previous studies indicated that neonatal exposure to GABA<sub>A</sub> receptor agonists including midazolam causes cognitive deficits later in life, mechanisms underlying these deficits have not been fully understood. In this study, we investigated whether exposing neonatal mice to midazolam affects NS/PC behavior and consequently impairs cognitive deficits in adulthood.

Postnatal day 7 (P7) mice received an injection of midazolam intraperitoneally for 3 consecutive days. We found that neonatally midazolam exposure decreased proliferation of NS/PCs and differentiation into neurons in the hippocampus in early postnatal stages. Interestingly, we also observed reduction of hippocampal NS/PC proliferation and neuronal differentiation in midazolam-exposed mice, even in the adulthood, associated with hippocampus-dependent cognitive deficits. Moreover, RNA sequencing of EGFP-positive hippocampal NS/PCs isolated from midazolam-exposed Nestin-EGFP mice revealed that neonatal midazolam exposure affects
expression genes involved in NS/PC proliferation in adulthood. These data suggest that midazolam has long-lasting adverse effects on NS/PC behavior and consequently causes cognitive deficits. Taken together, our findings may serve as a clue to elucidate the mechanism of anesthetic effects on neurogenesis and its association with memory dysfunctions.


Poster

277. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 277.05/A24

Topic: A.02. Postnatal Neurogenesis

Support: NIH RO1 NS094161-02

Title: Ganglioside GD3 is required for maintenance of mitochondrial dynamics and neuronal morphogenesis in adult neurogenesis

Authors: *F. TANG, J. WANG, Y. ITOKAZU, R. K. YU; Med. Col. of Georgia, Augusta Univ., Augusta, GA

Abstract: Gangliosides are abundantly expressed in the nervous system and are known to play important roles in neurodevelopment. We previously demonstrated that GD3 is a predominant ganglioside species (>80% of the total gangliosides) in mouse neural stem cells (NSCs). The presence of GD3 in NSCs coincides with its ability to facilitate EGF-induced cell proliferation and maintains NSC pools in adult brain. Notably, GD3 expression is not confined to NSCs but is observed throughout the adult DG, raising the possibility that GD3 have a function in later steps of the adult neurogenic sequence. Here, we describe deletion of GD3 from the hippocampal neurogenic lineage not only impairs stem cell maintenance, but also alters the dendritic structure as well as the number of excitatory synapses of adult-generated DG neurons. When examining the behavioral phenotypes of these animals, GD3-depleted mice display impairment in recognition memory. To gain insight into its cellular function, we examined GD3 binding partners from mouse-brain extract by using GD3 specific antibodies, followed by LC-MS/MS analysis and identify mitochondrial fission protein-Drp1 as a new GD3 binding protein. Biochemical and imaging analyses revealed aberrant mitochondrial fragmentation in GD3-depleted DG neurons. These results thus demonstrate GD3 may not only play a critical role in the regulation of NSCs function, but may also function during later stages of neurogenesis.

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277. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 277.06/A25

Topic: A.02. Postnatal Neurogenesis

Support:  DBT NER (BT/PR16164/NER/95/88/2015)
          DST PURSE- (Phase-II) (PAC-JNU-DST-PURSE-462)
          UGC RNW
          UGC DRS-II
          UPE-II, JNU (Project Id No. 247)
          CSIR-SRF to SKA (File no. 09/263(1101)/2016-EMR-I)

Title: The cellular and molecular attributes of adult neurogenesis and brain regeneration in zebrafish

Authors: *S. ANAND, A. MONDAL;
Sch. of Life Sci., Jawaharlal Nehru Univ., New Delhi, India

Abstract: In recent years, the adult zebrafish brain, owing to its tremendous neurogenic and regenerative ability, has emerged as a useful vertebrate model to study adult neurogenesis and brain regeneration. Several studies in recent years have been undertaken to characterize the cellular and molecular factors that mediate adult neurogenesis and brain regeneration in zebrafish. Many researchers have experimentally induced injury in the telencephalon in order to determine the cellular and molecular changes occurring in the brain during regeneration. Understanding reparative neurogenesis in the adult zebrafish brain may hold the key to the treatment of neurodegenerative diseases. Brain regeneration results from the proliferation and differentiation of adult neural stem cells (aNSCs). In a normal uninjured brain, the aNSCs spontaneously undergo cell division and differentiation to produce new cells, which replace the older dying cells in specific brain regions. In this study, we performed immunohistochemistry in 6 to 12 months old ASWT zebrafish brain to determine the precise location of the proliferating neural progenitor cells in the telencephalon of adult zebrafish brain. This was followed by double immunostaining experiments to characterize the proliferating progenitors using different differentiated and intermediate neuronal and glial cell markers. We then evaluated the response of these proliferating progenitors in a stab wounded adult zebrafish brain and how this response is mediated via the BDNF/TrkB signalling cascade using the selective TrkB receptor antagonist ANA-12. We found that the proliferating neural progenitors were distributed in distinct pallial and subpallial telencephalic regions with majority of them localized in the sub-pallium. Based on the results of double immunostaining experiments, we concluded that the progenitor cell population in the telencephalon, is not a homogenous cell population, rather it is a heterogeneous
cell population. When a stab wound injury was induced in the middle of the right telencephalic hemisphere, the proliferation of neural progenitors increased. However, the increase in proliferation was more or less eliminated when the injured zebrafish were treated with ANA-12. This suggests a major role of BDNF in mediating the brain regeneration process in zebrafish. This knowledge will contribute to the overall understanding of adult neurogenesis and brain regeneration in the zebrafish model.

Disclosures: S. Anand: None. A. Mondal: None.

Poster

277. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 277.07/A26

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant NS102365

Title: Jedi-1 is a novel regulator of postnatal neurogenesis

Authors: *K. A. KATDARE¹, M. N. TUGEND¹, F. E. HICKMAN¹, R. A. IHRIE², B. D. CARTER¹;

Abstract: Jedi-1 is an engulfment receptor involved in phagocytosis of apoptotic neuron corpses in the developing peripheral nervous system. However, Jedi-1 is also expressed in the brain and mRNA was detected in neurosphere cultures derived from perinatal ventricular-subventricular zone (V-SVZ) tissue. Previous studies indicated that neural progenitor cells (NPCs) in the V-SVZ could be phagocytic. To determine whether Jedi-1 contributes to this phagocytosis, we analyzed the uptake of carboxylated microspheres in NPCs derived from jedi-1−/− and jedi-1+/+ perinatal mice. Although we confirmed the phagocytic ability of these cells, no difference was detected between jedi-1−/− and jedi-1+/+ NPCs. However, we noted a marked increase in proliferation of the jedi-1−/− NPCs. Specifically, V-SVZ tissue was isolated from early postnatal mouse brains, dissociated and cultured for 7-10 days until neurospheres were formed. Neurospheres were then dissociated and plated as a monolayer and tested for EdU incorporation following a period of mitogen withdrawal. After 2-3 days of mitogen withdrawal, there was a 116% increase in proliferating cells from jedi-1−/− mice relative to jedi-1+/+. At 2 days, most cells in culture were GFAP+ and Nestin+ indicating a neural progenitor identity. We are currently identifying which subtype of NPCs is the proliferating cell. The NPCs from both genotypes maintained their collective multipotency, generating neurons, astrocytes and oligodendrocytes after 14 days in culture. We are currently investigating whether there is any difference in the
proportions of these cell types generated from jedi-1−/− vs jedi-1+/+ neurospheres. To examine the effects of jedi-1 deletion on neurogenesis in vivo, 4-week-old jedi-1−/− and jedi-1+/+ mice were injected with EdU (25mg/kg) and the brains isolated after 1 hour. EdU labelling and Ki67 immunostaining were quantified on sections sampled at every 50μm through the entire lateral ventricle. Surprisingly, in contrast to the in vitro results, we detected a 70% decrease in EdU+/Ki67+ cells in the V-SVZ of the jedi-1−/− mice relative to jedi-1+/+. Current studies are aimed at quantifying proliferation at earlier ages, to determine whether there is depletion of the NPC pool by 1 month in the jedi-1−/− brain. We are also exploring the mechanisms by which Jedi-1 modulates NPC proliferation. Together, our results reveal that Jedi-1 is a novel regulator of postnatal neurogenesis.


Poster

277. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 277.08/A27

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant NS089938

Title: Divergent recombination of floxed sequences undermines the accuracy of stop-floxed reporters as indicators of global genetic recombination within single cells: Implications for studies of cell autonomous effects of gene knockdown

Authors: *T. J. DAUSE1, E. D. KIRBY2;
1Dept. of Psychology, 2Dept. of Psychology, Dept. of Neuroscience, The Chronic Brain Injury Program, Ohio State, Columbus, OH

Abstract: A common question in cell biology is whether expression of a specific gene regulates cellular physiology autonomously or via paracrine effects. While gene function can be generally inferred from the effects of genetic ablation, determining whether gene knockdown impacts cells autonomously or via neighboring cell interactions requires a method to identify cells in which a target gene has been excised or suppressed versus those that are intact. To identify cells in which a gene has been knocked down, it is common practice to use Cre-lox system transgenic mouse models where flanking of essential exons of a target gene with loxP sites is combined with a stop-floxed fluorescent reporter under a universal promoter. Use of these models to determine cell autonomous effects of gene knockdown assume that recombination of both target and reporter genes occur simultaneously in the same cells with high probability. Because gene recombination in two loci are separate events, it is possible they may not correlate well cell by
cell. To our knowledge, this assumption—that recombination in one gene accurately predicts recombination in another at the single cell level—has not been tested. To test this assumption, we quantified co-expression of 2 separate Cre-dependent fluorescent reporters (Rosa EYFP Jackson #006148; Rosa tdTomato Jackson #007909) in the commonly used tamoxifen-sensitive NestinCreERT2 mouse line (Jackson #016261). In NestinCreERT2 mice, tamoxifen treatment activates Cre-mediated recombination in floxed sequences within adult neural stem and progenitor cells (NSPCs) with high specificity and efficiency. We analyzed fluorescent reporter recombination in NSPCs of the subventricular zone and dentate gyrus in NestinCreERT2(het);Rosa(EYFP/tdTomato) mice and found that EYFP recombination was a poor predictor of tdTomato recombination and vice versa. EYFP and tdTomato reporters recombined at different rates and in divergent NSPCs. These data imply that using inducible recombination of a fluorescent reporter to infer recombination of another gene at the single cell level is not a reliable methodology. It is important to weigh these limitations when considering the use of a transgenic mouse model where recombination of a target gene is inferred via the recombination of a fluorescent reporter on another gene.

**Disclosures:** T.J. Dause: None. E.D. Kirby: None.

**Poster**

277. Postnatal Neurogenesis: Molecular Mechanisms

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 277.09/A28

**Topic:** A.02. Postnatal Neurogenesis

**Support:** NINDS T32 Training Grant NS007224

**Title:** The role of microglia in neuroblast migration in the olfactory system

**Authors:** *S. J. MELLER, E. MARTÍN-LÓPEZ, T. LIBERIA, C. A. GREER; Dept. of Neurosurg., Yale Univ. Sch. of Med., New Haven, CT

**Abstract:** Understanding the mechanisms of neuroblast migration and integration into olfactory circuits will not only pave the way for therapies following smell loss, but may also have implications for the treatment of perinatal brain injury. The continued neurogenesis of the immature brain may provide opportunities for intervention by allowing recruitment of migrating neural precursor cells (neuroblasts) to sites of injury and functional recovery. Indeed, previous work has shown that migrating neuroblasts may be diverted from their migratory path to areas of injury where they can establish functional synapses with neighboring cells. We are interested in whether microglia, which are brain-resident immune cells, may help direct migrating neuroblasts during normal development and contribute to the recruitment of these cells to areas of injury. Microglia are first-line responders to brain injury and infection, but recent evidence indicates that
microglia are also critical for normal neurodevelopment. Microglia are involved in the formation of neural circuits through synaptic pruning, regulation of neurogenesis in stem cell niches, and promotion of myelin development. In addition, we are testing the hypothesis that microglia may influence neuroblast migration. In mice neuroblasts are generated in the subventricular zone throughout life and migrate long distances in the rostral migratory stream (RMS) to the olfactory bulb where they differentiate in interneurons. We find that microglia interact with migrating neuroblasts in the developing RMS prior to the establishment of the vascular and astrocytic scaffold that later guides neuroblasts. We also have evidence that microglia respond to a model of neuron injury in the olfactory bulb with an increase in the migration of neuroblasts to the areas of injury. In ongoing work we are developing strategies to eliminate microglia from migratory paths and investigating the molecular mechanisms mediating the effects of microglia on neuroblast migration, including the potential contribution of secretory factors. We hope that this work will eventually suggest new therapeutic strategies to promote normal neurodevelopment in infants following perinatal injury.

Disclosures:  S.J. Meller: None. E. Martín-López: None. T. Liberia: None. C.A. Greer: None.

Poster

277. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 277.10/A29

Topic: A.02. Postnatal Neurogenesis

Title: Microglia and complement signaling in dendritic morphogenesis and behavioral output of adult-born neurons

Authors: *K. MCDERMOTT, M. FRECHOU, E. WOOD, J. GONCALVES; Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: The dentate gyrus (DG) of the hippocampus, one of the few locations of adult neurogenesis, provides an opportunity to study new cells integrating into an existing circuit. The dendrites of dentate granule cells (DGCs) develop through trial and error, but the underlying mechanisms and functional implications of this growth process are not known. Microglia, the resident immune cells in the brain, prune synapses and eliminate apoptotic newborn neurons through phagocytosis. While microglia are known to affect adult neurogenesis, studies have focused on their role in cell survival rather than the development of adult-born neurons. Their contribution to the dendritic development of these neurons is not well understood. We hypothesize that microglia are necessary for the proper morphological and functional maturation of adult-born neurons in the dentate gyrus. We are investigating the role of microglia in adult-born DGC development through a combination of cell labeling, ablation, and in vivo imaging.
First, we are studying the effects of microglia ablation on the dendritic complexity of adult-born DGCs. We are also exploring the molecular mechanisms that underlie pruning in the DG through the complement pathway, which has been implicated in synaptic pruning in other brain areas. Finally, we are investigating how microglia and complement contribute to DG-dependent behavior. Our data suggest that ablation of microglia and loss of complement component C3 affect the dendritic growth of adult-born neurons. We also have preliminary data suggesting that C3 affects DG-dependent behavior.

**Disclosures:** K. McDermott: None. M. Frechou: None. E. Wood: None. J. Goncalves: None.

**Poster**

**277. Postnatal Neurogenesis: Molecular Mechanisms**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 277.11/A30

**Topic:** A.02. Postnatal Neurogenesis

**Support:** Hackett Fund

**Title:** Assessment of neurogenesis and brain connectome in MPS I mice with iduronidase deficiency

**Authors:** *C. PEARCE, W. ZHU, P. HACKETT, W. CHEN, X.-H. ZHU, W. C. LOW; Univ. of Minnesota, Minneapolis, MN

**Abstract:** Mucopolysaccharidosis type I (MPS I, a.k.a. Hurler’s Syndrome) is a genetic disease caused by mutations in the gene that encodes the enzyme alpha-L-iduronidase. This enzyme is required for the degradation of glycoaminoglycans (GAGs), and mutations result in lysosomal accumulation of GAGs in neurons and cognitive deficits in children with this disorder. Heparan sulfate is a GAG that binds to FGF receptors to form a trimer with FG, and this trimer is required for signaling in neural stem cells for proliferation and differentiation during development. We therefore postulate that alpha-L-iduronidase deficiency may lead to decreases in neurogenesis and alterations in brain connectivity as underlying causes of cognitive impairment. To test this hypothesis, we have studied the brain connectome in MPS I mice using resting-state fMRI (rs-fMRI) to evaluate the connectivity of the hippocampal formation and other brain regions and neurogenesis using the X-CLARITY system coupled with immunohistochemistry to visualize neural cell proliferation in whole-brain preparations. In these studies, we observed diminished brain connectivity between the hippocampus and other regions of brain in MPS I mice vs. WT controls. Quantification of neurogenesis presented in the context of the brain connectivity observations provides a more clear understanding of mechanisms responsible for cognitive impairment in MPS I-affected individuals.

Poster

277. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 277.12/A31

Topic: A.02. Postnatal Neurogenesis

Support: FNRS
Fondation Léon Fredericq

Title: Cdk1 is essential for long-term maintenance of neural stem cells in the postnatal hippocampus

Authors: *R. VANDENBOSCH*¹², Q. MARLIER¹, S. VERTENEUIL¹, P. KALDIS³, M. BARBACID⁴, D. SANTAMARIA⁵, L. NGUYEN¹, B. MALGRANGE¹;
¹GIGA-Stem Cells/Neurosciences, ²Div. of Histology, Dept. of Biomed. and Preclinical Sci., Univ. of Liège, Liège, Belgium; ³Inst. of Mol. and Cell Biol. (IMCB), Singapore, Singapore; ⁴Ctr. Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain; ⁵Univ. of Bordeaux, INSERM U1218, ACTION Laboratory, IECB, Pessac, France

Abstract: In contrast to the majority of neurons of the mammalian CNS, which are born during embryogenesis, hippocampal dentate granule neurons are continuously produced from mid-gestation to old age, including in humans. Postnatally born hippocampal neurons develop locally from neural stem cells (NSCs) anchored in a specialized niche of the dentate gyrus (DG), the subgranular zone (SGZ). We have previously shown that cyclin-dependent kinase (Cdk) 6, an important regulator of G1 progression, specifically drives the expansion of transit-amplifying progenitors in the postnatal hippocampus. However, it is not clear yet which Cdk is involved in the proliferation and long-term maintenance of hippocampal NSCs. Here, we investigated the role of Cdk1, an essential Cdk for M-phase, in postnatal hippocampal neurogenesis. Consistently with the crucial role of Cdk1 in the cell cycle, our results demonstrate that conditional loss of Cdk1 in hippocampal NSCs dramatically reduce their proliferation rate. Moreover, the total number of Sox2⁺ NSCs rapidly declines upon Cdk1 deficiency, and this is accompanied by a concomitant increase in the number of newborn neurons produced. Surprisingly, we also observed that the loss of one allele of Cdk1 was sufficient to phenocopy most of the phenotypes encountered in the complete absence of Cdk1. These results suggest a role for Cdk1 beyond cell cycle regulation, where Cdk1 dosage regulates hippocampal NSC fate choice between neuronal differentiation and stem cell maintenance. We are actually investigating the mechanisms by which Cdk1 control cell fate in hippocampal NSCs.

Poster

277. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 277.13/A32

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant 5R01HD083828-05

Title: A role for polycomb repressive proteins in acquisition of the mature NMDAR phenotype at hippocampal synapses

Authors: *H.-R. BYUN¹, J.-Y. HWANG², B. COURT-VAZQUEZ¹, R. ZUKIN¹;
¹Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY; ²Dept. of Pharmacol. and Neurosci., Creighton Univ. Sch. of Med., Omaha, NE

Abstract: NMDARs (N-methyl-D-aspartate receptors) mediate excitatory transmission and are critical to synaptogenesis, formation of neural circuitry and to higher cognitive functions. A hallmark feature of NMDARs is a developmental switch in receptor phenotype from primarily GluN2B- to GluN2A-containing during the critical period of postnatal brain development. This is significant in that the GluN2B subunit restricts the number of AMPARs, decreases the threshold and increases the magnification of LTP. The gene silencing transcription factor REST is critical to the developmenta11 switch in NMDAR phenotype at hippocampal synapses (Noh, Hwang, Proc Natl Acad Sci USA, 2012). The polycomb repressive protein Enhancer of zeste homolog 2 (EZH2) is a gene silencing factors and member of a large macromolecular complex known as polycomb repressive complex 2 (PRC2) that is predicted to coordinate with REST to silence >500 neuron-specific target genes. A fundamental mechanism by which EZH2 and REST silence genes is by epigenetic remodeling. EZH2 is a histone-lysine N-methyltransferase that confers a tri-methyl mark on histone H3 at lysine 27, a mark of gene repression. Moreover, EZH2 recruits the DNA methylation machinery, which confers methyl moieties at the 5’-position of cytosines within the DNA, consistent with the concept that EZH2 induces enduring repression of target genes. However, a role for polycomb proteins in the developmental regulation of synaptic proteins is as yet unclear. Here we show that EZH2 and other components of the PRC2 (EZH1, SUZ12, and EED) are developmentally regulated in the hippocampus during the first two weeks postnatal and are recruited to the grin2b promoter, prior to the switch in NMDAR phenotype at P15. In addition, H3K27m23, the functional readout of EZH2, is enriched at the grin2b promoter at P15, where it remains as late as P60. Moreover, DNMT1 and 3a, proteins that mediate DNA methylation, are recruited to the grin2b promoter, where DNA methylation is prominent at P15 and continues to increase as late as P30, the latest time point examined. We
further show that acute knockdown of EZH2 increases GluN2B expression in DG neurons. These findings demonstrate that EZH2 is causally related to *grin2b* repression and the switch in NMDAR phenotype. We are currently examining a possible causal role for EZH2 in the recruitment of REST and the DNA methylation machinery and the long term stabilization of the mature NMDAR phenotype at hippocampal synapses.

**Disclosures:** H. Byun: None. J. Hwang: None. B. Court-Vazquez: None. R. Zukin: None.

**Poster**

277. Postnatal Neurogenesis: Molecular Mechanisms

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 277.14/A33

**Topic:** A.02. Postnatal Neurogenesis

**Title:** Control of neural stem cell specification in the postnatal forebrain by antagonist function of Vax1 and Pax6

**Authors:** *N. CORÉ, A. ERNI, C. BÉCLIN, H. CREMER; IBDM CNRS-UMR7288, Marseille, France

**Abstract:** Neural stem cells (NSCs) in the postnatal mouse ventricular/subventricular zone (V/SVZ), that generate different types of interneurons for the olfactory bulb, are highly heterogeneous. Depending on their location along the ventro-dorsal axis of the lateral ventricles, they preferentially give rise to distinct neuronal subclasses with defined positions, connectivity or neurotransmitter phenotypes. Understanding the molecular mechanisms that determine the generation of the distinct neuron populations at the NSC level will be essential for directing the differentiation of NSCs into defined neuronal cell populations in therapeutic contexts. We found that the homeodomain transcription factor (TF) Vax1 is expressed in the lateral V-SVZ stem cell compartment in a ventro-dorsal gradient, suggesting a role in stem cell patterning and determination of neuronal phenotype. We used postnatal in vivo electroporation to miss-express Vax1 in the dorsal NSC aspects of the V-SVZ. Overexpressing Vax1 in dorsal or dorso-lateral NSCs led to a decrease in the dopaminergic interneuron population in the olfactory bulb. This phenotype is accompanied by a reduction of the pro-dopaminergic TF Pax6 in the stem cell compartment, suggesting that dopaminergic fate repression by Vax1 occurs through repression of Pax6. Moreover, conditional inactivation of Vax1 in NSCs along the lateral SVZ led to a reduced number of Calbindin+ neurons, showing that Vax1 is necessary for the production of this sub-set of OB neurons. This shows that antagonistic interactions between Vax1 and Pax6 control neuronal phenotype along the dorso-ventral axis of the forebrain stem cell compartment. If these interactions are direct or implicate intermediate regulators like, for example, miR-7a is currently under investigation.
Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Title: Ketamine treatment during late adolescence decreases the inhibitory synaptic transmission and reduces the adult neurogenesis in the rat dorsal dentate gyrus


Abstract: The brain growth occurs before birth or during childhood but it undergoes extensive remodeling in the adolescence period. Accumulating data from epidemiology and genetic to basic neuroscience showing that adolescence is a high-risk period for the onset neuropsychiatric diseases such as schizophrenia (SZ). Interestingly animal and human postmortem studies suggest that alterations in GABA interneurons contribute to neuropathological and clinical features of SZ. Inhibitory signaling from parvalbumin positive GABA interneurons (INsPV+) is a critical regulator of adult neurogenesis in the dentate gyrus. Recently in a pharmacological model of hypofunction of NMDA receptors through adolescence ketamine treatment (ket-treatment) that induce symptoms of SZ, we have observed a strong decrease of number of INsPV+ and a decrease of GABA transmission in prefrontal cortex but without effect on the ventral hippocampus. Considering that the contribution of the hippocampus in different brain functions varies along its septal-temporal axis, we used an immunofluorescent and electrophysiology approach to determine if adolescence ket-treatment, impairs the GABAergic synaptic transmission and decreases the adult hippocampal neurogenesis in rat dorsal dentate gyrus (DDG). Our results show that adolescence ket-treatment induced a strong decrease in the number
of INsPV+ and reduced spontaneous and evoked GABAergic transmission between INsPV+ and mature dentate granule cells (mDGC) in the DDG. Also, we observed that the ket-treatment did not affect the passive or active membrane properties of the mDGC. Using the expression level of BrdU/Ki67 and DCX markers, we found that the adolescence ket-treatment induced a significantly reduction of the proliferation rate and adult neurogenesis in the DDG. These results suggest that INsPV+ GABAergic transmission plays a key role in the synaptic and structural plasticity in DDG, and that the hypofunction of the INsPV+ in the adult DDG could be key to understand the pathogenesis of SZ.


Poster

277. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 277.16/A35

Topic: A.02. Postnatal Neurogenesis

Support: NSERC

Title: Effect of cannabinoid exposure during gastrulation on neuronal development in zebrafish

Authors: *M. AMIN, K. AHMED, D. ALI;
Biol. Sci., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Marijuana is one of the most commonly used illicit recreational drugs. It is reported that up to 14% of pregnant females aged between 12-44 have used cannabis during their first trimester. In this study we wanted to determine the effect of brief exposure of cannabinoids (THC, CBD and CBN) during early development. To do this, we exposed zebrafish embryos to different cannabinoids for 5 hours during a stage of development known as gastrulation (5.25 hr-10.75 hr). Cannabinoid exposed embryos exhibited a dose-dependent reduction in survival, body length, hatching and heart rates. Treated fish were less responsive to touch whereas controls exhibited robust escape responses upon touching the head or tail with forceps. Because the movement of treated fish was severely limited, we examined motor neuron (MN) development, muscle fiber morphology and activity at the neuromuscular junction (NMJ). Fluorescent labelling of primary and secondary MNs indicated a change in branching patterns and a reduction in the number of axonal branches in the trunk musculature. Further, to look at the structural details of muscle fiber morphology, we performed TEM (transmission electron microscopy) imaging of longitudinal sections of muscle fibers and observed the presence of large-sized mitochondria in several muscle layers in treated embryos but not in controls. Next, we found that whole cell recording of mEPCS from white muscle fibers were less frequent in
cannabinoid treated embryos vs controls. To determine if cannabinoid treatment altered the ability of fish to respond to sound at 5 dpf, we examined the C-start escape response in larvae. We found that larvae treated with cannabinoids exhibited a drastic reduction in the number of C-start escape responses to sound stimuli. To examine how cannabinoids mediate their activity, we carried out co-exposure of cannabinoids with the CB1R blockers AM251, CP9455 or the CB2R blockers AM630 and JTE907. Our results suggest that the effects of δ9(-) THC occur largely (but not exclusively) through CB1R whereas the effects of CBD and CBN occur mainly (but not exclusively) through activation of CB2Rs. These findings suggest that cannabinoids alter neuronal development via acting through either CB1R or CB2R.


Poster

277. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 277.17/A36

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant MH087473

Title: Regulated intramembrane proteolysis of neuregulin 1 is important for postnatal hippocampal neurogenesis and neuronal development

Authors: *A. JONE1, P. M. RAJEBHOSALE2, L. W. ROLE3, D. A. TALMAGE4;
1Neurobio. and Behavior, 2Ctr. for Nervous Syst. Disorders, 3Neurobio. & Behavior, 4Pharmacol. Sci., Stony Brook Univ., Stony Brook, NY

Abstract: The Neuregulin 1 (Nrg1) gene encodes a family of versatile signaling proteins extensively involved in neural development and synaptic plasticity. One neuron-specific isoform, Type III Nrg1, is critical for neuronal survival, neural fate determination, receptor trafficking, axon myelination, and synaptic transmission. Genetic deletion of Type III Nrg1 in mice results in deficits in synaptic plasticity and behavioral deficits involving working memory and sensorimotor gating. Upon ErbB4 binding or neuronal depolarization, Type III Nrg1 undergoes regulated intramembranous proteolysis (RIP), mediated in part by γ-secretase cleavage, to generate a carboxyl-terminal fragment. This soluble intracellular domain (ICD) of Type III Nrg1 is capable of translocating to the nucleus, where it possesses strong transcriptional transactivation properties. In vitro, a valine residue in the transmembrane domain (position 321) is necessary for proper RIP of Type III Nrg1 and appropriate dendritic arborization. Interestingly, a single-nucleotide polymorphism that has been associated with psychosis and schizophrenia in a human population in Costa Rica results in the substitution of a leucine for this valine residue.
We generated a valine-321-leucine (V321L) mouse line and asked whether this point mutation affects Nrg1 RIP and disrupts developmental events in vivo. We show through subcellular fractionation that Nrg1 RIP and subsequent ICD nuclear translocation are impaired by the V321L mutation. Because the ICD has been previously demonstrated to regulate neural stem cell proliferation and fate specification in vitro, we asked whether neurogenesis is affected by disruptions in Nrg1 RIP in vivo. Our results indicate that mice with the V321L mutation have aberrant hippocampal neurogenesis, with reduced cell proliferation, altered cell cycle dynamics, and disproportionate fate specification in the dentate gyrus compared to wild-type littermates. Furthermore, newborn neurons in V321L mutants appear to have impaired dendritic arborization. These results suggest that Nrg1 RIP plays an important role in regulating neural stem cell development and neuronal maturation in the hippocampus.

Disclosures:  A. Jone: None. P.M. Rajebhosale: None. L.W. Role: None. D.A. Talmage: None.

Poster

277. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 277.18/A37

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R01GM112591
The Welch Foundation BE-0017

Title: PITPNA and PITPNB cooperate in maintaining neural stem cells self-renewal via GOLPH3-dependent notch signaling during mouse brain development

Authors: *S. K. HUR1,2, V. BANKAITIS2;

Abstract: Phosphatidylinositol (PtdIns) transfer proteins (PITPs) stimulate PtdIns-4-P synthesis and signaling in eukaryotic cells. However, the precise nature of the associated signaling pathways, and of the biological outcomes associated with PITP activities remain unclear. Herein, we show that two type-1 START-like PITPs, PITPNA and PITPNB, cooperate in maintaining neural stem cell (NSC) self-renewal via a PtdIns-4-P and GOLPH3-dependent mechanism that promotes Notch signaling in embryonic NSCs. We have exploited an in utero electroporation approach to investigate the role of PITP-dependent inositol lipid signaling in the embryonic NSC pool. By silencing Pitpnb with shRNA in a Pitpna null mouse line we evoked a dramatic depletion of the NSC pool achieved through acceleration asymmetric differentiation of cell
division in the embryonic brain. In utero electroporation delivery of plasmids isogenic to wild-type PITPNA or PITPNB rescued the NSC depletion resulting from eviction of both PITPs. However, a PITPNA mutant clone deficient in phosphatidylinositol or phosphatidylcholine binding failed to rescue NSC depletion. Moreover, neither Sec14p, the major yeast PITP, which possesses the same lipid-binding/transfer activities but is structurally unrelated to type1 PITPs, nor PITPN1, a homolog of PITPNA/PITPNB that also shows PtdIns-transfer activity but which uses a different second lipid, PtdOH, restored the NSC pool. These data reveal that PITPNA/PITPNB are specifically required for maintaining the NSC pool and additionally demonstrate that the function role of PITPNA/PITPNB in NSC self-renewal cannot be accounted for by simple PtdIns-4-P transfer as proposed in PtdIns-gradient models. We observed GOLPH3-dependent Golgi positioning which was required for controlling NSC self-renewal, and for maintaining the NSC pool. Furthermore, we confirmed that PITP deficiencies evokes a significant reduction of the Notch intracellular domain (NICD) in embryonic NSC. We propose a mechanism where PITPNA/PITPNB drive PtdIns-4-P-dependent recruitment of GOLPH3 to Golgi membranes so as to promote an asymmetric Golgi network where the Notch receptor matures to control NSC self-renewal.

Disclosures: S.K. Hur: A. Employment/Salary (full or part-time); Texas A&M University. V. Bankaitis: None.

Poster

277. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 277.19/A38

Topic: A.01. Neurogenesis and Gliogenesis

Support: BMBF JenAge 0315581
IZKF Jena
TMWWDG RegenerAging-FSU-I-03/14

Title: A conditional knockout mouse model to study the specific role of cyclin D2 in brain development, adult neurogenesis and human brain pathologies

Authors: V. GOYAL1, S. A. ZAHRA1, C. HENNINGS3, G. ZIMMER-BENSCH4, C. BEETZ2, *C. W. SCHMEER1, C. HUEBNER3, O. W. WITTE1, A. URBACH1;
1Hans-Berger Dept. of Neurol., 2Dept. of Clin. Chem., Jena Univ. Hosp., Jena, Germany; 3Inst. of Human Genet., Jena, Germany; 4RWTH Aachen Univ., Aachen, Germany

Abstract: Cyclin D2 (D2) is one out of three homologous D-cyclins implicated in cell cycle progression and malignant transformation. D2 becomes detectable during gastrulation and shows dynamic expression patterns during neurulation and brain development. Loss- or gain-of-
function mutations of D$_2$ have been associated to circumscribed neurodevelopmental deficits resulting in loss of interneurons, microcephaly or megalencephaly. We previously showed that depletion of D$_2$ impairs formation of the adult hippocampal stem cell pool, which is the source of lifelong neurogenesis. Our studies further suggest that D$_2$ is critical for the proliferation of adult neural stem and progenitor cells. However, because of the aforementioned deficits and other developmental defects of conventional D$_2$ knockout (D2KO) mice, the precise role of D$_2$ in adults, e.g. for regulating neurogenesis, could not be evaluated so far. To bypass the limitations of the conventional D2KO, we engineered a mouse line in which exons I and II of the $ccnd2$ gene are flanked by loxP sites, to enable a spatiotemporally controlled deletion of D$_2$. In order to test the efficiency of the line, we crossed these animals to Cre deleter mice to achieve a ubiquitous conditional D$_2$ knockout (cD2KO). In situ hybridization as well as Western blot analysis of D$_2$-expressing tissues confirmed the lack of D$_2$ transcripts and protein in homozygous cD2KO mice. Macroscopic evaluation and brain volumetry revealed that cD2KO mice display a phenotype equal to that of constitutive D2KO mice: Compared to wildtype siblings, we observed a 17% reduction in total brain volume, with atrophies becoming most prominent in the dentate gyrus (38% smaller), the olfactory bulb and the cerebellum. Moreover, incorporation of the S-phase label Bromodeoxyuridine into hippocampal precursor cells was reduced by 90%, indicating a severe impairment of adult neurogenesis. Heterozygous cD2KO mice were normal for all evaluated parameters, despite having an intermediate neurogenesis phenotype (approx. 35% less Bromodeoxyuridine incorporation). Taken together, we generated and validated a new conditional D2KO mouse model useful for investigating the contribution of D$_2$ to brain development, stem cell self-renewal, adult neurogenesis and disease processes such as tumorigenesis.


Poster

277. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 277.20/A39

Topic: A.01. Neurogenesis and Gliogenesis

Support: IZKF Jena
BMBF JenAge 0315581
TMWWDG RegenerAging-FSU-I-03/14
Title: Differential effects of cyclin D2 ablation on the self-renewal capacity of postnatal and adult hippocampal stem cells and dynamic expression of D-cyclins during precursor differentiation in vitro

Authors: A. SYEDA ZAHRA, O. W. WITTE, *A. URBACH; Hans Berger Dept. of Neurol., Jena Univ. Hosp., Jena, Germany

Abstract: The neurogenic potential of the adult dentate gyrus (DG) relies on a pool of quiescent neural stem cells (NSCs) that become activated to generate new granule cells on demand. Our studies on cyclin D2 knockout (D2KO) mice suggest that cyclin D2 is required for the establishment of the adult NSC pool during the second to third postnatal week, and that cyclin D2 becomes increasingly important for hippocampal neurogenesis during this period. We showed that cyclin D2 is the only D-type cyclin of adult NSCs, suggesting an important role for their proliferation and self-renewal. On the contrary, developmental NSCs in the postnatal hilar germinative matrix as well as in the emerging subgranular zone also express cyclin D1, which might compensate for the loss of cyclin D2 during postnatal neurogenesis. To further examine these findings, we isolated neural precursors from postnatal day 7 (P7) and adult mouse dentate gyri and cultured them as neurospheres. We found that neurospheres could be established from both, P7 and adult D2KO precursors. However, precursors from D2KO mice formed less and in adults also smaller spheres during primary culture as compared to wildtype mice. The spheres of P7 D2KO mice were able to expand exponentially over at least ten passages, demonstrating that their DG contains bona fide self-renewing NSCs that do not require D2 for proliferation. On the contrary, the precursor population from adult D2KO mice displayed no long-term neurosphere-forming capacity and stopped growing after the third passage, suggesting that it is composed of only progenitor cells. Proliferation of D2KO precursors is likely accomplished by cyclin D1 which is expressed in spheres of both, P7 and adult mice (qRT-PCR and Western blot). To further evaluate the dynamics of D-cyclin expression during adult neurogenesis, we cultured DG precursors from wildtype mice as monoadherent cultures (with FGF2 and EGF) and differentiated them in mitogen-free media for 96 hrs. We observed similar quantities of cyclin D1 and D2 transcripts and proteins which were high in proliferating cultures and sharply decreased during the first 24 hrs of differentiation, simultaneously with a drop of the proliferation marker Ki67 and increasing levels of differentiation markers DCX, Fox3 and GFAP. Together, these findings confirm our in vivo data suggesting that cyclin D2 is dispensable for proliferation of developmental NSCs but required for the formation and maintenance of the adult NSC population.

Disclosures: A. Syeda Zahra: None. O.W. Witte: None. A. Urbach: None.
Title: Postnatal development and spatial distribution of neuromuscular junctions and innervated nerves in mouse skeletal muscles

Authors: *J. XU$^{1,2}$, T. YU$^{1,2}$, Y. LI$^{1,2}$, Y. YAO$^{1,2}$, D. ZHU$^{1,2}$;  
$^1$Britton Chance Ctr. for Biomed. Photonics, Wuhan Natl. Lab. for Optoelectronics, Huazhong Univ. of Sci. and Technology, Wuhan, China, Wuhan, China; $^2$MoE Key Lab. for Biomed. Photonics, Collaborative Innovation Ctr. for Biomed. Engineering, Sch. of Engin. Sciences, Huazhong Univ. of Sci. and Technology, Wuhan, China, Wuhan, China

Abstract: Neuromuscular junction (NMJ) is the typical chemical synaptic connection between lower motor neurons and muscle fibers, and control voluntary muscles contraction. NMJ is also a classical model of synapse development, which can provide references for the development of central nervous system. They are functional at birth and undergo numerous alterations postnatally, especially the change of innervation pattern (polyneuronal innervation to mononeuronal innervation) resulted from synapse elimination. And axon loss not only simplifies motor unit size, but also may influence the number of NMJs due to synapse elimination without takeover. However, the studies on NMJ development mainly focused on individual NMJ or a few NMJs innervated by a single motor neuron, which makes it difficult to understand the influence of synapse elimination for NMJ development in whole skeletal muscle. Currently, combining with optical clearing techniques, whole-mount analysis of the distribution and number of NMJs has been frequently used as an experimental paradigm. Here, combined this paradigm with the study of NMJ development, all NMJs and axon branches were detected and analyzed quantitatively in mouse skeletal muscles at different time points during postnatal development. We found that the spatial distribution pattern of NMJs and nerve branches was consistent after...
birth, but the number of NMJs in gastrocnemius and tibialis anterior increased continually during early postnatal development and reaches equal level to the adulthood at about P10. In addition, a phenomenon was observed by high-magnification confocal imaging that some new nerve terminals sprouting from the nerve branches would “share” with neighboring acetylcholine receptor (AChR) clusters, which would lead to the division of the neighboring AChR clusters. The above results indicated that NMJ formation still occurs in some muscles during the early postnatal stage, and synapse elimination do not reduce the number of NMJs in whole muscles, on the contrary, it could be associated with an increase in NMJ number.

Disclosures: J. Xu: None. T. Yu: None. Y. Li: None. Y. Yao: None. D. Zhu: None.

Poster

277. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 277.22/A41

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH T32 GM113846

Title: Thalamocortical axons regulate neurogenesis in sensory areas of the mouse neocortex

Authors: *T. R. MONKO, J. STOLLEY, S. DABRUZZI, P. GONCHAROV, Y. NAKAGAWA;
Neurosci., Univ. of Minnesota Twin Cities, Minneapolis, MN

Abstract: Connectivity between the mammalian thalamus and the neocortex is important for proper brain functioning and has been proposed to have an instructive role in shaping the structural and functional differences among neocortical areas. Thalamocortical axons are known to refine the dendritic arbors of upper layer neurons to produce unique cytoarchitectural features, such as barrels in rodent primary somatosensory cortex, and distinct areal borders such as those found between striate and extrastriate in primate visual cortex. These axons control gene expression patterns across areas of the thalamo-recipient layer 4 of neocortex, but it is unknown if thalamocortical axons are important in the generation of the increased number of upper-layer neurons in primary sensory areas. Axons from the thalamus reach the neocortex when upper layer neurons are being produced and may therefore have a role in spatiotemporal regulation of progenitor proliferation and differentiation. We address this by analyzing neurogenesis in thalamus-specific Gbx2 mutant mice, which lack thalamocortical axons. In these mice, the number of upper-layer neurons in primary somatosensory and visual areas was reduced in postnatal cortex. In addition, the number of radial glia and intermediate progenitor cells were reduced in putative sensory areas of the embryonic cortex. These results indicate that thalamocortical axons enhance production of upper-layer neurons in primary sensory areas by
interacting with progenitor cells. In addition, we investigate a molecular mechanism unique to sensory areas by examining the role of Vgf, which encodes a set of neuropeptides expressed specifically in principal sensory nuclei of embryonic thalamus. In thalamus-specific Vgf knockout mice, the number of dividing intermediate progenitor cells was reduced in putative sensory areas with a concomitant decrease in upper-layer neurons. These results collectively suggest that thalamic afferents play multiple complex roles in controlling neurogenesis in sensory areas of the neocortex. Because one of the Vgf-encoded peptides has a receptor exclusively expressed by microglia, our results may indicate an indirect role of the resident immune cells that mediate the thalamic regulation of neocortical neurogenesis.


Poster

277. Postnatal Neurogenesis: Molecular Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 277.23/A42

Topic: A.02. Postnatal Neurogenesis

Title: Cord blood-derived exosomal contactin-2 and BDNF: Biomarkers for brain health of neonates at risk for iron deficiency?

Authors: *T. M. MATVEEVA1, P. MARELL1, S. BLOHOWIAK1, P. KING1, M. GEORGIEFF1, P. V. TRAN2;
2Dept. of Pediatrics, 1Univ. of Minnesota, Minneapolis, MN

Abstract: Iron deficiency anemia (IDA), which affects approximately 20-30% of all pregnant women and their offspring worldwide, causes long-term impairments in cognition and socio-emotional behaviors in adulthood despite iron therapy following IDA diagnosis in infancy. The persistent abnormalities constitute a significant cost to society in terms of education attainment, job potential, and mental health. The underlying pathobiology for the persistent abnormalities has been ascribed to abnormal neural development and function (e.g., metabolism, dendritogenesis, and synaptogenesis) during critical periods in early life that are carried forward into adulthood. However, accumulating evidence also implicates iron-dependent epigenetic mechanisms, by which early-life ID induces long-lasting dysregulation of genes critical for neural function in adulthood. The epigenetic modifications can be mitigated with a methyl donor supplement, choline, suggesting therapeutic efficacy of the adjunctive diet. As such, it is important to establish early biomarkers that can index brain health of infants at risk for IDA. Given that iron is prioritized to the red cell production to support tissue oxygenation, when IDA is diagnosed using available blood-based markers (e.g., Ferritin, zinc-protoporphyrin [ZnPP]) the brain has already been deprived the severely needed iron for growth during critical periods of
development. We have explored the use of exosomes, which are secreted by cells and carry proteins and nucleic acids for cell-cell communication, from cord blood to identify potential brain-specific biomarkers. Our findings suggest Contactin-2, a neural specific cell-surface protein critical for axonal growth and myelination, and Brain-derived Neurotrophic Factor (BDNF), an important growth factor for neural development during fetal and postnatal life, as potential sex-specific markers that are remarkably correlated to infant iron status based on cord blood ferritin and ZnPP. Additional analyses of Contactin-2 positive exosomes from cord blood will potentially identify additional markers that can be readily translated into clinical tools for assessing developing brain health in neonates.


Poster

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 278.01/A43

Topic: A.03. Stem Cells and Reprogramming

Support: Leonard Wolfson Experimental Neurology Centre
Joy Bunker Scholarship

Title: iPSC derived engineered cerebral organoids as in vitro models of tauopathy

Authors: *C. E. J. LOVEJOY1, A. ALATZA1, C. ARBER1, J. HARDY1,2,3, S. WRAY1,2; 1Inst. of Neurol., UCL, London, United Kingdom; 2Queen Square Inst. of Neurol., London, United Kingdom; 3UK Dementia Res. Inst. at UCL, London, United Kingdom

Abstract: Introduction: Hyperphosphorylated, insoluble aggregates of the microtubule associated protein tau are a pathological hallmark of a range of clinically diverse neurodegenerative diseases termed tauopathies. Mutations in the tau gene (MAPT) cause Frontotemporal Dementia, and a subset of these mutations disrupt splicing of the MAPT gene. Induced pluripotent stem cell (iPSC) carrying MAPT mutations have been differentiated into neurons to study tau expression. However, iPSC-neurons closely resemble foetal neurons and predominantly express 3R tau isoforms rather than equimolar amounts of 3R/4R tau observed in the adult human CNS. We examined tau expression and splicing 3D cerebral organoids to test the hypothesis that 3D cultures mature quicker in vitro, and therefore express 4R tau earlier than 2D neurons. Methods: iPSC lines with the MAPT 10+16 mutation were used to generate 3D engineered cerebral organoids (EnCORs) using the protocol described in (Lancaster et al., 2017). Tau expression, splicing and phosphorylation were examined using RT-PCT and western blot. Results: Successful differentiation of iPSC into 2D and 3D cultures was confirmed by staining
for the neuronal markers phospho-vimentin, FOXG1, TBR1, and βIII-tubulin. Control EnCORs expressed predominantly 3R tau, while both 3R and 4R tau was expressed in cultures containing the MAPT 10+16 mutation. Tau isoform diversity increased over time, and a direct comparison of 2D and 3D cultures revealed a robust expression of 4R tau in 3D cultures from controls after 200 days in vitro, which was absent in 2D cultures. Tau was phosphorylated at multiple epitopes in EnCORs but no differences were observed between control and mutant genotypes. Conclusions: 2D and 3D neuronal models predominantly express the 0N3R foetal tau isoform. Multiple isoforms of tau are expressed after extended in vitro culture time points, and the appearance of 4R tau occurs earlier in 3D cultures than 2D cultures. The presence of the MAPT 10+16 splicing mutation results in expression of both 0N3R and 0N4R isoforms in both 2D and 3D. Cerebral organoids therefore provide a physiologically-relevant culture system to study the consequences of tau splicing.

Disclosures: C.E.J. Lovejoy: None. A. Alatza: None. C. Arber: None. J. Hardy: None. S. Wray: None.

Poster

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 278.02/A44

Topic: A.03. Stem Cells and Reprogramming

Support: PhD studentship grant from the UK Medical Research Council

Title: Repeat associated non-AUG translation of dipeptide repeat proteins in C9orf72 ALS/FTD patient iPSC-derived neurons

Authors: *S. E. SALOMONSSON1,2, K. M. WILSON1,2, B. MURALIDHARAN1,2, I. GLARIA1,2, M. BICTASH3, A. MONAGHAN3, P. J. WHITING3, A. M. ISAACS1,2; 1UK Dementia Res. Inst. at UCL, 2UCL Queen Square Inst. of Neurol., Univ. Col. London, London, United Kingdom; 3Alzheimers Res. UK UCL Drug Discovery Inst., London, United Kingdom

Abstract: Introduction
A G4C2 repeat expansion in the gene C9orf72 is the most common known cause of frontotemporal dementia and amyotrophic lateral sclerosis. Transcription of sense and antisense repeat mRNA leads to intraneuronal nuclear RNA foci and production of five dipeptide repeat proteins (DPRs), through non-canonical repeat-associated non-AUG (RAN) translation. DPRs form neuronal aggregates in the patient brain. Previous studies using overexpression cell models showed that activating the integrated stress response upregulated RAN translation. Furthermore, eIF4A inhibition reduced RAN translation in such models. We investigate if these factors also
regulate endogenous RAN translation in patient induced pluripotent stem cell (iPSC)-derived neurons.

Methods
N=3 patient iPSC lines with paired CRISPR/Cas9 corrected isogenic controls were used. Differentiation of motor neuron progenitor cells and mature spinal cord motor neurons was induced through a 2D dual SMAD inhibition protocol. iPSC-derived motor neurons were transiently treated with vehicle or sodium arsenite to activate the integrated stress response. Poly-GP RAN translation was quantified through an MSD immunoassay. Rocaglamide was used to inhibit eIF4A activity in patient iPSC-derived neuronal progenitor cells, to examine its potential for inhibiting endogenous RAN translation.

Results
Baseline poly-GP expression was greater in neuronal progenitor cells than in the iPSCs they were derived from. Baseline levels of poly-GP RAN translation differed among the patient lines but did not correlate with repeat length. There were donor-dependent differences in response to eIF4A inhibition. Preliminary data suggest that poly-GP translation was upregulated following transient activation of the integrated stress response in patient iPSC-derived motor neurons.

Conclusion
Baseline poly-GP levels were donor-dependent and neuronal differentiation increased RAN translation. The patient lines responded differently to Rocaglamide, which may be due to differences in DPR turnover rates, or sense versus antisense RAN translation of poly-GP. Sensitive DPR quantification in C9orf72 patient cells with endogenous RAN translation is a potential tool in the search for therapeutic targets.


Poster

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 278.03/A45

Topic: A.03. Stem Cells and Reprogramming

Title: Profiling bioenergetics in iPSC-derived brain cells from Alzheimer's disease patients and non-demented control individuals

Authors: *W. Ryu¹, R. A. Healy¹, M. Bormann¹, L. Wang¹, M. Shen¹, B. M. Cohen¹, K. C. Sonntag²;
¹Mclean Hospital/Harvard Med. Sch., Belmont, MA; ²McLean Hospital, Harvard Med. Sch., Belmont, MA
Abstract: Although toxic molecules, such as beta amyloid peptides (Aβ) and phosphorylated tau (p-tau) have been suggested as critical mediating factors in Alzheimer’s disease (AD), the underlying cause of sporadic or late-onset AD (LOAD) is still not known. Bioenergetics is the production of energy through metabolism of organic compounds in living organisms. Neural activity is heavily dependent on energy production and substantial changes in bioenergetics have been observed in aging and neurodegenerative diseases, including LOAD. A major energy product is adenosine triphosphate (ATP) which cells make through several processes, predominantly glycolysis and mitochondrial respiration, the latter consisting of the tricarboxylic acid (TCA) cycle and oxidative phosphorylation. In a previous study on skin fibroblasts derived from LOAD patients and non-demented control individuals, we found an increase in glycolysis and a decrease in oxidative metabolism in the patients’-derived cells. We have continued our investigations using induced pluripotent stem cell (iPSC)-derived neural cells, which would be more suitable to define neuronal defects and adapt potential therapeutic developments. We focused our investigations on LOAD and control iPSC-differentiated proliferative cells, including neural precursor cells (NPCs) and astrocytes. NPCs are multipotent progenitors that can be differentiated to any brain cell type. Astrocytes, play important roles in brain metabolism, including energy production. In particular, they produce lactate which is used by neurons as a substrate for mitochondrial respiration. Therefore, the investigation of iPSC-derived astrocytes could be important in understanding the role of bioenergetics in LOAD. To this end, we characterized NPCs and astrocytes from n=5 control and n=7 LOAD iPSCs. To confirm their bioenergetics profiles, we screened the cells using Seahorse XFp Cell Mito Stress Tests. The tests showed that mitochondrial respiration, glycolysis, and ATP production were elevated in both LOAD NPCs and astrocytes but their mitochondrial mass was similar to controls. The growth rates of NPCs and astrocytes, however, were slower in the LOAD patients’-derived cells than in cells from non-demented controls. These data suggest that bioenergetics changes also occur in brain cells from patients with LOAD and may be inherent pathophysiologic features in LOAD. Altogether, we have developed a cellular platform consisting of “personalized cell systems” to identify changes in key metabolic pathways that may be LOAD-specific. These models can lead to the development of novel diagnostic and/or therapeutic strategies.


Poster

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 278.04/A46

Topic: A.03. Stem Cells and Reprogramming

Support: Prinses Beatrix Spier Fonds
Title: Inflammatory neuropathies in vitro: From detecting autoantibodies to measuring conduction in an iPSC-based model


Abstract: Multifocal Motor Neuropathy (MMN) is a rare chronic inflammatory neuropathy characterized by asymmetrical weakness in distal limbs. It shares important features with the Acute Motor Axonal Neuropathy (AMAN) form of Guillain-Barré syndrome (GBS), namely conduction block in motor axons, and autoantibodies against the ganglioside GM1 which is abundant in neuronal membranes. We have previously established the first disease model for MMN by treating iPSC-derived motor neurons (MN) from control donors with patient sera, and showed that IgM-anti-GM1 raised intracellular Ca^{2+} levels by itself, and produced structural damage by activating the classical complement pathway. More recently we have performed a more detailed characterization of the model, investigated the mechanism of antibody pathogenicity in MMN, and expanded the application to the related neuropathies GBS, Lewis Sumner Syndrome (LSS) and Chronic Inflammatory Demyelinating Polyneuropathy (CIDP). Comparison of ganglioside expression in iPSC-derived motor- and sensory neurons did not reveal differences in antigen expression that could explain MN specificity in MMN, indicating there may be a MN-specific disease mechanism downstream of antibody binding. Motor- and sensory neurons also displayed equal expression of the complement-inhibiting proteins CD46, CD55 and CD59, but we did find common genetically encoded differences in protein expression between control iPSC donors that might have a disease-modifying effect in patients. MN-binding IgM autoantibodies were also detected in sera from GBS, LSS and CIDP patients, at varying subcellular localizations. Intracellular antibodies were even detected in some controls, but seemed particularly prevalent in CIDP. It is unclear whether such antibodies could be pathogenic. While Ca^{2+}-imaging previously indicated that IgM-anti-GM1 from MMN patients could influence MN function, it was unclear how the cytosolic Ca^{2+}-increase was induced, what effect it had on electrophysiological properties, and how this would relate to conduction block observed in patients. A pilot high-resolution microelectrode array experiment (MaxWell Biosystems) allowed simultaneous recording of multiple neurons individually, visualizing action potential propagation and measuring conduction velocity. Interestingly, we observed that different neurons in the culture responded differently to the addition of MMN serum, which should be followed up with post-recording correlation with neuron identity. If MN-specific conduction abnormalities are conserved in the model, it would greatly strengthen its reliability for diagnostic or drug testing purposes.

Establishing a centralized repository of human pluripotent stem cells for neurodegeneration research


**Abstract:** The Indiana University Genetics Biobank (IUGB) was established in 1990 and currently houses more than 20,000 lymphoblastoid cell lines and peripheral blood mononuclear cells. In partnership with the National Institute on Aging (NIA) through the National Centralized Repository for Alzheimer’s Disease and Related Dementias (NCRAD) and the Michael J. Fox Foundation (MJFF), the IUGB has been funded to create a centralized repository of human pluripotent stem cells (hPSCs) and fibroblasts. These cell lines are derived from patients with Alzheimer’s disease and related dementias (ADRD), Parkinson’s Disease (PD) and healthy controls. hPSCs provide a remarkable tool to study early human development, disease modeling, and drug development. However, much variability exists between hPSC lines, which is compounded by the diversity of culture conditions that exist among laboratories. Additionally, many labs do not have the resources to perform thorough quality control measures on cell lines or to distribute high demand lines to other investigators. Due to these factors, the IUGB works to centralize hPSC and fibroblast lines in one location and perform rigorous quality control to ensure cells can be offered in a standardized manner to researchers worldwide. hPSCs are first screened for multiple species of mycoplasma and other sources of bacterial and yeast contamination. A 94 single nucleotide polymorphism (SNP) fingerprint is obtained to assign an identity to every cell line. G-band karyotype is performed to assess genetic stability. hPSCs are differentiated to each of the three germ layers. Pluripotency as well as differentiation efficiency is determined by qRT-PCR analysis of more than 20 genes corresponding to each lineage. Additional quality control measures performed on NIA funded lines include pluripotency
confirmation by immunocytochemistry. Any pathogenic mutations are verified by PCR amplification followed by Sanger sequencing. Lines that pass all quality control measures are made available to approved researchers. Through these efforts, the IUGB has established a standardized facility to advance the study of neurodegenerative disorders through the distribution of hPSCs and fibroblasts.


Poster

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 278.06/A48

Topic: A.03. Stem Cells and Reprogramming

Title: Generation of two different human microglia cells separately from iPSC derived primitive or definitive hematopoietic progenitors

Authors: *K.-D. CHOI¹, M. L. HENDRICKSON², Z.-W. DU²;
¹Brainxell, Madison, WI; ²BrainXell, Inc., Madison, WI

Abstract: Human microglia are vital residents of the brain, where they play important roles in development, injury and disease, such as Alzheimer’s (AD) disease. Arising from yolk sac primitive progenitors that colonize in the brain during embryogenesis, microglia are unique among tissue macrophages in that they are thought to remain primitive progenitors derived throughout life, without contribution from the definitive hematopoiesis. After insults such as stroke and neurodegenerative disease, however, microglia dramatically change their phenotype and are joined by infiltrating macrophages from blood. These definitive hematopoietic progenitors-derived occupants can resemble microglia in morphology and surface marker expression but appear to participate differently in disease pathogenesis, making it essential to further clarify their functions. Currently, there is no method reported to generate these two microglia cells from the same iPSCs. To understand how naive microglia and infiltrating microglia cells affect the brain in disease condition, we developed a novel protocol to efficiently produce two different microglia cells separately from primitive or definitive hematopoietic progenitors. By manipulating WNT signaling and Activin-Nodal signaling, human iPSCs were first induced to separate hematopoietic fates, primitive hematopoietic progenitors (CD43⁺, CD235a⁺) and definitive hematopoietic progenitors (CD34⁺, CD43⁺, CD235a⁻). Next, these two hematopoietic progenitors were grown in serum-free differentiation medium containing CSF-1 and IL-34 to differentiate into macrophages, then cocultured with our BrainXell cortical neuron
and astrocyte mixed culture to induce microglia identity. We identified the different gene expression between these two microglia cells by RNA-seq profiling, and validated by qPCR, immunostaining and FACS analysis. In summary, two different microglia cells, derived separately from iPSC derived primitive or definitive hematopoietic progenitors, will provide useful models to understanding microglia function in neurological diseases like AD disease.


**Poster**

**278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 278.07/A49

**Topic:** A.03. Stem Cells and Reprogramming

**Title:** Maturation of iPSC-derived dopaminergic and cortical neurons with Neuro MQ medium, in a model of Parkinson’s disease

**Authors:** *J.-P. RICHARD*, I. SILVA, L. REN, M. HONDA, Y. OKUDA, S. EMINLI-MEISSNER, R. MODALI, M. RAO; 1REPROCELL USA, Beltsville, MD; 2REPROCELL Inc, Yokohama, Japan; 3REPROCELL Europe Ltd, Glasgow, United Kingdom

**Abstract:** Neurodegenerative diseases such as Alzheimer’s disease, ALS or Huntington Disease, affect millions of people worldwide. They are usually characterized by treatments available only to alleviate symptoms and no therapies to cure, and by a critical lack of biomarkers. Here, we focused on Parkinson’s disease, which is affecting mainly the dopaminergic neurons of the substantia nigra. The etiology of Parkinson’s disease is still unclear and genetic causes are usually estimated to represent 10% of cases, while most cases are sporadic (mix of environmental and genetic factors). This diversity explains that animal models don’t fully represent the disease spectrum and research involving induced Pluripotent Stem Cells (iPSCs) disease modeling capabilities are filling that void.

REPROCELL uses a unique cocktail of non-modified RNAs, immune evasion mRNAs and double stranded miRNAs in our reprogramming 3rd generation kit (stemRNA) to reprogram primary cells into high quality iPSC, in a short time (as short as 10 days) and with an efficiency of up to 4%. Our reprogramming platform is GMP compatible to produce clinically relevant iPSC cells and can be easily used to reprogram commonly isolated cell types (fibroblasts, endothelial progenitor cells and urine-derived cells). We present full characterization of the primary fibroblasts from a Parkinson’s disease patient and a control individual with no known neurological diseases (including Whole Exome Sequencing) and fully characterized the iPSC
We differentiated iPSC lines to dopaminergic neurons and astrocytes to assess differentiation potential. We kept dopaminergic neuronal monocultures for 8 weeks after the beginning of differentiation and we compared adapted published protocols with and without maturation with Neuro MQ medium. Neuro MQ medium helped neuronal cultures to mature faster, a very important feature when investigating late onset neurodegenerative diseases. Neuro MQ medium induces electrophysiological activity earlier in neuronal monocultures. To further investigate the maturation of neurons, we are using gene expression panels and neurite outgrowth assays and compare control and Parkinson’s disease cultures. We are also comparing maturation obtained via direct coculture with astrocytes.

**Disclosures:** J. Richard: A. Employment/Salary (full or part-time); REPROCELL USA. I. Silva: A. Employment/Salary (full or part-time); REPROCELL USA. L. Ren: A. Employment/Salary (full or part-time); REPROCELL USA. M. Honda: A. Employment/Salary (full or part-time); REPROCELL INC. Y. Okuda: A. Employment/Salary (full or part-time); REPROCELL INC. S. Eminli-Meissner: A. Employment/Salary (full or part-time); REPROCELL Europe Ltd. R. Modali: A. Employment/Salary (full or part-time); REPROCELL USA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); REPROCELL USA. M. Rao: A. Employment/Salary (full or part-time); REPROCELL USA.

**Poster**

**278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program#/Poster#:** 278.08/A50

**Topic:** A.03. Stem Cells and Reprogramming

**Title:** 3D midbrain floorplate model for differentiation of PSC-derived dopaminergic neurons

**Authors:** *R. E. JOSEPHSON¹, J. SAGAL¹, S. SHIN², M. DERR¹, D. KUNINGER¹; ¹Thermo Fisher Scientific, Frederick, MD; ²Primary and Stem cell Systems, Thermo Fisher Scientific Inc, Frederick, MD

**Abstract:** Accurate *in vitro* modeling of neurological diseases requires multiple cell types of the brain to interact and develop toward mature functionality. When human pluripotent stem cells (PSC) undergo neural differentiation in 3D, self-organization of progeny cells results in organoids with brain-like structures and functions that are not observed in 2D culture. However, the increased complexity of neural organoids often comes with the costs of low throughput and poor reproducibility. Disease models for drug discovery may therefore have to temper self-organized complexity with inductive specification of desired cell types. To model Parkinsons Disease (PD), we have developed a method for differentiation of human PSC to midbrain
dopaminergic (DA) neurons that combines elements of 2D dissociated culture and 3D organoid culture. Cells are efficiently specified as midbrain floor plate (FP) in 2D via an established protocol using a combination of small molecules and growth factors. FP cells are then seeded into suspension culture in defined numbers for spheroid formation and expansion, then maintained in suspension for two to five weeks of differentiation. Early differentiation of the 3D cultures is marked by morphological change and the appearance of NurrI- and tyrosine hydroxylase (TH)-expressing DA neurons at the organoid surface. Single-cell analysis demonstrates that many neurons co-express Sox6 and TH and thus resemble midbrain DA neurons of the Substantia Nigra pars compacta (SNc). Replating of spheroids on extracellular matrix results in neurite outgrowth and outward migration of DA neurons. Multielectrode array (MEA) recording of replated spheroids shows spontaneous burst activity within a relatively short time, followed by gradual refinement toward coordinated rhythmic bursting. To test disease modeling in the hybrid 2D/3D system, CRISPR was used to make PD-related mutations (LRRK2 G2019S and SNCA A30P) in a Cas9-expressing iPSC line. These lines were differentiated to generate DA neurons as above, and to demonstrate sensitivity of the mutant SNc-like DA neurons to oxidative stress. In short, a hybrid 2D/3D culture system for iPSC-derived midbrain floor plate improves maturation of DA neurons and makes promising steps toward a reproducible in vitro disease model for Parkinsons.


**Poster**

**278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 278.09/A51

**Topic:** A.03. Stem Cells and Reprogramming

**Support:** NCN Grant No. 2016/22/M/NZ2/00548 to PL
NCN Grant No. 2017/27/B/NZ1/02401 to PL

**Title:** Integration of clinical WGS supported by patient specific hiPS cell-based disease modeling and functional genomics translational approach for decoding of unknown neurodevelopmental disorders (UNDS)

**Authors:** M. WYPCHLO1,3, T. HAHN4, M. RYDZANICZ2, A. PRIGIONE4, R. KUHN4, A. RYBAK-WOLF4, S. DIECKE4, R. PLOSKI2, *P. LISOWSKI4;
1Dept. of Med. Genet., 2Med. Univ. of Warsaw, Warsaw, Poland; 3Postgraduate Sch. of Mol.
Abstract: BACKGROUND: Neurodevelopmental disorders (NDs) are characterized by the lack of effective diagnostic procedures and therapeutic strategies, due to limited knowledge of the underlying pathogenetic mechanisms. Whole-Genome-Sequencing (WGS) is the state of the art approach for detecting rare-disease-causing variants. However results interpretation and then functional verification is a daunting task.

AIM: Thus, we aim to establish a clinical diagnosis for patients with undiagnosed NDs (UNDs) applying clinical WGS. To support WGS studies functional significance is addressed by extensive transcriptome and epigenome profiling of the iPS and their isogenic controls along the neuronal lineage.

METHODS AND RESULTS: To support the interpretation of the WGS results allowing to find novel causative genetic variants, we developed hiPSCs technology platform by generation of mutation-specific neuronal subtypes, in vitro for whole genome transcriptome and methylome screening of patients derived neurons to unravel deregulation in genes expression and explain the genetic basis of rare neurodevelopmental diseases. This is achieved through genomic datasets integration from patient derived stem cells, neuronal precursors and matured neuronal cells including 2D neurons and 3D brain organoids. Experimental confirmation are finally carried out on the hiPSC-derived isogenic controls after correction of putative mutations specified by combination of WGS, transcriptomic and DNA methylation profiles.

CONCLUSIONS: Proposed approach for decoding of UNDs by integration of clinical WGS supported by patient specific induced pluripotent stem cell based modeling, transcriptomic and epigenomic translational approach, and finally in-vitro validation, is applicable to establish an efficient and effective methodology for genomic biomarkers discovery and plausible treatment of investigated undiagnosed neurodevelopmental diseases.


Poster

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 278.10/A52

Topic: A.03. Stem Cells and Reprogramming

Title: Chromatin dynamics in a 3D organoid model of human forebrain development
Abstract: Human forebrain development is characterized by a series of highly synchronized cellular events, which, if perturbed, can cause neurodevelopmental disease. To chart the regulatory activity that may coordinate these events, we generated a map of accessible chromatin in human stem cell-derived 3D neural cultures. In particular, we probed glial and neural cell lineages from 3D organoids resembling either dorsal or ventral forebrain over ~20 months of in vitro differentiation. We found that, at matched developmental time points, accessibility in forebrain organoids closely resembled that in primary tissue. We integrated accessibility with transcriptomics to infer specific transcription factors that regulate lineages and identify putative enhancers for developmentally expressed genes. Moreover, we used this resource to map genetic risk for neurodevelopmental disorders to specific lineages and developmental stages, and uncovered sequence motifs enriched in human-specific regulatory DNA regions. Overall, this organoid platform brings novel insights into chromatin regulatory dynamics of inaccessible stages of human forebrain development and could be used to model neuropsychiatric disorders and study human brain evolution.


Poster

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 278.11/A53

Topic: A.03. Stem Cells and Reprogramming

Support: Department of Biotechnology, Government of India

Title: Altered network activity of human cortical neurons lacking FMRP

Authors: *S. DAS SHARMA\textsuperscript{1,2}, R. PAL\textsuperscript{1}, N. RAJ\textsuperscript{3}, B. T. SELVARAJ\textsuperscript{4}, B. K. REDDY\textsuperscript{1}, K. K. SAMAGA\textsuperscript{1}, D. J. SRINIVASAN\textsuperscript{1,2}, L. ORNELAS\textsuperscript{5}, D. SAREEN\textsuperscript{5}, G. J. BASSELL\textsuperscript{3}, C. N. SVENDSEN\textsuperscript{6}, P. C. KIND\textsuperscript{1,7,8,9}, S. CHANDRAN\textsuperscript{1,8,9,10}, S. CHATTARJI\textsuperscript{1,2,9,11}, D. J. A. WYLLIE\textsuperscript{1,7,8,9},
\textsuperscript{1}Ctr. for Brain Develop. and Repair, Inst. For Stem Cell Sci. and Regenerative Med., Bangalore, India; \textsuperscript{2}The Univ. of Trans-Disciplinary Hlth. Sci. and Technol., Bangalore, India; \textsuperscript{3}Emory Univ., Atlanta, GA; \textsuperscript{4}Ctr. for Clin. Brain Sciences, Univ. of Edinburgh, EH16 4SB, Edinburgh, United Kingdom; \textsuperscript{5}The Board of Governors Regenerative Med. Inst. and Dept. of Biomed. Sciences,
Abstract: Fragile X syndrome (FXS), an X-linked disorder, the most common form of inherited intellectual disability and autism, is caused by the silencing of the FMR1 gene. Data from rodent models of FXS indicate that loss of FMRP leads to an increase in neuronal excitability. In order to assess whether increased excitability is present in human neurons we generated cortical neurons from human pluripotent stem cells and using whole-cell patch-clamp recording techniques assessed whether loss of FMRP expression leads to altered network activity of these excitatory neurons. We have recorded from two control induced pluripotent stem cell (iPSC) lines, three FXS iPSC lines and a pair of isogenic embryonic stem cell line in which we deleted 5’UTR and exon 1 of the FMR1 gene using CRISPR/Cas9. For each set of ‘control’ or ‘FMRP-lacking’ lines the data were internally consistent and data reported here are from the isogenic pair, FMR1+/y and FMR1−/y. In current-clamp recordings FMR1+/y neurons displayed spontaneous firing of bursts of action potentials with mean duration of 50.4 ± 2.9 s (n=16 cells). The mean number of such events observed (in 10 minutes) was 5.0 ± 0.65. In contrast FMR1−/y neurons displayed burst durations that were significantly shorter in duration (12.8 ± 0.8 s, n=15 cells; P<0.0001) but which occurred more frequently with an average number (in 10 minutes) of 22.5 ± 2.6 (P<0.0001). We hypothesize that this altered network excitability in FXS neurons may potentially be due to altered activity of persistent sodium conductance (Na\textsubscript{P}) and/or large conductance calcium activated potassium channel dysfunction (BK\textsubscript{Ca}). To confirm this we assessed the effect of riluzole (persistent Na\textsubscript{P} blocker) and paxilline (BK\textsubscript{Ca} blocker) on the burst properties. Burst properties of FMR1−/y neurons did not show significant change in presence of blockers with mean burst duration of 15.2 ± 2.0 s (n=5 cells; P=0.69) and mean burst number (in 10 minutes) of 30.8 ± 4.2 (P=0.32). In presence of riluzole and paxilline FMR1+/y neurons displayed a decrease in mean burst duration 19.2 ± 2.1 s (n=7 cells; P=0.017) and increase in mean burst number 11.3 ± 1.0 (P=0.0011) thereby resembling the bursting profile of FMR1−/y neurons. Current density measurements of Na\textsubscript{P} and BK\textsubscript{Ca} channels from FMR1−/y neurons show a decrease in persistent sodium (INa\textsubscript{P} at -40 mV: FMR1+/y -0.96 ± 0.13 pA/pF, n=18 cells; FMR1−/y, -0.3002 ± 0.03 pA/pF, n=18 cells; P < 0.0001) and BK\textsubscript{Ca} (IBK\textsubscript{Ca} at +40 mV: FMR1+/y 13.1 ± 1.8 pA/pF, n=11 cells; FMR1−/y, 4.48 ± 0.76 pA/pF, n=11 cells; P=0.0003). These findings suggest that lack of FMRP leads to Na\textsubscript{P} and BK\textsubscript{Ca} channel dysfunction causing aberrant network excitability in human stem cell derived cortical neurons.

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 278.12/A54

Title: Characterization of the activity-dependent development of IPSC-derived neurons from fragile X patients

Authors: *G. MAUSSION, C. ROCHA, V. SOUBANNIER, T. M. DURCAN;
Montreal Neurolog. Inst., Montreal, QC, Canada

Abstract: Fragile X syndrome is a form of syndromic autism whose genetic causes have been relatively well uncovered. It is actually mainly caused by a CGG triplet expansion in the 5’ UTR sequence of FMR1 gene, affecting mostly men. FMR1 encodes a mRNA binding protein which is involved in the regulation of local translation at the synaptic level. The mechanisms leading from such gene mutations to a neurodevelopmental disorder still need to be investigated. While several studies have shown that the neuronal development is driven by cellular activity and connectivity, we aim to further investigate the effect of FMR1 repression on the neuronal activity taking advantage of IPSC-derived neurons from patient’s cells.

IPSC-derived neurons will be investigated through calcium imaging to characterize their pattern of spontaneous activities, as well as their capability to respond to neurotransmitter through extrasynaptic receptors. A multi-electrod array approach is going to be used to analyse the overall network activities.

Those studies should provide further information on the impairment of activity-dependent neuronal development in Fragile X syndrome.

Disclosures: G. Maussion: None. C. Rocha: None. V. Soubannier: None. T.M. Durcan: None.
Title: Analysis of neuronal development in patients with KCNJ11/Kir6.2 mutations using cerebral organoids

Authors: *G. DALGIN*¹, A. J. GARCIA, III², A. K. TRYBA³, A. COHEN⁴, L. PHILIPSON¹, S. A. W. GREELEY¹;
¹Kovler Diabetes Ctr., Univ. of Chicago, Chicago, IL; ²Emergency Med., ³Pediatric Neurol., The Univ. of Chicago, Chicago, IL; ⁴Rosalind Franklin Univ., North Chicago, IL

Abstract: The fundamental question in brain research is to understand how the complex human brain is developed. Recent advancements in human induced pluripotent stem cell and 3D cell culture technologies made it possible to generate cerebral organoids. This technology made it possible to explore the human brain development in a dish, and increased our opportunities for personalized medicine. Neonatal diabetes mellitus (NDM) is a rare type of monogenic diabetes that is caused by a single gene abnormality and patients develop diabetes early in life. Heterozygous activating mutations in the ATP-sensitive potassium (K<sub>ATP</sub>) channel gene <i>KCNJ11</i> are the most common cause of permanent NDM. In addition to diabetes patients exhibit range of neurodevelopmental dysfunctions, from learning disorders to cognitive dysfunction and seizures. Our clinical data suggest that neurodevelopmental disorders in KCNJ11 mutant patients may start during fetal and postnatal brain development that is largely inaccessible for direct molecular and functional analysis. To model KCNJ11 dependent brain development and to understand the pathophysiology of neurodevelopmental disorders in patients we developed patient induced pluripotent stem cell derived cerebral organoids. The molecular and electrophysiological signatures of cerebral organoids developed from non-pathogenic/normal ipscs suggest that these organoids established a stable neural network with synchronized bursting. In contrast extracellular recording of local population activity revealed decreased activity of KCNJ11 mutant brain organoids. Therefore, we developed a reliable platform to analyze brain organoids from patients with neurological disorders to predict the outcome and determine personalized treatments. We are testing potential therapeutics that may improve outcome and examine how genetic variations affect the phenotype.


Poster

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 278.14/A56

Topic: A.03. Stem Cells and Reprogramming
Support: Nebraska Stem Cell Grant

Title: Probing the role of astrocytes in fragile X syndrome using human induced pluripotent stem cell-derived astrocytes

Authors: *B. REN*¹, Y. JUNG², B. OLDHAM², A. ARMSTRONG², R. GOUGH², P. RAGUNATHAN², A. DUNAEVSKY²;
¹Biochem. and Mol. Biol., ²Neurolog. Sci., Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Fragile X syndrome (FXS), the most common monogenic cause of autism spectrum disorder, results from the hypermethylation of CGG expansion in the FMR1 gene locus, transcriptional silencing of the gene and the absence of fragile x mental retardation protein (FMRP). The fmr1 mutant mice have been useful in elucidating the mechanisms of neural impairments, however, attempts to translate outcomes to humans have been challenging. Here we investigate the role of human astrocytes in the pathogenesis of FXS by differentiating human induced pluripotent stem cells (hiPSCs), derived from fibroblasts of FXS patients, to astrocytes. We found that FXS astrocytes showed altered transcriptional and functional properties, including shortened cell cycle duration and enhanced calcium signaling. To examine the phenotypes of FXS astrocytes in the brain, we generated a chimeric mouse model by neonatal implantation of hiPSC-astrocytes expressing a fluorescent protein. Three months post engraftment, transplanted cells maintained astrocytic lineage. Analysis of astrocyte morphologies revealed that FXS astrocytes have longer processes and altered complexity in different brain regions. Analysis of astrocyte morphologies at 6 and 9 months post-engraftment is ongoing. Preliminary data suggest that FXS astrocytes exhibit altered Ca²⁺ signaling properties in cortical slices four months post engraftment. To further understand whether structural synaptic plasticity is altered, repeated in vivo imaging of hiPSC-astrocytes and dendritic spines in the chimeric mice is performed. Our studies suggest the contribution of human astrocytes in FXS pathogenesis and provide therapeutic targets for personalized FXS treatment.


Poster

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 278.15/A57

Topic: A.03. Stem Cells and Reprogramming

Title: Exploring Smith-Magenis syndrome-associated phenotypes in induced pluripotent stem cell (iPSC) - derived neural cells
Authors: *B. K. ENGLAND, F. J. GORDOVEZ, J. CROSS, N. AKULA, W. R. CORONA, A. SMITH, *S. D. DETERA-WADLEY, F. MCMAHON; NIH, Bethesda, MD

Abstract: Smith-Mageni Syndrome (SMS) is a rare neurodevelopmental disorder characterized by behavioral, neurocognitive, circadian rhythm, and craniofacial abnormalities. The majority of cases are caused by a heterozygous deletion on 17p11.2. Non-deletion cases carry damaging mutations on RAI1, a gene that is encoded by this region. RAI1 is highly expressed in brain and is thought to function as a transcription factor although its exact biological role remains to be well defined. To dissect the genetic and phenotype features of SMS, we are studying a family that includes parents, a daughter with SMS and her unaffected sister. Sanger sequencing of the region that includes the SMS mutation in all four family members confirmed the presence of a frameshift mutation in the patient and its absence in her parents and sister. To identify potential defects in the entire coding region we are conducting whole exome sequencing on all family members. SMS symptoms that include behavioral problems and intellectual disability strongly suggest defects in brain function. We chose to study iPSC-derived neural cells to reveal defects in neurobiological mechanisms associated with RAI1 mutation. Peripheral blood mononuclear cells (PBMCs) from the entire family are reprogrammed to iPSCs. After assessing karyotype and pluripotency, three iPSC clones from each subject are differentiated into neural progenitor cells. Initial studies will involve RNA-sequencing and evaluation of cellular morphology. These results will be used to guide future research, including differentiation into other cell types, such as neurons and astrocytes. Ultimately, this study aims to further elucidate the function of RAI1 with the potential of illuminating the role of this gene in other neuropsychiatric illnesses.


Poster

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 278.16/A58

Topic: A.03. Stem Cells and Reprogramming

Support: R01MH104593
Brace Cove Foundation
Pitt Hopkins Research Foundation
Maryland Stem Cell Research Foundation

Title: An in-depth analysis of disrupted molecular pathways in Pitt-Hopkins syndrome patient derived neural progenitor cells (NPCs)
**Authors:** D. J. HILER\(^1\), *S. SRIPATHY RAO\(^1\), M. T. NGUYEN\(^3\), Y. WANG\(^1\), M. WARNER\(^1\), H.-Y. CHEN\(^2\), G. R. HAMERSKY\(^4\), S. C. PAGE\(^1\), O. SOUDRY\(^1\), R. E. STRAUB\(^1\), K. MARTINOWICH\(^1,5,6\), A. JAFFE\(^1,6,7,3,8\), B. J. MAHER\(^1,5,6\); 
\(^2\)Developmental Electrophysiology, \(^1\)Lieber Inst. for Brain Develop., Baltimore, MD; \(^3\)Dept. of Human Genetics, Johns Hopkins Sch. of Med., Baltimore, MD; \(^4\)Developmental Electrophysiology, OHSU, Portland, OR; \(^5\)The Solomon H. Snyder Dept. of Neuroscience, Johns Hopkins Sch. of Med., Baltimore, MD; \(^6\)Dept. of Psychiatry and Behavioral Sciences, Johns Hopkins Sch. of Med., Baltimore, MD; \(^7\)Dept. of Mental Health, Johns Hopkins Sch. of Med., Baltimore, MD; \(^8\)Dept. of Biostatistics, Johns Hopkins Sch. of Med., Baltimore, MD

**Abstract:** Pitt-Hopkins Syndrome (PTHS) is a rare neurodevelopmental disorder caused by an autosomal dominant mutation or deletion in the gene transcription factor 4 (*TCF4*). *TCF4* is a basic helix-loop-helix (bHLH) transcription factor that plays a critical role in neuronal development through known interactions with other proneural bHLH proteins. Human *TCF4* expression peaks during corticogenesis and patients with *TCF4* mutations have Pitt Hopkins Syndrome (PTHS), which is characterized by profound developmental delays and autistic behaviors. To study the role of *TCF4* in human cortical development, we have developed a platform to differentiate PTHS patient and control induced pluripotent stem cells (iPSC) into human neural progenitor cells (NPCs). In this study, we have reprogrammed iPSCs and differentiated NPCs from 6 control individuals and from 4 PTHS patients with a point mutation in the bHLH domain of *TCF4* and 2 PTHS patients with large truncations of *TCF4*. Differentiation of NPCs was validated with immunocytochemistry and Fluidigm qPCR. Over the course of 20 days of NPC differentiation, we have identified over 500 differentially expressed genes (DEGs) between PTHS and controls. GO-term enrichment analysis of these DEGs has identified a downregulation in terms associated with axonal development along with additional up-regulation/down-regulation of terms associated with cellular metabolism, trafficking, and respiration in PTHS NPCs. We have performed metabolomic analysis of PTHS and control NPCs to further assess specific molecular pathways identified by our GO-terms analysis. With this stem cell-based platform, we aim to better understand the role of TCF4 in cortical development and underlying pathophysiology of PTHS in a human context.


**Poster**

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 278.17/A59
Title: The role of KIAA0319 in neuronal development and developmental dyslexia

Authors: *S. PANIAGUA, B. CAKIR, Y. XIANG, I. H. PARK, J. R. GRUEN; Yale Univ., New Haven, CT

Abstract: Reading Disability (RD), also known as dyslexia, is defined as difficulty in processing written language in individuals with normal intellectual capacity and educational opportunity. The prevalence of RD is between 5% and 17% (Shaywitz, SE et al., 2013) and the heritability ranges from 44% to 75% (DeFries, JC et al., 1987; Hart, SA et al., 2010). Genetic linkage analysis and genome-wide association studies (GWAS) have identified several genes and regulatory elements linked to RD and reading ability, although their functions and molecular mechanisms are not well understood. Prominent among these are DCDC2 and KIAA0319, two genes encoded in the DYX2 locus of human chromosome 6p22 (Eicher, JD et al., 2014). Association of both genes has been independently replicated in multiple independent studies and languages (Fisher, SE, DeFries, JC, 2002; Thapar, A et al., 2015; Stergiakouli E, Thapar, A, 2010; Carrion-Castillo, A et al., 2013). Rodent models suggest that both genes are involved in neuronal migration, but their precise function is unknown (Paracchini, S et al., 2016). Additionally, glutamatergic synaptic transmission is elevated in Dcdc2-mutant mice, and this elevation is at least in part through an NMDAR-mediated, presynaptic mechanism at layer 4 synaptic connections (Che, A et al., 2016).

The goal of this study is to determine the mechanisms by which KIAA0319 and DCDC2 influence reading and language performance. We hypothesize that both genes play a critical role in neuronal development. To test this hypothesis, we knocked down mRNA expression of targets using CRISPRi in a human embryonic stem cell line in which a doxycycline-inducible dead Cas9 (dCas9) gene has been stably integrated. We then examined changes in morphology through immunofluorescence, synaptic signaling by electrophysiology, and neuronal differentiation through qRT-PCR targeting genes specific to neuronal subtypes and cortical layers. Preliminary data from KIAA0319 knockdown showed an increase in neuronal morphology and a decrease in proliferation compared to controls, after 20 days of neuronal differentiation through an extracellular-factor protocol. These results are consistent with previous studies in rats and mice (Franquinho, F et al., 2017), implicating KIAA0319 in regulating neuronal development by acting as an inhibitor of terminal differentiation. In aggregate, these studies suggest that differential expression of KIAA0319, within cortical layering and neuronal cell types, plays a crucial role in regulating brain development and affecting reading performance.

**Poster**

**278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #: Poster #:** 278.18/A60

**Topic:** A.03. Stem Cells and Reprogramming

**Support:** CQDM/MESI FACS 2019-2021

**HBHL platform funding**

**Title:** Quantitative characterization of variability in cortical neurons between healthy human induced pluripotent stem cell lines

**Authors:** *R. A. THOMAS*¹, E. CAI², X. C. CHEN¹, N. ABDIAN¹, E. A. FON¹, T. M. DURCAN¹;


**Abstract:** When studying developmental neurological diseases such as autism spectrum disorder, human tissue samples are only available postmortem. The breakthrough advances allowing reprogramming of human blood or skin cells into pluripotent stem cells (iPSCs), which can be differentiated into numerous cell types including neurons and astrocytes, allow for human cell models of neurodevelopmental disorders. However, iPSC are difficult to maintain and many different protocols for culturing and differentiation have been developed. How different cell lines behave under different culturing conditions has not been compared in parallel. To be able to compare between patient lines and controls there needs to be a bench-mark of normality. Standardized reproducible quality control checks must be established and shared across different research groups. Quantitative characterization of iPSC lines and neurons is critical to detecting disease phenotypes and identifying the sources of disease etiology. Here, we aim to create a panel of measurements using automated analysis programs to characterize 12 iPSC control lines. We characterize 2 commercial and 10 in house lines, analyzing 5 different stages. We quantified markers of pluripotency and cortical neurons using custom scripts. Additionally, we compared two types of growth media and two neuronal induction protocols. We find variation in rates of differentiation between protocols and between iPSC lines. The amount that iPSCs aggregate together, the proportion of non-neuronal cells in cultures and many markers vary across cell lines. We summarize the variation across iPSC and provide analysis tools and a reference data set for the range of normality in healthy iPSC and their differentiation into cortical neurons. We conclude, that to be confident in phenotypes found using iPSCs from patients, multiple control lines should be included and iPSC quality control characterization should always be performed for each line.

Poster

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 278.19/A61

Topic: A.03. Stem Cells and Reprogramming

Title: Engineering human cerebral organoid as a model for studying Down syndrome

Authors: *Y.-T. LIN¹, J. DAVILA², H. MATHYS¹, H. WOOLF¹, H. CAM¹, C. YU¹, L.-H. TSAI¹;
¹Picower Inst. for Learning and Memory, ²Broad Inst., MIT, Cambridge, MA

Abstract: Down syndrome (DS) associated with trisomy of chromosome 21 is a developmental disorder characterized by brain hypotrophy, intellectual disability and high risk for developing Alzheimer’s disease (AD) later in life. Approximately one in every 700 births worldwide is diagnosed as DS. Tissue culture and mouse models have yielded significant insight regarding the pathological phenotypes of DS. However, species differences between mouse and human and the limited utility of tissue cultures hamper advances in elucidating the mechanisms given rise to DS, and thereby hamstring the development of effective therapeutic interventions. Recent advances in generating human induced pluripotent stem cells (iPSCs) from individuals with DS provide a powerful platform that might better recapitulate certain DS phenotypes. We have used DS patient-derived iPSCs and its isogenic iPSCs disomic for chromosome 21 to investigate how the extra chromosome 21 affects brain development. We reported that our DS organoid model recapitulates multiple relevant phenotypes. Moreover, single-cell RNA sequencing identifies different cell population clusters and signature transcription factors unique to DS organoid compared to its isogenic disomy. We are using this DS organoid model to investigate the effect of trisomy 21 on the development of various brain cell types and pathological hallmarks of AD, and as potential platform for therapeutic interventions.

**278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases**

**Location:** Hall A  
**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM  
**Program #/Poster #:** 278.20/A62  
**Topic:** A.03. Stem Cells and Reprogramming  
**Support:** NIH NS111965  
CT Regenerative Medicine Research Fund 14SCDIS-01  

**Title:** Exploring the role of UBE3A in electrophysiological phenotypes of human Dup15q syndrome neurons

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

**Abstract:** Autism is one of the most prevalent developmental disorders worldwide. Affected children often display impairments in communication, difficulties in social interactions, repetitive behaviors, and learning disabilities. One of the most common genetic variations associated with autism is duplication in the 11.2-13.1 region of the long arm of chromosome 15. Children carrying this duplication develop Dup15q syndrome, a distinct neurodevelopmental disorder characterized by autism, epilepsy, and a range of intellectual and motor deficits. Using neurons derived from patient-specific induced pluripotent stem cells (iPSCs), we found that Dup15q neurons had an increased frequency of excitatory synaptic events and increased spontaneous action potential firing, as well as increased dendritic protrusions, compared to unaffected controls. It is important to note that only maternal duplications of this region result in Dup15q syndrome, while paternal duplications do not. There are many genes in the duplicated region, but only UBE3A, which encodes a ubiquitin ligase, is paternally imprinted in neurons and therefore only expressed from the maternal allele. This strongly affirms the relevance of UBE3A to Dup15q syndrome. Although UBE3A overexpression has been used to model Dup15 syndrome in mice, the contribution of UBE3A to the cellular phenotypes identified in human neurons is unknown. The goal of the present study is to define the role of increased UBE3A expression in the development of Dup15q cellular deficits. Antisense oligonucleotides (ASOs) will be used to lower UBE3A levels and the electrophysiological and morphological phenotypes will be examined during *in vitro* development. Preliminary data obtained in 15-week old Dup15q neurons indicate that reducing UBE3A to control levels rescues the elevated synaptic activity and spontaneous action potential firing rate. Decreasing UBE3A below control levels also disrupts neuronal and synaptic development, as seen in Angelman syndrome neurons, suggesting that appropriate regulation of UBE3A is critical for neuronal development. Elucidating the relationship between UBE3A and these electrophysiological phenotypes may shed light on the relevant downstream targets of UBE3A and provide a basis for exploring potential contributing factors...
roles of other duplicated genes in the affected region. Overall, these studies may identify novel therapeutic targets for treating Dup15q and related neurodevelopmental disorders.

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

Poster

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 278.21/A63

Topic: A.03. Stem Cells and Reprogramming

Support: Jordan’s Guardian Angels
Stewart and Dake’s Family Foundation
CDKL5–16–107–01 UPenn Orphan Disease Center: LouLou Foundation
CIRM DISC2-09032
NINDS R24 201603716
NINDS 1R01NS102486-01

Title: siRNA and CRISPR/dCas13b-mediated allele specific reduction of mutant RNA associated with Jordan’s syndrome

Authors: *J. L. CARTER\textsuperscript{1,2,3}, J. HALMAI\textsuperscript{1,2,3}, P. DENG\textsuperscript{1,2,3,4}, J. WALDO\textsuperscript{1,2,3}, S. DEL CAMPO\textsuperscript{1,2,3}, D. CAMERON\textsuperscript{1,2,3}, J. ANDERSON\textsuperscript{2}, J. NOLTA\textsuperscript{1,2}, K. FINK\textsuperscript{1,2,3};
\textsuperscript{1}Ctr. for Interventional Genet., Univ. of California Davis Hlth., Sacramento, CA; \textsuperscript{2}Stem Cell Program and Inst. for Regenerative Cures, \textsuperscript{3}Dept. of Neurol., Univ. of California Davis Hlth. Systems, Sacramento, CA; \textsuperscript{4}Genome Center, MIND Institute, and Biochem. and Mol. Med., Univ. of California, Davis, CA

Abstract: Jordan’s syndrome is a rare neurodevelopmental disorder arising from \textit{de novo} missense mutations in protein phosphatase 2 regulatory subunit b’delta (\textit{PPP2R5D}). \textit{PPP2R5D} encodes for B56δ, a protein that regulates protein phosphatase 2A (PP2A) substrate specificity and catalytic activity at serine/threonine sites. Dephosphorylation at these sites requires three structural subunits to complete the heterotrimeric PP2A enzyme: a scaffolding (PR65) and catalytic subunit (PPP2CA) form the core holoenzyme which interacts with variable regulatory subunits, such as B56δ. While studies have shown B56δ is highly expressed in the brain and genetic variants arising in its gene (\textit{PPP2R5D}) are linked to Jordan’s Syndrome, appropriate disease models are necessary to understand neuronal dysfunction and subsequently employ novel techniques to target the variant. Targeting the mutant \textit{PPP2R5D} transcript is a promising avenue to assess the consequence of these variants on B56δ and PP2A function. In our proof of principle studies, we have implemented small interfering RNAs (siRNA) and the dCas13b-ADAR2\textsubscript{DD}(E488Q/T375G) system to reduce and edit the mutant \textit{PPP2R5D} transcript,
respectively. PPP2R5D siRNA resulted in a potent knockdown of mutant and healthy PPP2R5D mRNA suggesting that the seed region was not able to discriminate the variant RNA. Interestingly, we observed an increase in PR65 and PPP2CA expression when siRNA were targeted to PPP2R5D in patient-derived fibroblasts. These preliminary studies by our group support a dominant negative mechanism of action, where variants in PPP2R5D affect PP2A holoenzyme subunits. To achieve allele specificity, we used dCas13b fused to an RNA adenosine deaminase, ADAR2dd (dCas13b-ADAR2ddE488Q/T375G) paired with a guide RNA (gRNA) to edit the mutant PPP2R5D transcript. We have employed this RNA editing system in patient-derived fibroblasts and induced pluripotent stem cells (iPSCs) and started assessing on-target editing efficiency. Our future work is focused on understanding the developmental implications of PPP2R5D variants in iPSC-derived neural progenitor cells (NPCs) and neurons by analyzing the underlying molecular mechanisms giving rise to altered signal transduction pathways and neuronal dysfunction in Jordan’s syndrome. These studies support the potential for RNA targeting strategies to enable molecular rescue and advance precision therapeutics for individuals with neurodevelopmental disorders such as, Jordan’s syndrome.


Poster

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 278.22/A64

Topic: A.03. Stem Cells and Reprogramming

Support: F30MH108321
U19MH104172

Title: Modelling synaptic mechanisms of autism-associated neuregulin-3 mutations using human neurons

Authors: *L. Wang\textsuperscript{1,2}, V. R. Mirabella\textsuperscript{1}, Z.-P. Pang\textsuperscript{1};\textsuperscript{1}Neurosci. and Cell Biol., Child Hlth. Inst. of NJ, New Brunswick, NJ; \textsuperscript{2}Ctr. for Med. Genet., Sch. of Life Sciences, Central South Univ., Changsha, China

Abstract: Synaptic transmission controls information flow in the brain and synaptic dysfunction is likely a biological basis for several neurodevelopmental disorders including autism spectrum disorders (ASDs), Down syndrome, and neuropsychiatric disorders such as schizophrenia. Recent human genetic studies revealed that an increasing number of mutations in neurexins (NLGNs) and neurexins, synaptic cell-adhesion proteins, are linked to ASDs and schizophrenia. The first defined gene mutation identified in idiopathic autism was an arginine (R) to cysteine
(C) missense mutation at position 451 of NLGN 3 (NLGN3 R451C). Numerous studies using knock-in animals and heterologous overexpression systems have suggested that NLGN 3 R451C may act as both a loss and gain-of-function mutation, however the detailed molecular mechanism(s) by which it causes behavioral pathology and synaptic dysfunction remain unclear. Recent advances in stem cell biology have allowed the efficient conversion of human stem cells into defined neural subtypes and we hypothesized that studying the R451C mutation using this simplified and species-specific approach may yield new insight into its molecular etiology. To test this hypothesis, we have generated isogenic knock-in human embryonic stem cell lines harboring NLGN 3 R451C allele. Initial characterization showed no major impact of NLGN3 R451C on isolated excitatory synaptic transmission in relatively homogeneous glutamatergic human neuronal cultures, suggesting the possibility that the NLGN3 R451C mutation could preferentially impact subtype-specific neuronal crosstalk between excitatory and inhibitory neurons, overall network properties and E/I balance. Ongoing experiments are defining correlating functional, morphometric and biochemical parameters in co-cultured excitatory and inhibitory human induced neurons to determine a molecular and synaptic basis for NLGN3 gene association with the pathogenesis of autism.

Disclosures:  L. Wang: None. V.R. Mirabella: None. Z. Pang: None.

Poster

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 278.23/A65

Topic: A.03. Stem Cells and Reprogramming

Support: Ricerca Corrente 2019 Italian Ministry of Health Project: #1758 - Cycle 2018A Lejeune Foundation

Title: Smith-Magenis syndrome: In vitro and in vivo evaluation of IPS-derived human neural stem cells for disease modeling and therapeutic approaches


1Fondazione IRCCS Casa Sollievo della Sofferenza, Cell. Reprogramming Unit, 2Fondazione IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy; 3Dep. of Biotech. and Biosci., Univ. of Milan Bicocca, Milan, Italy; 4Univ. of Rome La Sapienza, Roma, Italy; 5Univ. Policlinico Tor Vergata, Roma, Italy; 6Azienda Ospedaliera Antonio Cardarelli, Napoli, Italy
Abstract: Rationale: Smith-Magenis syndrome (SMS; MIM 182290, incidence 1/25000) is a complex genetic disorder characterized by multiple congenital anomalies, including dysmorphic craniofacial features, brachydactyly, hypotonia, developmental delay and sleep abnormalities. Usually after puberty, SMS patients develop mood instability and anxiety that worsen during adolescence. Most individuals present a moderate range of intellectual disability; however, the substantial deleterious behaviours leads to altered adaptive function (communication and socialization) and a lower perceived cognition. Autistic features and/or autistic spectrum disorder have been reported in as many as 90% of subjects affected by SMS and the severity ranges from mild to severe autism. SMS has been associated to 4-Mb deletion Copy Number Variation on chromosome 17p11.2. More recently it has been shown that rare nucleotide mutations in RAI1 gene - in the absence of Chr17p11.2 deletions - reproduce 21 out of 30 SMS features. Thus it was recognized that RAI1 haploinsufficiency by itself is the primary cause of the syndrome. Little is known about RAI1 molecular function and its role in neural development. Methods: We investigated the role of the novel RAI1 mutation (NM_030665.3:c.1194) on the functional properties of human patient’s fibroblasts derived from a SM patient and, subsequently, of Neural Stem Cells generated upon fibroblast reprogramming followed by neural differentiation (SMS-hiNSCs). Results: The mutation generated a RAI1 protein incapable of entering the nucleus and acting as transcription factor. Fibroblasts analysis showed a strong alteration in circadian cycle associated with an impairment in cell growth caused by an altered metabolism. Based on these results, we produced induced pluripotent stem cells and differentiated them into SMS-hiNSC in order to evaluate how the truncated form of RAI1 might influence the cell fate, the proliferation and differentiation pattern of these cells in vitro and in vivo, by transplanting the SMS-hiNSCs into the brain of immundeficient mice, in long term experiments. Our results showed that hiNSC cells carrying RAI1 mutation showed a low ability to differentiate, compared to the control lines, both in vitro and in vivo, consistent with the SMS phenotype.


Poster

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 278.24/A66

Topic: A.03. Stem Cells and Reprogramming

Support: Stanley Center, Broad Institute
NIH. R01-MH112940
Klarman Cell Observatory
Howard Hughes Medical Institute

**Title:** Individual brain organoids reproducibly generate cell diversity of the human cerebral cortex

**Authors:** *S. VELASCO*¹,², A. J. KEDAIGLE¹,², S. K. SIMMONS², A. NASH¹,², M. ROCHA¹,², G. QUADRATO³, B. PAULSEN¹, L. NGUYEN², X. ADICONIS², A. REGEV², J. Z. LEVIN², P. ARLOTTA¹;
¹Harvard Univ., Cambridge, MA; ²Broad Inst., Cambridge, MA; ³USC, Los Angeles, CA

**Abstract:** Experimental models of the human brain are needed for basic understanding of its development and disease. Human brain organoids hold unprecedented promise for this purpose; however, they are plagued by high organoid-to-organoid variability. This has raised doubts as to whether developmental processes of the human brain can occur outside the context of embryogenesis with a degree of reproducibility comparable to the endogenous tissue. Here, we show that an organoid model of the dorsal forebrain can achieve reproducible generation of a rich diversity of cell types appropriate for the human cerebral cortex. Using single-cell RNA sequencing of 166,242 cells isolated from 21 individual organoids, we find that 95% of the organoids generate a virtually indistinguishable compendium of cell types, through the same developmental trajectories, and with organoid-to-organoid variability comparable to that of individual endogenous brains. Furthermore, organoids derived from different stem cell lines show consistent reproducibility in the cell types produced. The data demonstrate that reproducible development of complex central nervous system cellular diversity does not require the context of the embryo, and that establishment of terminal cell identity is a highly constrained process that can emerge from diverse stem cell origins and growth environments.


**Poster**

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:/Poster #:** 278.25/A67

**Topic:** A.03. Stem Cells and Reprogramming

**Title:** Modeling schizophrenia and its polygenic risk using induced pluripotent stem cell-derived neurons

**Authors:** *S. C. PAGE*¹, D. J. HILER¹, S. SRIPATHY RAO¹, Y. WANG¹, C. V. NYUGEN¹, E. A. PATTIE¹, Z.-Y. YE¹, H.-Y. CHEN¹, M. TIPPANI¹, M. T. NGUYEN², F. FARINELLI¹, N. J.
Abstract: Progress towards a better understanding of schizophrenia (SZ) etiology, and in developing novel treatments has been hindered by a lack of animal models with high validity for the disorder. Moreover, current animal models cannot effectively model polygenic risk contributing to schizophrenia. The advent of induced pluripotent stem cell (iPSC) provides a unique opportunity to study human cells that retain the entire genetic architecture of schizophrenia risk, thus opening the door to developing in vitro models that can improve our ability to develop novel treatment strategies. We have established a systematic and quantifiable iPSC platform to model common risk variation associated with SZ. To help overcome heterogeneity associated with common variation, we generated iPSCs from 16 SZ patients with high polygene risk scores (PRS) and 16 neurotypical individuals with a low PRS. To control for technical variability, iPSCs underwent four independent rounds of directed differentiation into cortical neurons, a process that was monitored at each stage using a variety of quality control assays, including qPCR and immunocytochemistry followed by high-content imaging. To identify phenotypes between SZ cases and controls an array of assays were performed on neural progenitor cells (NPCs) and cortical neurons in a double-blind manner. These assays include immunocytochemistry, electrophysiology, \( \text{Ca}^{2+} \) imaging, metabolomics, qPCR, and RNA sequencing. This varied approach for measuring functional and dynamic properties associated with neuronal development will assist in the identification of phenotypic variation between SZ cases and controls. Overall, we have established a robust in vitro platform to study polygenic risk for schizophrenia, and we anticipate this model system will advance our ability to identify therapeutic targets for drug development.


Poster

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program#/Poster #: 278.26/A68
Title: Improving functional maturation of human neurons derived from the inducible pluripotent stem (IPS) cells - Effects of vitamin E and DHA on synaptic connectivity and ionic conductances

Authors: *K. M. GLANOWSKA*, C. J. DELONG, J. L. MCMAHON, K. S. O'SHEA, G. G. MURPHY;

Mol. and Behavioral Neurosci. Institute, Dept. of Neurol., Dept. of Cell and Developmental Biol., Mol. and Behavioral Neurosci. Inst., Dept. of Cell and Developmental Biology, Dept. of Psychiatry, Mol. and Behavioral Neurosci. Institute, Dept. of Mol. and Integrative Physiol., Univ. of Michigan, Ann Arbor, MI

Abstract: Patient-derived IPS cell models present invaluable opportunity to gain insights into human nervous system development, function and mechanisms underlying its disorders. Many neuropsychiatric disorders, including Bipolar Spectrum Disorder (BSD), are believed to be associated with altered neuronal excitability and synaptic connectivity, which require disease models to recapitulate electrophysiological properties of mature human neurons. Existing neuronal differentiation protocols generate neurons that express appropriate neuronal markers and appear to be relatively mature. However, their ability to reproduce firing behavior observed in tissue from human brains has been difficult to obtain. We showed previously that vitamin E (vit E) improves health and maturation of the membrane properties of neurons derived from control individuals and from patients diagnosed with the BSD. Interestingly, vit E treatment also improved firing properties of both types of neurons, which were further significantly improved by addition of the docosahexaenoic acid (DHA), but not by other omega-3 or omega-6 fatty acids. In the present work we further demonstrate effects of vit E and DHA on functional maturation of IPS cells-derived neurons with particular focus on synaptic connectivity and major conductances underlying proper electrophysiological function. Human neural progenitor cells (NPCs) were derived from IPS cells via dual inhibition of BMP and TGFβ. Neuronal differentiation was induced by culturing NPCs in complete BrainPhys supplemented with vit E, DHA or the combination of both. Electrophysiological recordings and high spatial/temporal resolution calcium imaging were performed after 8 weeks of differentiation in 2D neuronal cultures. We performed patch-clamp recordings in the voltage clamp mode to detect sodium and potassium currents by using a series of voltage steps from -80 to +100 mV. Spontaneous glutamatergic postsynaptic currents were monitored by clamping Vm at -60 mV. We also examined spontaneous and evoked Ca²⁺ transients using high resolution Ca²⁺ imaging. The effects of vit E, DHA and the combination of both were compared to control conditions. Results indicating higher ionic conductances similar to those observed in adult neuronal tissue and more frequent and/or higher amplitude synaptic events were interpreted as improved neuronal maturation. Further studies are necessary to establish the mechanisms by which DHA and vit E promote neuronal maturation *in vitro*. Our results demonstrate the utility of culture media augmentation with vit E and DHA in studies of patient-derived neurons in which biophysical properties are of primary interest.

Poster

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 278.27/A69

Topic: A.03. Stem Cells and Reprogramming

Support: U19MH10643403

Title: Omega 3 fatty acid and vitamin e promote maturation of neurons derived from induced pluripotent stem cells of bipolar disorder patients

Authors: *C. DELONG1, K. GLANOWSKA2, G. G. MURPHY3, K. O'SHEA1; 1Cell & Dev Biol, 2Neurol., 3MBNI/Physiology, Univ. of Michigan, Ann Arbor, MI

Abstract: Bipolar disorder (BD) is a hereditary neuropsychiatric disorder that is characterized by pathological fluctuations in mood from mania to depression, due to an underlying dysfunction in neuronal excitability that may in part be due to abnormal synaptic properties in neurons. The use of induced pluripotent stem cells provides a model to study neuronal excitability, however current protocols lack the ability to generate a majority of mature neurons that reflect those of the adult human brain. Neurons in the brain are enriched with long chain polyunsaturated omega 3 fatty acids, in particular docosahexaenoic acid (DHA), that are necessary for normal brain development, and previous studies in mouse neurons suggest that DHA enhances synaptic properties. We therefore looked at the effect of DHA or other fatty acids in the presence of the antioxidant alpha-tocopherol on passive and active electrophysiological properties and synaptic density in human neurons. Neural precursor cells (NPCs) were derived from iPSC via inhibition of BMP and TGFbeta signaling using Dorsomorphin and SB431542. Neuronal differentiation was induced by culturing NPCs in complete BrainPhys medium, which was supplemented once a week for 8 weeks with alpha-tocopherol alone or with oleic acid (OA), arachidonic acid (AA), eicosapentaenoic acid (EPA), or DHA. In all conditions, the ratio of GAD67-positive to vGlut1-positive cells was approximately 1:4, and the percentage of non-neuronal cells was 3-5%, suggesting a similar composition of cell types across conditions. In control and bipolar neurons, the enrichment of media with alpha-tocopherol alone improved both passive and active electrophysiological firing properties over control, several of which were further improved by the addition of DHA, but not OA, AA, or EPA. Synaptic density (number of synapsin 1+ puncta/micron of neurites) was increased over control by all treatments, with the greatest increase by alpha-tocopherol and alpha-tocopherol+DHA. Current studies are focused on DHA +/- alpha-tocopherol treatment of control vs bipolar neurons to measure effects on fatty acid
composition, single-cell and network electrophysiological properties, gene expression, neurite outgrowth, and calcium signaling.

**Disclosures:** C. Delong: None. K. Glanowska: None. G.G. Murphy: None. K. O'Shea: None.

**Poster**

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 278.28/A70

**Topic:** A.03. Stem Cells and Reprogramming

**Support:** MH106434

**Title:** Human induced pluripotent stem cell (hiPSC) derived GABAergic and glutamatergic neuron development and function in bipolar disorder

**Authors:** *D. J. SCHILL*, K. F. CAMPBELL, K. M. GLANOWSKA, C. J. DELONG, M. G. MCINNIS, G. G. MURPHY, K. O'SHEA;

1Cell & Dev Biol, 2Dept. of Neurol., 3Psychiatry, 4MBNI/Physiology, Univ. of Michigan, Ann Arbor, MI

**Abstract:** Bipolar I Disorder (BP) is a serious, recurrent mood disorder that is characterized by alternating episodes of mania and depression that affects nearly 5.7 million Americans. Considerable evidence suggests that changes in the development and function of several neural subtypes may be responsible for these shifts in mood state, however the underlying mechanisms remain unknown. There is an interplay in communication and network assembly governed by inhibitory GABAergic interneurons and excitatory glutamatergic neurons which modulate neuronal excitability during cortical differentiation. However, little is known about how these populations contribute to the BP phenotype. In the current investigation we have derived stem cells from BP patients who share a single nucleotide polymorphism, rs1006737, in the L-type calcium channel gene \( CACNA1C \) known to be associated with BP. We generated four independent lines of both C and BP human induced pluripotent stem cells (hiPSC) and differentiated them to GABAergic and glutamatergic neurons using dual Smad inhibition followed by exposure to dorsal or ventral patterning factors, harvesting cells at sequential stages of differentiation. GABAergic neural progenitor cells (NPCs) were > 90% positive for the ventral marker Nkx2.1 and yielded > 80% GABA+ neurons that co-express mature neuronal markers SV-2, Map2, and NeuN protein. GABAergic neurons robustly express \( GAD67 \) and \( SLC12A5 \) mRNA, indicative of a switch to an inhibitory state. Glutamatergic NPCs were > 90% Pax6, Nestin and FoxG1 positive while continued neuronal differentiation yielded > 90% vGlut2 and vGlut1 positive neurons that co-express the mature neuronal marker NeuN. Interestingly, preliminary data suggests that BP glutamatergic NPCs display a premature
differentiation phenotype as compared to their Control counterparts in that glutamatergic NPCs express significantly more EMX2, FOXG1 and CACNA1C mRNA. Electrophysiology, live cell calcium imaging, neurite outgrowth analysis, and RNA-seq of C and BP GABAergic and glutamatergic neurons is in progress. Understanding the developmental and functional differences in BP GABAergic and glutamatergic cortical neurons may help identify novel therapeutic targets to treat bipolar disorder.


Poster

278. Pluripotent Stem Cells and Organoid Models of Degenerative Diseases

Location:  Hall A

Time:  Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:/Poster #:  278.29/A71

Topic:  A.03. Stem Cells and Reprogramming

Support:  NIH Grant NIMH-106434

Title:  Characterization of exosomes isolated from bipolar patient plasma and iPSC, roles in neuronal development and plasticity


Cell and Developmental Biol.,  Psychiatry, Univ. of Michigan Med. Sch., Ann Arbor, MI

Abstract:  Bipolar I Disorder (BP) is a serious, recurrent mood disorder that is characterized by alternating episodes of mania and depression. To begin to identify novel approaches and pathways associated with BP, we have differentiated BP and undiagnosed control (C) induced pluripotent stem cells (iPSC) to astrocytes and performed RNA-seq. In comparing differentially expressed genes using hierarchical cluster analysis, “Exosome” was the most highly significant cluster identified. Astrocytes have been shown to release exosomes in culture and importantly, miRNAs have been shown to be differentially expressed in exosomes derived from BP vs C postmortem brain tissue. However, little is known regarding what transcripts and proteins are carried from astrocytes to neurons, how they regulate biological functions of the recipient cell, and in turn how that may be altered in mood disorders. Astrocyte-derived exosomes have been suggested to promote neuronal plasticity, as well as to remove toxic proteins in brain, and alterations in function or content may be involved in neurodevelopmental, neuropathological and neuropsychiatric conditions. To examine the characteristics of BP patient and Control exosomes, their cargo and interactions with neural precursor cells, astrocytes were differentiated from BP and C iPSC lines. Immunocytochemical analysis demonstrated that differentiated astrocytes expressed CD44, S100b, and GFAP. Supernatants from these cultures were collected, exosomes
were isolated using differential ultra-centrifugation, and analyzed using NanoSight technology, demonstrating that the mean size of exosomes derived from the bipolar media was significantly smaller than that of control astrocytes, but concentrations were similar. Western blot analysis demonstrated the presence of exosomal markers: CD63, HSP70, CD9, and CD81, and proteomic analyses of both spontaneously secreted exosomes and following stimulated release are in progress. Checkerboard analyses of exosome contribution to neuronal differentiation is being carried out by addition of BP and C astrocyte derived exosomes to BP and C neural progenitor cells (NPC). Neurite outgrowth, miRNA array, electrophysiology, and immunocytochemistry/RTqPCR for differentiation makers are being carried out on recipient cells. Utilizing PKH26 to fluorescently label exosomes demonstrates uptake by recipient NPC in both live cell imaging and immunocytochemical analysis. Additionally, proteomic analysis of exosomes isolated from peripheral blood of patients who contributed fibroblasts for stem cell derivation is currently underway.


Poster

279. Molecular Mechanisms of Synaptogenesis and Activity-Dependent Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 279.01/A72

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH R01 ES028738

Title: Neurodevelopmental disruptions by PBDES are mediated in part by inhibition of neuronal MEK-ERK signaling

Authors: *R. POSTON¹, K. RIENECKER¹, L. MURPHY², K. MULLIGAN², A. REJEPOVA¹, M. GHANINEJAD-ESFAHANI¹, R. N. SAHA¹;
¹Mol. and Cell Biol., Univ. of California Merced, Merced, CA; ²Dept. of Biol. Sci., California State Univ. Sacramento, Sacramento, CA

Abstract: The developing nervous system is particularly sensitive to influence by environmental signals, including dysregulation by toxins. Polychlorinated diphenyl ethers (PCDDs), one such type of toxins, are an environmentally pervasive class of brominated flame retardants that have been extensively used as coatings on a wide range of consumer products. Their environmental stability, propensity for bioaccumulation, and known potential for neurotoxicity have evoked interest regarding their effects on the developing nervous system. Concerningly, there is a growing body of evidence correlating human exposure levels to behavioral deficits related to neurodevelopmental disorders. Understanding the molecular and cellular mechanisms by which
PBDEs affect the developing brain may help clarify this association and provide insight into the disorders. Using primary cultures of cortical neurons from embryonic rats (*R. norvegicus*), we found that chronic exposure to a hydroxylated metabolite of BDE-47, one of the most abundant PBDE congeners, suppressed both spontaneous and evoked electrical activity. We also made the fortuitous observation that some ortho-hydroxylated metabolites share key structural similarities to Type-III non-ATP competitive MEK1 inhibitors. This led to the development of a mechanistic hypothesis for how these compounds may partially inhibit MEK1, a central kinase in the MEK-ERK pathway. Modeling of protein-ligand docking and observed attenuation of pERK induction and MAPK-driven gene transcription following acute PBDE exposures support this hypothesis. Further, when we exposed flies (*D. melanogaster*) via feeding, we found that both 6-OH-BDE-47 and the MEK1 inhibitor PD-0325901 caused increased frequency of mushroom body β-lobe midline crossing, a metric of axonal guidance *in vivo*. Our data support the hypothesis that certain ortho-hydroxylated PBDE metabolites can act as MEK1 inhibitors and disrupt spontaneous and evoked electrical activity, pre-synaptic composition, and axonal guidance. These data indicate that partial inhibition of MAPK signaling by specific ortho-hydroxylated PBDEs could impact neurodevelopmentally critical processes.


**Poster**

279. Molecular Mechanisms of Synaptogenesis and Activity-Dependent Development

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 279.02/A73

**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** JSPS 16K07339
JSPS 16KK0158
Astellas Foundation for Research on Metabolic Disorders

**Title:** Regulation of RNA splicing associated with environmental stimulation in the postnatal brain

**Authors:** *F. TSURUTA*¹, J. KIM¹, T. TAKETOMI², C. HAMADA², J. NAKAMURA¹, B. SATO¹, T. CHIBA¹;
¹Grad. Sch. of Life and Env. Sci., ²Col. of Biol. Sci., Univ. of Tsukuba, Tsukuba, Japan

**Abstract:** In mammals, a wide variety of environmental stimuli play important roles in establishing the functions of the postnatal brain. During postnatal stages, these stimuli enforce the diversity and complexity of synaptic connectivity and neuronal circuit, resulting in affecting various critical periods. Recently, it has been reported that RNA splicing has effects on the brain
function a time- and region-dependent manner, regulating synaptic connectivity and neuronal circuit. However, it has not been elucidated the molecular mechanisms linking environmental stimuli to the neuronal function involved in RNA splicing after birth. Here, we report that ubiquitin specific peptidase 15 (USP15) is accumulated in the nucleus after visual stimulation. Previously we found that USP15 deubiquitinates Terminal Uridylyl Transferase 1 (TUT1), contributing U6-snRNA stabilization and proper RNA metabolism. In the brain, USP15 exists in both nucleus and cytoplasm in layer V cortical neurons. On the other hand, USP15 is not accumulated in the nucleus in the absence of light exposure under the dark condition. Using the microarray analysis, we identified substantial genes, which are involved in ER stress and membrane trafficking. One of the interesting candidates influenced by USP15 deficiency is a mutant of Hevin, which is a secreted protein from astrocyte and accelerate the synaptic formation. This mutant lacks a calcium binding domain, EF-hand at the C-terminal region. We also found that this mutation hampers the trafficking pathway and is accumulated in the endoplasmic reticulum in not only astrocyte but also specific neurons, inducing a mild ER stress. Taken together, our results suggest that visual stimulation during postnatal stages promotes USP15 accumulation in the nucleus in layer V cortical neurons, followed by controlling the proper RNA splicing in the nucleus. A defect in this machinery produce multiple splicing mutants like Hevin and causes neuronal dysfunctions. These finding may provide the possibility that USP15 act as a key mediator that link environmental stimuli to the establishment of the proper neuronal circuit.


Poster

279. Molecular Mechanisms of Synaptogenesis and Activity-Dependent Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 279.03/A74

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: DFG SFB 1134/A03
      PMU FFF: R-16/04/084-BEN
      PMU FFF: R-14/04/063-KÖN
      FWF: F44010
      FWF: F4413-B23 (SFB-F44 – Cell Signaling in chronic CNS disorders)
      German National Academic Foundation

Title: Structural and functional maturation profile of rat primary motor cortex layer 5b neurons

Authors: *D. DANNEHL*1,2, B. BENEDETTI2, M. JANSSEN1, C. CORCELLI1, S. COUILLARD-DESPRES2, M. ENGELHARDT1;
Abstract: Layer 5b pyramidal neurons (L5bPN) are the major output population of the primary motor cortex (M1), playing a key role in motor circuit integration and initiation of voluntary movement. L5bPN are among the most active excitatory neurons in the CNS. However, axonal maturation patterns and firing properties of M1 L5bPN during development remain elusive. Here, we characterized the structural and functional maturation of the action potential (AP) initiation site, the axon initial segment (AIS), of rat M1 L5bPN. AIS maturation from P1 to P150 was investigated via morphometrical analysis applying confocal-based immunofluorescent detection of the AIS scaffolding proteins βIV-spectrin and ankyrin-G. The elongation rate of L5bPN AIS was highest during the first postnatal week (0.6 ± 0.1 µm/day, oneway ANOVA, p<0.001). At P10 to P15, the elongation rate began to slow significantly (0.4 µm ± 0.06 µm/day, p<0.001), until negligible AIS elongation was observed at adult age (< 0.06 µm/day). Recent studies have shown that AIS elongation contributes to increased neuronal excitability. Hence, we analyzed the relation between AIS maturation and cellular excitability via whole cell patch-clamp recordings in acute slices prepared from 5 age groups: (i) P2-5, (ii) P10-15, (iii) P20-25, (iv) P50-56, and (v) > P150 (average n = 20 cells per group). AP parameters indicated that with the exception of higher maximal firing frequencies, the overall excitability of M1 L5bPN decreased with age despite corresponding AIS elongation. Cells from older age groups showed a significantly decreased input resistance in comparison to the two youngest age groups (P20-25 = 371 ± 280 MΩ; P50-56 = 249 ± 69 MΩ; P>150 = 365 ± 242 MΩ, p<0.001; P2-5 = 1609 ± 878 MΩ; P10-15 740 ± 308 MΩ), correlating to higher rheobase and smaller gain. Remarkably, all significant physiological development occurred right after P2-5 or P10-15, namely at phases of fast or intermediate AIS elongation rate. Our data suggest that notwithstanding overall longer AIS, older neurons are not more excitable than younger neurons with significantly shorter AIS. Possibly, progressive elongation of AIS with age might have a function in counterbalancing the impact of decreased input resistance on the overall neuronal excitability to balance neuronal excitability in the developing cortical network.


Poster

279. Molecular Mechanisms of Synaptogenesis and Activity-Dependent Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 279.04/A75

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Title: Dynamic regulation of synaptopodin and the cisternal organelle in the axon initial segment of retinal ganglion cells during postnatal development

Authors: M. JANSSEN¹, A. SCHLÜTER¹-², S. ROSSBERGER¹-³, D. DANNEHL¹, S. VORWALD¹, J. HANNE⁴, C. SCHULTZ¹, D. MAUCERI², *M. ENGELHARDT¹; ¹Inst. of Neuroanatomy, Med. Fac. Mannheim, Heidelberg Univ., Mannheim, Germany; ²Neurobio., ³Kirchhoff Inst. of Physics, Heidelberg Univ., Heidelberg, Germany; ⁴Abberior Instruments GmbH, Heidelberg, Germany

Abstract: The axon initial segment (AIS) is a key component of normal cellular function in neurons. The AIS constitutes the site of action potential generation and plays an important role for establishing neuronal polarity. In visual cortex pyramidal neurons, the AIS undergoes periods of activity-dependent structural plasticity during development. However, it remains unknown how AIS morphology is organized during development for downstream cells in the visual pathway, namely retinal ganglion cells (RGCs) and whether these AIS retain the ability to dynamically adjust to changes in network state in the retina. Here, we investigated the maturation of RGC AIS during mouse retinal development, and tested putative activity-dependent mechanisms by applying visual deprivation with a focus on the AIS-specific cisternal organelle (CO), a presumed Ca²⁺-store. Whole-mount retinas from wildtype and Thy1-GFP transgenic mice were processed for multi-channel immunofluorescence using antibodies against AIS scaffolding proteins Ankyrin-G, βIV-spectrin and the CO marker synaptopodin (synpo).

Confocal microscopy and morphometrical analysis of AIS length and position as well as synpo cluster size was performed. Data show that RGC AIS undergo dynamic length maturation during development, with longest AIS during the early postnatal phase (P10: 24.45 ± 0.42 µm SD, P15: 24.11 ± 0.28 µm SD), followed by a significant length reduction from P28 until adulthood (P21: 20.68 ± 0.36 µm SD; P28: 16.92 ± 0.28 µm SD; P>55: 16.82 ± 0.25 µm SD; oneway ANOVA, p≤0.05). Furthermore, a subset of RGC AIS contains synpo clusters and data show that the size, but not the number of synpo clusters in RGC AIS undergoes significant changes during retinal development (P21: 0.59 ± 0.01 µm² SD vs. P28: 0.50 ± 0.02 µm² SD and P35: 0.52 ± 0.004 µm² SD; oneway ANOVA, p≤0.05). Similar to previous data from visual cortex, we found that both RGC AIS and CO maturation seem to be activity-regulated events during retinal development, since both are impaired under visual deprivation conditions. Using superresolution microscopy, we addressed the subcellular localization of synpo in RGC axons. Similar to cortical neurons, RGCs show a periodic distribution of AIS scaffolding proteins. A previously reported scaffold-deficient nanodomain correlating with synpo localization is not evident in all RGC AIS. In summary, our work demonstrates a dynamic regulation of both the AIS and synpo in RGCs during retinal development and after visual deprivation, providing evidence that the AIS and CO in RGCs can undergo structural plasticity in response to changes in network activity.

Poster

279. Molecular Mechanisms of Synaptogenesis and Activity-Dependent Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 279.05/A76

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: Deutsche Forschungsgemeinschaft, DFG (SFB 1134, TP03)
Deutsche Forschungsgemeinschaft (DR 326/12-1)

Title: Characterization of a transgenic mouse line to study the axon initial segment in living neurons

Authors: *C. THOME*¹, S. KARABULUT², C. ACUNA GOYCOLEA³, E. D'ESTE⁴, A. D. NELSON⁵, C. SCHULTZ⁷, V. BENNETT⁸, P. M. JENKINS⁶, M. ENGELHARDT⁹;

Abstract: The axon initial segment (AIS) is the cellular compartment where most neurons integrate synaptic inputs and generate their primary output signal: the action potential. However, it was only recently that its structure and molecular composition have been untangled. Such studies mostly relied on immunofluorescent staining in fixed neuronal tissue using antibodies against elements of the AIS cytoskeleton. For example, the scaffolding proteins Ankyrin-G and βIV-spectrin cluster voltage-gated ion channels at the AIS and provide a cellular anchor between axonal membrane and cytoskeleton. Recent approaches allow life labeling of the AIS using antibodies against the extracellular domain protein neurofascin-186 or by using transfection of cultured neurons with AIS-specific constructs. However, these techniques are somewhat limited in their application and have, so far, only been established for in vitro applications. Here we present and characterize a transgenic mouse line, in which the AIS is intrinsically labeled with a genetically encoded fluorophore. In this model, a modified sequence generates an Ankyrin-G-GFP fusion protein that is activated by Cre recombinase and only visualizes endogenous Ankyrin-G. This allows AIS labeling in subsets of neuronal populations and time-points in the living animal, depending on which Cre-line is selected as a cross or which virus is
We investigated whether the fusion of Ankyrin-G with GFP and its insertion at the AIS affects AIS morphology, development, molecular composition, and electrophysiological function after activation of the construct in different neurons (interneurons, excitatory neurons, dopaminergic neurons) and by different techniques (virus transfection, breeding). Using confocal and superresolution (STED) microscopy as well as whole cell patch clamp recording in vitro, ex vivo and in vivo, we confirm that the subcellular scaffold of the AIS and basic electrophysiological parameters of labeled cells are not changed after the fusion of GFP to Ankyrin-G. Now, we use this mouse line to study the time course of AIS development and plasticity in individual living neurons using confocal life imaging in combination with whole cell patch clamp recordings.


**Poster**

279. Molecular Mechanisms of Synaptogenesis and Activity-Dependent Development

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program#/Poster #:** 279.06/A77

**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** KAKENHI 18K06530
KAKENHI 19K06962

**Title:** Voltage-sensitive dye recording of glossopharyngeal nerve-related synaptic networks in the embryonic mouse brainstem

**Authors:** *K. SATO*¹, Y. MOMOSE-SATO²;
¹Komazawa Women's Univ, Fac. of Human Hlth., Tokyo, Japan; ²Dept. of Nutr. and Dietetics, Kanto Gakuin University, Col. of Nutr., Yokohama, Japan

**Abstract:** The glossopharyngeal nerve (N.IX) transfers motor and sensory information related to visceral and somatic functions, such as salivary secretion, gustation and the control of blood pressure. N.IX-related neural circuits are indispensable for these essential functions. Compared with the strenuous analysis of morphogenesis, we are still at the entrance to elucidate the functiogenesis of these neural circuits during ontogenesis. In the present study, we applied voltage-sensitive dye recording to the embryonic mouse brainstem, and examined the functional development of the N.IX-related neural circuits. First, we optically identified the motor nucleus (the inferior salivatory nucleus (ISN)) and the first-order sensory nucleus (the nucleus of the tractus solitarius (NTS)). We also succeeded in recording optical responses in the second/higher-order sensory nuclei via the NTS, including the parabrachial nucleus. Second, we pursued
neuronal excitability and the onset of synaptic function in the N.IX-related nuclei. The neurons in the ISN were excitable at least at E11, and functional synaptic transmission in the NTS was first expressed at E12. In the second/higher-order sensory nuclei, synaptic function emerged at around E12-13. Third, by mapping optical responses to N.IX and vagus nerve (N.X) stimulation, we showed that the distribution patterns of neural activity in the NTS were different between N.IX and N.X from the early stage of ontogenesis. We discuss N.IX-related neural circuit formation in the brainstem, in comparison with our previous results obtained from the chick and rat embryos.

**Disclosures:** K. Sato: None. Y. Momose-Sato: None.

**Poster**

279. Molecular Mechanisms of Synaptogenesis and Activity-Dependent Development

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 279.07/A78

**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** KAKENHI 19K06962
KAKENHI 18K06530

**Title:** Exposure to nicotine during development disrupts synaptic network formation by inhibiting correlated spontaneous wave activity

**Authors:** *Y. MOMOSE-SATO*¹, K. SATO²;
¹Kanto Gakuin University, Col. of Nutr., Yokohama, Japan; ²Dept. of Hlth. and Nutr. Sci., Komazawa Women's Univ, Fac. of Human Hlth., Tokyo, Japan

**Abstract:** Correlated spontaneous activity propagating over a wide region of the central nervous system is expressed during a specific period of embryonic development. In a previous study using the optical imaging technique with a voltage-sensitive dye, we demonstrated that this wave-like activity, which we referred to as the depolarization wave, plays a fundamental role in the early process of synaptic network formation. We found that in ovo application of bicuculline/strychnine or d-tubocurarine, which blocked neurotransmitters mediating the wave, significantly reduced functional synaptic expression in the brainstem sensory nucleus. This result, especially for d-tubocurarine, an antagonist of nicotinic acetylcholine receptors, raised the possibility that prenatal nicotine exposure associated with maternal smoking affects the development of neural circuit formation by interfering with the depolarization wave. In the present study, we tested this hypothesis by examining the effects of nicotine on the depolarization wave and assessing the chronic action of nicotine on functional synaptic expression. The application of nicotine transiently increased electrical bursts and embryonic motility associated with the depolarization wave, but subsequently inhibited these activities.
Furthermore, chronic exposure to nicotine *in ovo* markedly reduced functional synaptic expression in the brainstem sensory nucleus, the parabrachial nucleus. This study suggested that prenatal nicotine exposure disrupts the initial creation of neural circuitry by inhibiting the correlated spontaneous activity.

**Disclosures:** Y. Momose-Sato: None. K. Sato: None.

**Poster**

279. Molecular Mechanisms of Synaptogenesis and Activity-Dependent Development

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 279.08/A79

**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** CIHR
Alfred P. Sloan Foundation
NSERC

**Title:** Morphological annotations reveal the trajectory of cerebellar interneuron diversification

**Authors:** W. X. WANG, *J. L. LEFEBVRE;
Mol. Genet., Hosp. For Sick Children/ U. of Toronto, Toronto, ON, Canada

**Abstract:** Inhibitory interneurons comprise diverse classes of cells that target specific subcellular compartments of their postsynaptic neurons. Even within the same class, interneurons exhibit significant heterogeneity in their morphological and electrophysiological features, suggesting that connectivity patterns are shaped by extrinsic cues during development. To investigate inhibitory interneuron diversification, we focused on the cerebellar cortex in which the sole projection neurons, the Purkinje cells, receive nearly all inhibitory inputs from one class of interneurons, the molecular layer interneurons (MLIs). MLIs derive from a common progenitor pool but give rise to divergent types that target distinct Purkinje cell compartments, including dendrites, somata, and axon initial segments. Based on these axon targeting motifs and other morphological characteristics, MLIs have classically been divided into two types, the stellate cells and the basket cells. However, observations of intermediate and overlapping morphologies have led to the alternate model that MLIs represent one class of interneurons with continuously varying characteristics. To assess MLI diversity, we established genetic strategies to label MLIs with single-cell resolution, sampling across birth dates and final laminar positions. We reconstructed the morphologies of 147 mature MLIs, including their complete dendritic and axonal arbors. Following unsupervised clustering of 24 morphological features, we show that the MLIs cluster into two subpopulations—basket cells and stellate cells. Interestingly, the stellate cell cluster is further represented by a continuum of differences, revealing a cellular heterogeneity within this population. We next analyzed the morphological trajectory of MLIs...
during the first postnatal weeks to determine the progression of diversification. Using imaging-based pseudo time analysis of axonal features from 1001 reconstructed MLIs, we mapped the developmental bifurcation of MLIs into basket and stellate cells during the final stages of MLI migration. We demonstrate that MLIs undergo considerable remodeling to establish their final wiring patterns, and that cerebellar interneuron patterning is highly regulated by local signaling rather than by birthdate. Further studies bridging form to molecular signatures will set the stage to identify mechanisms that influence MLI identity and synaptic targeting.

Disclosures: W.X. Wang: None. J.L. Lefebvre: None.

Poster

279. Molecular Mechanisms of Synaptogenesis and Activity-Dependent Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 279.09/A80

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH grant R01NS091234

Title: SCN heterogeneity revealed through developmental patterning of neuropeptide expression

Authors: *V. CARMONA-ALCOCER, K. E. ROHR, F. SAABEDRA, A. VALENCIA, N. BURRIESCJ, J. A. EVANS;
Biomed. Sci., Marquette Univ., Marquette University, WI

Abstract: Neuropeptide signaling modulates the function of master clock neurons in the suprachiasmatic nucleus (SCN). Both arginine vasopressin (AVP) and vasoactive intestinal peptide (VIP) regulate the SCN network during adulthood, but when these two neuropeptides are first expressed during development has been difficult to establish precisely. To address this important issue, we used a transgenic approach to define the developmental patterns of neuropeptide expression across the SCN network. Specifically, we crossed Avp-Cre+/- or Vip-Cre+/- males to Ai9+/- females that express floxed tdTomato (tdT). In the offspring of this genetic cross, the fluorescent protein tdT is stably expressed after initiation of Avp or Vip transcription. Here we use this approach to profile the spatiotemporal patterning of neuropeptide expression by examining tdT expression at critical developmental time points spanning from embryonic age (E) 15.5 to adulthood. Our results indicate that the onset of neuropeptide transcription begins shortly after SCN neurogenesis is complete, with initiation of Avp and Vip transcription occurring in a cell-type specific manner. Further, this work reveals that there are spatial gradients in the developmental patterning of neuropeptide expression across the anteroposterior SCN. These results suggest that SCN neurons of the same neurochemical class can be distinguished into further sub-clusters, which may relate to functional differences detected along the anteroposterior axis of the mature SCN network.

Poster

279. Molecular Mechanisms of Synaptogenesis and Activity-Dependent Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 279.10/A81

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: CIHR

Title: Non-ionotrophic NMDAR signaling promotes spontaneous and evoked neurotransmitter release in the developing retinotectal system

Authors: *M. VAN HORN, L. TIMMINS, A. SCHOLH, V. HIGENELL, S. GLASGOW, P. KESNER, E. RUTHAZER;
McGill Univ., Montreal, QC, Canada

Abstract: The NMDA receptor plays an important role in activity-dependent refinement of sensory connections, which occurs during normal neural circuit development. Recently, presynaptic NMDA receptors (preNMDARs) have been found to regulate many forms of plasticity at several synapses. Here we investigated the potential role of preNMDARs on retinal ganglion cells (RGCs) in the developing Xenopus laevis tadpole visual system. To test for the presence of functional NMDARs in RGC axons, we performed two-photon imaging of RGC axon terminals in isolated whole-brain preparations from animals in which RGCs were expressing the genetically-encoded calcium indicator GCaMP6s. Using this preparation, where the RGCs were separated from their somata, we found application of NMDA produced Ca\(^{2+}\) transients in RGC axon terminals 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 (PPRs). Interestingly, raising the tadpoles in MK801, to block Ca\(^{2+}\) influx through the ion channel of the NMDARs, did not prevent the APV-induced decrease in mEPSC frequency or the increase in PPRs. These results suggest that NMDARs can regulate both spontaneous and evoked synaptic release independent of Ca\(^{2+}\) influx through the ion channel. Additional studies are required to determine the downstream signaling pathways mediating these non-ionotrophic mediated changes. Taken together these results suggest an unappreciated role of NMDARs on RGCs in the developing retinotectal system and identify a novel, non-ionotropic mechanism by which NMDARs can modulate evoked and spontaneous neurotransmission and contribute to neural circuit development.

Poster

279. Molecular Mechanisms of Synaptogenesis and Activity-Dependent Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 279.11/A82

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: F32EY028869-01
R01 EY015788
U01 NS094358
P30 EY026878
R01 MH111424

Title: Learning to see with closed eyes: Retinal waves tune cortical neurons before eye opening

Authors: *A. S. HAMODI, X. GE, M. C. CRAIR;
Yale Univ., New Haven, CT

Abstract: Development of neural circuits depends on a combination of molecular and activity-dependent mechanisms. The activity can be spontaneous—such as retinal waves that course across the mammalian perinatal retina—or sensory-evoked. Spontaneous waves provide essential instructions for the development and refinement of visual circuits, and disruption of early spontaneous activity has been implicated in numerous neuropsychiatric disorders, such as autism and schizophrenia. Although much is known about the retinal circuitry responsible for retinal wave generation, it is unclear how retinal waves shape the development of functional cortical architecture. It is also completely unknown which subtypes of neurons participate in waves in visual cortex and when they are recruited during development. Here we determine the roles and dynamics of excitatory and inhibitory neuron activity in visual cortex during the period of retinal waves (before eye-opening). To do this, we use simultaneous cellular-resolution calcium imaging of genetically defined neurons within a local circuit and mesoscopic imaging of neuronal activity across cortex to assess the function of retinal waves in cortical circuit formation in awake, head-fixed mice. In addition, we developed a novel method for ultra-fast whole-brain vector-driven gene delivery. Using this novel method to obtain pan-neuronal expression of GCaMP6s before eye-opening, we observe that all interneuron subclasses in visual cortex are recruited by retinal waves as early as P4. However, the degree of participation depends on the subclass of neurons, cortical layer, and developmental age. Additionally, we are using our dual-imaging approach to examine the role of retinal waves in emergence of functional receptive field properties already present at eye-opening, such as orientation- and direction-selectivity. To do this, we are directly linking retinal wave properties to the number and identity of cortical neurons...
recruited, and testing whether retinal waves instruct the development of direction selectivity in cortical neurons prior to eye-opening. Our work enables the generation of novel insights into the role of specific populations of individual neurons in the development of functional organization of brain circuits in health and disease.

**Disclosures:**  A.S. Hamodi: None. X. Ge: None. M.C. Crair: None.

**Poster**

279. Molecular Mechanisms of Synaptogenesis and Activity-Dependent Development

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 279.12/A83

**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Title:** Epigenetic modulation of auditory system critical period plasticity

**Authors:** *B. SCHWARTZ, W. WANG, S. BAO;
Univ. of Arizona, Tucson, AZ

**Abstract:** The critical period is a time of maximal plasticity within the cortex. The progression of the critical period is marked by experience-dependent transcriptional alterations in cortical neurons, that in turn shifts the excitatory-inhibitory balance in the brain and accordingly, reduces plasticity. Epigenetic mechanisms, such as DNA methylation, control the transcriptional state of neurons, and has been shown to be dynamically regulated during the critical period. Here we show that adult animals have a significantly higher concentration of DNA methylation than critical period animals. Pharmacological reduction of DNA methylation in adult animals re-establishes critical period auditory map plasticity. Furthermore, the reduction of DNA methylation in adult animals, reverted intrinsic characteristics of inhibitory synapses to an immature state. Our data suggests that accumulation of DNA methylation during the critical period confers a mature phenotype to cortical neurons, which in turn, facilitates the reduction in plasticity seen after the critical period.

**Disclosures:** B. Schwartz: None. W. Wang: None. S. Bao: None.
Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: ERC 319456

Title: Functional frequency maturation across the perinatal time window

King's Col. London, London, United Kingdom

Abstract: Adult neural activity as studied by resting state functional magnetic resonance imaging (rs-fMRI) occurs at a relatively low frequency spectrum (0.001 to 0.3Hz). In the first year of postnatal life, there is a drastic shift from global slow frequencies (0.025 Hz) to adult like peak frequencies (0.1 Hz) in rs-fMRI. However, whilst the spatial distribution of neural networks are known to rapidly evolve across the perinatal period, it is not known how changes in frequency may modulate network development. We used high temporal resolution fMRI data to characterise changes in frequency power in diverse cortical regions across the perinatal time window. A total of 150 term born neonates were recruited as part of the developing human connectome project and were scanned at median age 41.57 postmenstrual weeks. fMRI data were acquired using a 3T scanner and a multi-slice echo planar imaging sequence with multiband excitation (MB factor 9; TR 0.39s). Data pre-processing was performed using FSL. Signal artefacts were identified using independent component analysis and regressed out using FIX. Spatial distortion effects were then corrected. Power spectral density was calculated in native space for each subject and then warped into standard space. In-house scripts generated in matlab were used to calculate the fractional amplitude of lower fluctuations (fALFF). The proportion of spectral power in lower relative to higher frequencies was studied in 6 different frequency bands ranging from 0.0016 to 0.3 Hz. A general linear model was used to assess the effect of age in each frequency band in 7 different cortical regions. A significant effect (FDR corrected) of increasing power with age was seen in the somatosensory, motor and auditory cortices at 0.075 to 0.1 Hz (figure 1). Whole brain analysis revealed a significant increase of power in 0.05 to 0.075 Hz in posterior cortices. These results suggest early developing sensory networks are the first to acquire the ability to function at higher frequency ranges and indicate adult like frequency power follows a posterior to anterior maturation trajectory.

Poster 279. Molecular Mechanisms of Synaptogenesis and Activity-Dependent Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 279.14/A85

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: Howard Hughes Medical Institute (#55007652), NIH (R01NS103758) PICT2015-3814 PICT2016-0675

Title: Long lasting remodeling of hippocampal networks by adult neurogenesis
Authors: *A. I. GROISMAN*, S. M. YANG, A. F. SCHINDER; 
Leloir Inst. (IIBBA-CONICET), Capital Federal, Argentina

**Abstract:** Adult-born granule cells (GCs) are continuously generated in the dentate gyrus of the hippocampus of all mammals, including humans. They are known to participate in information processing in the dentate gyrus which is involved in memory and learning. Developing GCs undergo a period of high excitability that is regulated by the local GABAergic network, generating heterogeneous functional profiles. In order to better understand the integration dynamics between GCs and the different types of GABAergic interneurons, we built a spatio-temporal map from INs to GCs, and from GCs to INs. We combined presynaptic optogenetic stimulation and whole-cell recordings of fluorescently-labeled postsynaptic cells. GABAergic interneurons expressing parvalbumin (PV-INs) and somatostatin (SST-INs) were identified by genetic labeling. Retroviruses were used to transduce developing GCs. Optogenetic stimulation of PV-INs or SST-INs elicited postsynaptic responses with small amplitude and slow kinetics in young developing GCs, characteristic of immature synapses. Mature synapses were only observed in 6- to 8-week-old GCs, well beyond the critical period of high excitability. Development of GC outputs occurred at a slower pace, with synapses requiring up to 11 weeks to reach functional maturation. PV-INs formed perisomatic synapses onto GCs and contributed to both feedforward and feedback loops within the dentate gyrus, while SST-IN contacted proximal and distal dendrites in GCs and contributed to feedback but not feedforward inhibition. Thus, perisomatic inhibition arises both from feedforward and feedback loops, while dendritic inhibition comes primarily from feedback circuits, and it is the loop demanding the longest maturation time. These data demonstrate that integration of new GCs within the preexistent dentate GABAergic network is specific of each INs population and that adult neurogenesis takes a large period of time for remodeling and circuit integration.

**Disclosures:** A.I. Groisman: None. A.F. Schinder: None. S.M. Yang: None.

**Poster**

279. **Molecular Mechanisms of Synaptogenesis and Activity-Dependent Development**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 279.15/DP01/A86

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**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** NIH/NINDs National Research Service Award F31NS089223
NIH Grant 1DP2OD006514-01
NIH Grant TR01 1R01NS076467-01
NIH Grant 1U01NS090449-01
Title: The rich get richer: Serial section electron microscopy reveals that climbing fiber-Purkinje cell synapse rearrangement in the developing mouse cerebellum is economical and begins with positive feedback addition of synapses

Authors: *A. M. WILSON¹, R. SCHALEK², A. SUISSA-PELEG², T. JONES⁴, S. KNOWLES-BARLEY⁵, H. PFISTER², J. W. LICHTMAN³;
¹Princeton Univ., Princeton, NJ; ²Harvard Univ., Cambridge, MA; ³Broad Inst., Cambridge, MA; ⁴Google, Seattle, WA

Abstract: During postnatal development, cerebellar climbing fibers become strong synaptic inputs to a subset of their original Purkinje cell targets, and eliminate their connections from the rest. As a result, in the adult cerebellum each climbing fiber innervates a small number of Purkinje cells (often separated by large distances of several mm in a sagittal zone). Furthermore, each Purkinje cell is innervated by a single climbing fiber. This remapping has been broken down into various stages (Kano et al., 2018). However, the processes responsible for the observed changes in climbing fiber-Purkinje cell synaptic connectivity have not been well described. In order to get insight about the nature of these processes, we reconstructed two serial section electron microscopy datasets from mice at different time points during the first postnatal week (190 x 120 x 50 μm³ at postnatal day 3 and 190 x 120 x 75 μm³ at postnatal day 7). Between postnatal days 3 and 7, Purkinje cells retract long dendritic branches while local dendritic processes grow. On this changing dendritic landscape, individual climbing fibers selectively add many synapses onto a subset of their Purkinje cell targets, without pruning synapses from their other Purkinje cell targets. The active zone areas for individual synapses associated with powerful versus weak connections are indistinguishable. These results show that increases in synapse number, rather than changes in synapse size, are the predominant form of early developmental plasticity. Finally, although multiple climbing fibers innervate each Purkinje cell in the first postnatal week, the number of climbing fiber axons and Purkinje cells in a local region of cerebellum appear to be almost the same (likely due to exuberant climbing fiber branching). Thus, over-innervation of Purkinje cells by climbing fibers in early postnatal development is economical: the number of axons innervating Purkinje cells within a local region is about the amount required to assure that each axon ends up with a postsynaptic target.

Poster

279. Molecular Mechanisms of Synaptogenesis and Activity-Dependent Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 279.16/B1

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: Culshaw Family Heart Lung Award, Children's Hospital Colorado Foundation Center for Women's Health Research Seed Grant, University of Colorado Anschutz Medical Campus

Title: Antenatal sFLT1 overexposure induces neurodevelopmental milestone delay, cortical thinning and synapse reorganization in juvenile offspring

Authors: D. EFFINGER1, G. SEEDORF2, A. J. LAW3, S. H. ABMAN2, *C. PATERSON1; 1Dept. of Psychiatry, 2Pediatric Heart and Lung Center, Dept. of Pediatrics, 3Departments of Psychiatry, Cell & Developmental Biology, Med., Univ. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: Epidemiologic studies have shown that complications during gestation are strongly associated with an increased risk for neurodevelopmental and neuropsychiatric disorders in offspring. While maternal insults during pregnancy directly impact fetal development, mechanisms leading to abnormal offspring neurodevelopmental reprogramming are not fully understood. Clinical studies suggest that an imbalance of pro- and anti-angiogenic factors may be pathogenic in multiple gestational complications. Particularly evident are elevated maternal and fetal soluble fms-like tyrosine kinase 1 (sFLT1) levels, however whether elevated sFLT1 levels are sufficient to impair structure and function of the developing brain is uncertain. We have established a model of elevated antenatal sFLT1 levels via intra-amniotic injection of sFLT-1 in gestational day 20 male and female rat embryos. To determine the potential biological processes driving the association between antenatal sFLT1 elevations and increased incidence of neurological disorders in offspring, we examined neurodevelopmental milestone attainment as well as prefrontal cortex (PFC) structure and synapse marker expression at the juvenile stage. Behavioral milestone analysis from postnatal days (PND) 3-14 revealed that sFLT1 overexposure delayed offspring growth and neuroreflex attainment with a tendency to be more evident in male offspring. Histological analyses revealed that at the juvenile stage (PND 14) cortices of offspring exposed to sFLT-1 were thinner in specific lamina of prefrontal areas associated with cognitive outcomes than saline treated offspring. Moreover, we found an imbalance in cortical protein levels of excitatory and inhibitory synapse proteins in sFLT1 exposed offspring. Together these findings implicate that elevated antenatal sFLT1 levels are sufficient to cause sustained structural, behavioral and synaptic disruption of the juvenile rat brain that are reflective of clinical findings in offspring born from complicated pregnancies.
These data bring to attention the neurobiological importance of regulated gestational sFLT1 levels, and highlight the potential contribution of sFLT1 to high risk for neurodevelopmental and neuropsychiatric disorders.


Poster

280. Genetic and Environmental Factors for Autism Spectrum Disorders

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 280.01/B2

Topic: A.07. Developmental Disorders

Support: Deanship of Scientific Research, The University of Jordan

Title: Examine the association between gluten sensitivity and the pathophysiology of autism

Authors: *L. ALZGHOUL;
The Univ. of Jordan, Amman, Jordan

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder. It characterized by a socio-communicational impairment with restricted or repetitive interest and activity. In addition, gastrointestinal (GI) disturbances are also noticed in many patients with ASD, leading to the suggestion that dietary sensitivity may play a role in the pathophysiology of ASD. Several studies have focused on immunological responses to dietary proteins, such as gluten and casein. One study found that peripheral blood mononuclear cells from children with ASD responded to gliadin by producing higher levels of inflammatory cytokines, compared with peripheral blood mononuclear cells from typically developing children. Studies looking at mucosal immune responses in children with ASD who have GI symptoms have shown increased infiltration of T-cell, monocyte, and eosinophil in the gut mucosa, prominent mucosal T-cell activation with increased TNF but lower IL-10 production, compared with non-inflamed control subjects or compared with children with celiac disease or inflammatory bowel diseases. Hence the aim of this study was to examine the association between blood levels of anti gliadin antigens and autism in Jordan. To study that, blood levels of anti-gliadin IgA and IgG as well as anti- Tissue transglutaminase IgA and IgG were measured in 120 ASD patient, 117 siblings, and 130 age and gender matched healthy controls using ELISA kits. Furthermore, the association between the levels of levels of antibodies and GI symptoms were also examined. Our data revealed 1) no statistically significant defference of anti-gliadin IgA, anti- Tissue transglutaminase IgA and anti-Tissue transglutaminase IgG levels were found between the three groups, 2) levels of anti-gliadin IgG that was statistically higher in the ASD group compared to healthy controls but not the
sibling group was also detected no association between any of the tested markers with GI symptoms. These data suggest no role of gluten sensitivity in the pathophysiology of ASD.

Disclosures: L. Alzghoul: None.

Poster

280. Genetic and Environmental Factors for Autism Spectrum Disorders

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 280.02/B3

Topic: A.07. Developmental Disorders

Title: Prevalence, etiology, and treatment of sleep disorders in autism spectrum disorder

Authors: *B. CHANG, M. L. BAUMAN;
Dept. of Anat. and Neurobio., Boston Univ. Sch. of Med., Boston, MA

Abstract: Autism Spectrum Disorder is a range of neurodevelopmental disorders that typically manifest as social deficits, delayed or impaired communication skills, and repetitive behaviors in day-to-day life. Patients with Autism Spectrum Disorder (ASD) often present with other concurrent clinical disorders. Sleep disorders (SD) and sleep issues are highly prevalent in ASD children and rank as one of the most common concurrent clinical disorders. Sleep problems can have an impact on daytime health and may result in neurocognitive dysfunction and behavioral disruptions. Therefore, sleep disorders may have wide-ranging effects on daytime functioning, developmental progress, and quality of life for children with ASD. A literature review of studies, abstracts, and clinical trial data relating to ASD, SD, and other comorbidities observed in ASD was performed to provide a review of the research status of ASD, SD, the interplay between these two disorders, and therapeutic interventions that have been researched or are currently being investigated. Future areas of investigation based on the current state of autism research are also suggested. Current models and theories on the relationship between ASD and SD suggest that the underlying etiology of autism itself may contribute to sleep troubles, and might even have wide-reaching impacts on other unrelated aspects of ASD (Figure 1). Gastrointestinal, otolaryngologic, and psychiatric comorbidities are observed in autism and may affect sleep in these patients, but the mechanism by which this occurs is unclear. There are many treatments for sleep troubles in ASD such as melatonin and behavioral interventions, with varying success. Much work is required to understand the underlying mechanism between both autism and sleep disorders. There are multiple ongoing clinical trials underway which may have promising results, but there is still a need for more efficacious therapeutic interventions. Future studies should also incorporate robust data-collection instruments such as polysomnography to validate findings.
Disclosures: B. Chang: None. M.L. Bauman: None.

Poster

280. Genetic and Environmental Factors for Autism Spectrum Disorders

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 280.03/B4

Topic: A.07. Developmental Disorders

Title: A multicentric, randomised, single-blind phase 2 trial to evaluate the pharmacokinetics and PK/PD of trazodone in children and adolescents with neurodevelopmental disorders and insomnia

Authors: *F. CALISTI, V. TELLONE, A. DEL VECCHIO, M. ROSIGNOLI, R. PICOLLO, S. OLIVIERI, F. GAROFOLO, A. CATTANEO, S. TONGIANI; Angelini RR&D (Research, Regulatory & Development) - Angelini S.p.A., Rome, Italy

Abstract: Background: Insomnia is the most common sleep disorder presented to paediatric healthcare providers and it is reported in otherwise healthy children as well as in children with a range of neurological conditions, including headache, epilepsy or neurodevelopmental disorders (NDDs). NDDs include attention deficit hyperactivity disorder (ADHD), Autism Spectrum Disorders. Good sleep practices and proven behavioural strategies are the first-line treatments. However, in the specific group of children with NDDs, there are children who do not respond to
beha viual interventions who are candidates for a pharmacological management for their insomnia. Trazodone (TZD) is a multimodal antidepressant, a serotonin antagonist and reuptake inhibitor, with a good safety profile. TZD has a clear and ameliorating effect on sleep architecture and can improve the quality of sleep in depressed patients. These characteristics may suggest that TZD is an ideal candidate to treat insomnia in patients with NDD. **Aim and Objectives:** The aim of this study is the characterisation of the PK parameters, the assessment of linearity in drug exposure and the collection of preliminary efficacy data for a pharmacokinetic-pharmacodynamic (PKPD) model. The primary objective of this study is to assess the PK of TZD after single and repeated oral doses in patients aged from 2 to ≤17 years.

The secondary objectives of this study are:

- PKPD relationship of TZD
- Concentration-QT interval correlation
- Dose rationale in children and adolescents
- Safety and tolerability
- Palatability

**Study Design**

Multi-centre, single-blind, parallel-group, randomised Phase II clinical trial designed to assess the PK and PD of 3 dose levels of TZD in children and adolescents with NDDs (autism, intellectual disability or ADHD). A minimum of 36 patients will be randomised to one of the following 3 treatment arms:

- Arm 1: 0.25 mg/kg/day
- Arm 2: 0.4 mg/kg/day
- Arm 3: 0.5 mg/kg/day

At the start of the screening period, an actigraphy device will be delivered to the patient and used for at least 1 week to ensure that the patient has become familiarised with the device use before treatment phase. Sleep latency and total sleep time will be recorded by actigraphy starting from 3 consecutive days prior to first drug intake to the end of the treatment.

**Conclusions:** Insomnia in patients with NDD is an unmet medical need with a substantial impact and burden on functioning and quality of life of both patients and caregivers. TZD is a reasonable candidate for the treatment of this condition based on its mode of action and safety profile. The results from this study will provide new information which may be relevant to further development programs.

Title: Maternal immune challenge enhances neural progenitor cell proliferation and increased hippocampus volume in neonatal rats

Authors: *Y. Pang, K. Carter, M. Loayza, L.-W. Fan, A. Bhatt; Univ. of Mississippi Med. Ctr. / Dept of Pediatrics, Jackson, MS

Abstract: Epidemiological studies suggest that maternal infection is a risk factor for Autism Spectrum Disorder (ASD). A causative link is supported by experimental studies showing that maternal exposure to bacterial pathogen lipopolysaccharide (LPS) produce ASD-like behavioral characteristics in offspring animals. However, the underlying mechanisms by which LPS-triggered inflammatory response leads to abnormal brain development are not fully understood. Here we test a novel hypothesis that maternal LPS exposure disrupts cellular programs that controls neuron numbers, leading to excessive neurogenesis in the offspring, and this may explain why ASD infants experience a period of brain overgrowth. Pregnant rats at embryonic day 12.5 (E 12.5) were injected with LPS (50 ug/kg body weight, i.p.). On E17 and postnatal day 21 (P21), expression of microglial pro-inflammatory and anti-inflammatory markers, programmed neuronal death (PND), and neural progenitor cell proliferation were assessed. The sizes of hippocampus and striatum were assessed by stereology in Nissl-stained sections. Our data showed that maternal LPS significant suppressed PND in the hippocampal region at E17, which was associated with increased neural progenitor cell proliferation in the dentate gyrus of postnatal rats. LPS-induced microglia activation is characterized with a mixed M1 and M2-like phenotype, as low levels of both pro-inflammatory (iNOS, CD68 and MHC-II) and anti-inflammatory (TGFb and CD206) markers were detected by immunohistochemistry. Volume assessment showed that the size of hippocampal was specifically increased in male, but not female rats with maternal LPS exposure, whereas no changes were observed in the striatal volume. In conclusion, this study may provide a significant implication regarding underlying neurobiological mechanisms of brain overgrowth in ASD infants.

Title: Acute p-cresol induces autism-like behaviors and activates dopamine turnover in BTBR mice: A gene X environment interaction paradigm for autism spectrum disorder

Abstract: Autism Spectrum Disorder (ASD) is a severe neurodevelopmental disorder characterized by deficits in social interaction and communication, stereotypic behaviors, restricted interests, and abnormal sensory processing. Its incidence has dramatically risen during the last few decades and many cases remain unexplained even after advanced genetic testing. P-cresol is an aromatic compound either of environmental origin or produced by specific gut bacterial strains. It is a known uremic toxin, able to negatively affect brain function. Urinary and foecal levels of p-cresol have been found significantly elevated in ASD children over matched controls in at least five published reports. In the present study, we have assessed the effects of a single acute injection of low- or high-dose (1 or 10 mg/kg i.v., respectively) p-cresol in BTBR mice, a reliable animal model of human ASD. Low-dose p-cresol significantly increased anxiety in the elevated plus maze (P<.001) and hyperactivity in the open field (P<0.05). In addition, high-dose p-cresol also produced stereotypic behaviors in the open field (P<.001) and complete loss of preference for social interaction in the three-chamber test (P<.01). No effect was recorded on object recognition. Significantly elevated tissue dopamine, HVA and DOPAC levels were found by HPLC in the amygdala (P<0.05) following low-dose p-cresol, as well as in dorsal (P<0.001) and ventral striatum (P<0.01) after high-dose p-cresol, while no effect was recorded in medial prefrontal cortex and hippocampus. Marginal effects on norepinephrine and 5-HT were recorded. Our study (a) supports a gene x environment interaction model, whereby p-cresol can acutely induce autism-like behaviors acting upon a susceptible genetic background; (b)
underscores dopaminergic roles in ASD, especially abnormal activation of the reward circuitry; (c) spurs ongoing follow-up studies to clinically characterize 216 ASD children and 58 unaffected siblings based on urinary p-cresol levels; (d) raises interest in the correction of chronic constipation and in microbiota transfer therapy as potential strategies to lower p-cresol absorption and clinically ameliorate autistic behaviors, anxiety and hyperactivity in young children with ASD.


**Poster**

**280. Genetic and Environmental Factors for Autism Spectrum Disorders**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 280.06/B7

**Topic:** A.07. Developmental Disorders

**Support:** NIH Grant MH112861

Nancy Lurie Marks Family Foundation

**Title:** Gene-environment interactions in an experimental mouse model of neurodevelopmental disorders and stress

**Authors:** *M. L. PHAN*¹, N. JADAV¹, T. LIU¹, C. YOHN¹, J. LUNDEN², X. ZHOU², M. VOLLBRECHT², E. M. DICICCO-BLOOM², B. A. SAMUELS¹;


**Abstract:** Autism spectrum disorders (ASD) are pervasive neurodevelopmental disorders characterized by impairments in social interactions and the presence of repetitive or stereotyped behaviors and interests. Compounding these challenges are findings indicating that adults with ASD experience more stressful life events, greater perceived stress, and increased comorbidity with anxiety disorder and depression. Stress exposure contributes to multiple disorders, and chronic stress is a major risk factor for mood disorders. However, it is unknown whether adult exposure to stress can exacerbate the neurobiological and behavioral phenotypes associated with ASD. Using Engrailed-2 knockout (En2-KO) mice, we developed an innovative hypothesis to examine a novel gene x environment interaction with ecological validity for ASD at cellular, circuit, and behavioral levels. En2 genetic polymorphisms are associated with ASD in multiple
distinct human populations, and En2-KO mice display behavioral disturbances in social interaction assessments and memory tasks, as well as reductions in juvenile play, social sniffing, and aggressiveness. We exposed adult wild-type (WT) and En2-KO mice to either chronic social defeat stress (CSDS) or control and quantified the effects on social interactions, negative affective behaviors, and neuroanatomical changes. A social interaction test, following CSDS, showed that the En2-KO mice spend more time in a defined interaction zone surrounding a plexiglass enclosure when an aggressive mouse strain was contained as compared to an empty enclosure and that CSDS decreased the time spent in the social interaction zone. This altered behavior was accompanied by reduced noradrenergic axonal fibers in the nucleus accumbens and amygdala but not in the prefrontal cortex in En2-KO mice exposed to CSDS. These findings are consistent with our hypothesis that stress exposure alters not only behavior and stress hormones, but this effect may be mediated by altered LC fiber innervation into critical regions that underlie stress responses.


Poster

280. Genetic and Environmental Factors for Autism Spectrum Disorders

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: 280.07/B8

Topic: A.07. Developmental Disorders

Support: NIH

Title: IBA1 immunohistochemistry, peripheral cytokine levels and bacterial profiles in mouse models of ASD

Authors: *S. M. PARKINSON¹, B. BEASLEY¹, K. SCOTT¹, W. GILL², R. W. BROWN³, M. CHANDLEY¹;

Abstract: Autism spectrum disorder is a neurodevelopmental disorder marked by social deficits and repetitive actions. Communication is thought to occur between the bacterial collections known as the microbiota in the gut and the resident immune cells in the brain, microglia. It has been postulated that bacteria in the gut are capable of secreting signaling molecules that can indirectly induce peripheral inflammatory cytokine release or directly elicit microglia activation to alter neurotransmission in the brain. Tissues from male mice were used to determine if microglial activation, increased peripheral cytokine levels and altered fecal profile panels were
present in mice that demonstrate social behavior deficits. Brain tissue and blood were collected at postnatal day 21 from wildtype control mice (C57BL/6J) and maternal immune activation (MIA), valproic acid (VA), and BTBR mouse models. Behavioral, 16S, and fecal short-chain fatty acid profiles were evaluated in adult male mice from two models (VA and BTBR) and the wildtype control group. Immunohistochemistry in males for the microglial marker ionized calcium binding adaptor molecule 1 (IBA-1) found no difference in area fraction or microglia cell counts in the caudate or cingulate cortex between the wild type and social deficit groups (N=4). Peripheral cytokine analysis demonstrated that BTBR mice had increased IL-1 (p<0.05) and IFN- levels (p<0.05) that were statistically significant from the control group, but the valproic acid and MIA models did not differ in cytokine expression levels from the control group. Behavioral analysis of the adult mice revealed significantly reduced marble burying in all three models and decreased sociability time in the VA and BTBR groups when compared to wild type control mice. Short chain fatty acid expression of acetate (p<.001, p<.005) and valerate (p<.005, p,.05) in feces were significantly different between wild type controls and social deficit groups (VA, BTBR). The 16S rRNA sequencing of fecal matter demonstrated differences in bacterial profiles between the BTBR and VA groups when compared to wild type control mice. These studies are instrumental in the creation of future mechanistic strategies or peripheral markers that may illuminate treatable signaling pathways for ASD.


Poster

280. Genetic and Environmental Factors for Autism Spectrum Disorders

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: 280.08/B9

Topic: A.07. Developmental Disorders

Title: Examining a novel gene-environment interaction in autism spectrum disorder

Authors: *K. TESSEMA¹, L. ELAHI¹, R. GAU¹, J. A. MARTINEZ-AGOSTO², J. E. LE BELLE³, H. KORNBLUM⁴;
¹Semel Inst., ²Human Genet., ³Psychiatry, UCLA, Los Angeles, CA; ⁴UCLA Med. Ctr., Los Angeles, CA

Abstract: Autism spectrum disorder (ASD) accounts for a large individual, familial, and societal burden. The lives of patients and their families are also significantly impacted, as the biopsychosocial effects generally impair functioning in multiple settings. Currently, treatment approaches focus on behavioral therapy since developing biological treatments has been challenging due to the variability in causative mechanisms and lack of reliable biomarkers. To address this challenge, it is crucial to uncover common pathogenic mechanisms underlying
multiple ASD risk factors, as such understanding would help develop therapeutic strategies for larger groups of patients. One candidate pathway that has been studied in a subset of ASD patients is the PI3K-AKT-mTOR pathway. Evidence suggests that this pathway can become dysregulated in response to both genetic and environmental influences, often resulting in enlarged brains and abnormal connectivity. Here, we describe initial studies that investigate a novel gene-environment interaction that converges on the mTOR axis, specifically that of exposure to maternal inflammation in utero and heterozygous PTEN mutations. We observe that media conditions associated with maternal inflammation differentially affect proliferation in both 2-D (neural progenitor) and 3-D (organoid) cultures in heterozygous PTEN mutant patient-derived cell lines compared to familial control-derived cell lines. We are continuing to characterize this phenotype by comprehensively examining the consequences of mTOR dysregulation, currently focusing on single-cell proteomic and transcriptomic profiling to identify the specific molecular alterations underlying this effect.


Poster

280. Genetic and Environmental Factors for Autism Spectrum Disorders

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 280.09/B10

Topic: A.07. Developmental Disorders

Support: SFARI 401457

NIH 1K99MH115143

CIHR Postdoctoral Fellowship

Title: Oxytocin normalizes altered social circuit connectivity in the Cntnap2 knockout mouse

Authors: *K. Y. CHOE¹, M. SAFRIN², R. A. BETHLEHEM³, N. G. HARRIS⁴, D. H. GESCHWIND¹;

¹Neurosc., ¹UCLA, Los Angeles, CA; ³Dept. of Psychiatry, Univ. of Cambridge, Autism Res. Ctr., Cambridge, United Kingdom; ⁴Neurosurg., Dept. Neurosurgery, UCLA, Los Angeles, CA

Abstract: Aberrant functional connectivity (FC) is frequently found in autism spectrum disorders (ASD), notably correlating with the degree of social impairment (Supek et al., 2013). In our previous work, exogenous administration of oxytocin (OXT) or DREADD activation of paraventricular nuclei (PVN) OXT neurons improves social deficits in mice lacking an ASD risk gene, Cntnap2 (Penagarikano et al., 2015). Given the ability of OXT to increase circuit signal-to-noise via modulating interneuron function (Owen et al., 2013), we hypothesized that OXT might exert its prosocial effects via rescuing alterations in FC potentially present in the Cntnap2 KO
mouse. To test this, we used high field (7T) fMRI to measure resting-state FC at the baseline and after exogenously administered OXT in dexmedetomine-sedated wild-type (WT) and Cntnap2 KO mice (n=15/group). In line with observations made in individuals with ASD (Rudie et al., 2013), we observed significantly lowered mean FC between regions with established roles in social behavior in the KO mouse (e.g. PVN, nucleus accumbens (NAcc), medial prefrontal cortex; p<0.001 vs WT, Monte Carlo exact permutation test), and higher mean FC between these and other regions not typically involved in social functions (e.g. sensory cortices, thalamus) (p<0.001 vs WT). Strikingly, both FC phenotypes were normalized by i.p. administration of OXT, significantly elevating the mean FC between social regions and attenuating that between social and other regions (p<0.001). In-depth investigation using pairwise ROI and independent component analysis revealed that OXT induced a KO-specific modification of functional circuit connectivity involving the NAcc, a result that was confirmed by immunohistological quantification of neurons expressing c-Fos (immediate early gene product commonly used as a marker of increased neuronal activity). These results suggest that the observed social deficits in KO mice are potentially related to lowered FC between social brain regions, which can be temporarily normalized by OXT administration. To further investigate the functional significance of OXT-induced NAcc circuit activation in social rescue of the KO mouse, we are currently testing the impact of optogenetic activation of this circuit on FC with optogenetic fMRI as well as social behavior.


Poster

280. Genetic and Environmental Factors for Autism Spectrum Disorders

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program#/Poster #: 280.10/B11

Topic: A.07. Developmental Disorders

Support: The Project is supported by the European Union and co-financed by the European Social Fund (grant agreement no. EFOP-3.6.2- 16-2017- 00008; title: The role of neuro-inflammation in neurodegeneration: form molecules to clinics). BR is supported by the ÚNKP-18-4 New National Excellence Program of the Ministry of Human Capacities and by the János Bolyai Research Fellowship from the Hungarian Academy of Sciences

Title: Investigating the social brain of the CNTNAP2 mouse model of autism with quantitative electron microscopy
Authors: *G. M. MARCELLO¹, K. Y. CHOE², P. SOTONYI¹, B. RACZ¹, P. GOLSHANI⁴,⁵,⁶,⁷, D. H. GESCHWIND³;
¹Univ. of Vet. Med. Budapest, Budapest, Hungary; ²Semel Inst., ³UCLA, Los Angeles, CA; ⁴UCLA Dept. of Neurol., Los Angeles, CA; ⁵David Geffen Sch. of Medicine, UCLA, Los Angeles, CA; ⁶Dept. of Neurology, Integrative Ctr. for Learning and Memory, Brain Res. Institute, UCLA, Los Angeles, CA; ⁷Intellectual Develop. and Disabilities Res. Center, UCLA, Los Angeles, CA

Abstract: Social behavioral difficulties are at the core of autism spectrum disorder (ASD). The loss of the brain-wide distributed contactin associated protein-like 2 (CNTNAP2) is implicated in a syndromic form of ASD. Trans-synaptic structural scaffolding and facilitating neuron-glia interactions are major functions of the transmembrane protein CNTNAP2. Along with investigating the synaptic ultrastructure of social behavior implicated brain regions, we quantified glial-neuron interactions in the hypothalamic paraventricular nucleus (PVN). The PVN contains magnocellular oxytocin (OXT) producing cells. There is a growing body of evidence for OXT’s involvement in neuropsychiatric disorders such as ASD. We used the parameter of astrocyte end-foot coverage of OXT neuron cell body to quantify neuron-glial interaction. We found significant differences in glial endfoot coverage of OXT neurons in Cntnap2 knockout (KO) mice as compared to WT. The marked structural difference in astrocyte endfeet - OXT neuron interactions in the KO PVN implicates a potential dysregulation in the synaptic and glial input to these neurons that may underlie the observed social deficits present in these mice. We reason that a potential alteration in the structural relationship between oxytocinergic neurons and their neighboring astrocytes in addition to marked differences in synaptic ultrastructure of socially implicated brain areas may serve as neuroanatomical correlates of underlying deficits in social behavior previously reported in the Cntnap2 KO mouse.


Poster

280. Genetic and Environmental Factors for Autism Spectrum Disorders

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 280.11/B12

Topic: A.07. Developmental Disorders

Support: NIH Grant MH100510
Swiss National Science Foundation P400PB_180785

Title: Diminished sodium channels impair dendritic spike generation in layer 5 prefrontal neurons in fragile x syndrome
Authors: F. BRANDALISE¹, B. E. KALMBACH², E. P. COOK³, *D. H. BRAGER⁴;
¹Basic Neurosciences, Ctr. Médical Universitaire, Geneva, Switzerland; ²Human Cell Types, Allen Inst. For Brain Sci., Seattle, WA; ³McGill Univ., Montreal, QC, Canada; ⁴Neurosci., Univ. Texas at Austin, Austin, TX

Abstract: The prefrontal cortex (PFC) sits at the top of the hierarchy associated with cognitive function by exerting top-down control over numerous cortical and subcortical regions. Patients with Fragile X syndrome, the leading monogenetic cause of autism, suffer from cognitive impairment, including working memory and attention deficits, all directly related to functions of the PFC. Extratelencephalic-projecting L5 neurons (ET) of the PFC project to multiple cortical and downstream structures including thalamic and brainstem nuclei and are therefore well positioned to integrate task-relevant sensory signals and guide motor actions. In ET neurons, distal synaptic inputs are too weak to overcome the dendritic filtering properties to influence the somatic membrane potential and produce action potential output. To overcome this, synaptic inputs in the distal dendrites are transformed into local dendritic Na⁺ spikes, which can propagate to the soma and trigger action potentials. We investigated this dendritic input-output transformation in L5 ET neurons of the fmrl⁻/⁻ mouse model of Fragile X syndrome. Using dual somatic-dendritic current clamp, we found that attenuation of both back propagating action potentials and subthreshold inputs was significantly greater in fmrl⁻/⁻ neurons compared to wild type. Using either current injection or optogenetic stimulation, we found that the threshold for dendritic spike initiation was more depolarized in fmrl⁻/⁻ neurons compared to wild type. Using a systems-based analysis, we found that the dendritic input that preceded dendritic spikes and somatic APs was larger in fmrl⁻/⁻ neurons. Outside-out patch clamp recordings revealed that the dendritic sodium current was significantly smaller in fmrl⁻/⁻ ET neurons compared to wild type. Together, these data suggest that a loss of dendritic sodium channel function in ET neurons of the PFC contributes to a fundamental breakdown in the input-output transformation process in Fragile X syndrome.


Poster

280. Genetic and Environmental Factors for Autism Spectrum Disorders

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 280.12/B13

Topic: A.07. Developmental Disorders

Support: NIH Grant MH100510

Title: Dysfunctional temporoammonic pathway LTP in a mouse model of fragile X syndrome
**Authors:** *G. ORDEMANN*¹, R. A. CHITWOOD², D. H. BRAGER³;  
¹Ctr. for Learning and Memory, The Univ. of Texas, Austin, TX; ²Ctr. for Learning and Memory, The Univ. of Texas, Austin, TX; ³Inst. for Neurosci., Univ. Texas at Austin, Austin, TX

**Abstract:** Fragile X syndrome (FXS) is the most common monogenetic cause of autism and intellectual disability. Although the genetic underpinnings of FXS are well understood, the neurophysiological basis for the intellectual deficits remain largely unknown. Our lab previously identified dendritic channelopathies in FXS affecting synaptic integration and Schaffer collateral (SC) synaptic plasticity in CA1 neurons of the hippocampus, a structure critical for learning and memory. Here, we use the *fmr1*⁻/⁻ mouse model of FXS and a combination of somatic and dendritic current clamp recordings paired with 2-photon calcium imaging to investigate properties and plasticity of temporoammonic (TA) synapses from the entorhinal cortex onto CA1 neurons. Following theta burst stimulation, LTP at TA synapses in *fmr1*⁻/⁻ CA1 neurons was significantly smaller (8.8 ± 21.8%) compared to wild type CA1 neurons (324.4 ± 74.4%). Using 2-photon imaging we found that dendritic calcium signals during 100 Hz burst stimulation of TA inputs was significantly smaller in *fmr1*⁻/⁻ CA1 neurons (14.29% αF/F₀) compared to wild type CA1 neurons (57.36% αF/F₀). Using both calcium imaging and current clamp recordings, we found no significant differences in NMDA receptor function at TA synapses between wild type and *fmr1*⁻/⁻ neurons. Interestingly, and in contrast in TBS, simultaneous pairing of both TA and SC synapses results in comparable levels of LTP between wild type and *fmr1*⁻/⁻ neurons. This suggests that TA synapses in *fmr1*⁻/⁻ neurons can be potentiated. Dendritic recordings of complex spikes were no different between wild type and *fmr1*⁻/⁻ neurons. However in *fmr1*⁻/⁻ neurons, unlike in wild type, complex spikes were relatively insensitive to high concentrations of extracellular Ni²⁺, suggesting a difference in the functional expression of voltage gated calcium channels in *fmr1*⁻/⁻ CA1 dendrites. We hypothesize that in *fmr1*⁻/⁻ neurons, a combination of insufficient dendritic depolarization and activation of voltage-gated calcium channels, impairs LTP at TA synapses in *fmr1*⁻/⁻ mice.

**Disclosures:** G. Ordemann: None. R.A. Chitwood: None. D.H. Brager: None.

**Poster**

**280. Genetic and Environmental Factors for Autism Spectrum Disorders**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 280.13/B14

**Topic:** A.07. Developmental Disorders

**Support:** NIMH Grant R01 MH100510  
NSF GRFP

**Title:** Dysfunction of hippocampal inhibitory interneuron function in fragile-X syndrome
Authors: *L. T. HEWITT*, D. H. BRAGER;

1The Univ. of Texas at Austin, Austin, TX; 2Inst. for Neurosci., Univ. Texas at Austin, Austin, TX

Abstract: Fragile X Syndrome (FXS) is the most commonly inherited form of intellectual disability and the leading genetic cause of autism. Patients with FXS exhibit increased incidence of epilepsy, sensory hypersensitivity, and anxiety among other cognitive impairments. Recent data suggests that a lack of inhibitory/excitatory balance in the brain may be a major contributor to the debilitating cognitive deficits in FXS. While there is an extensive body literature investigating excitatory pyramidal neurons and synaptic transmission, the impact of aberrant inhibitory activity is largely unknown. We focused on two integral inhibitory interneuron subtypes in the hippocampus: somatostatin expressing (SOM) and parvalbumin expressing (PV) interneurons. SOM interneurons specifically target pyramidal cell dendrites and control synaptic plasticity, while PV interneurons target pyramidal cell bodies and control cell ensemble activation. Using whole-cell current clamp recordings, we measured subthreshold (i.e. input resistance, resting membrane potential) and suprathreshold (i.e. action potential threshold) properties of SOM and PV cells in wild-type and fmr1/-y mice. Preliminary data suggests that subthreshold and suprathreshold properties in SOM interneurons of the fmr1/-y mouse are altered with no apparent phenotype in PV interneurons. Due to their distinct connections, SOM and PV cells to contribute to different functional aspects of the hippocampal microcircuit and alterations of either interneuron subtype in FXS would impair information processing in the hippocampus.


Poster

280. Genetic and Environmental Factors for Autism Spectrum Disorders

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 280.14/B15

Topic: A.07. Developmental Disorders

Support: NIH NS088776

Title: Sex-specific effects of prenatal omega-3 fatty acids on vocalization production in the Fmr1 knockout mouse

Authors: *S. O. NOLAN*, S. L. HODGES, M. BINDER, P. D. WOMBLE, J. N. LUGO, JR;

1Psychology and Neurosci., 2Inst. of Biomed. Studies, Baylor Univ., Waco, TX; 3Pharmacol., Vanderbilt Univ. Sch. of Med., Nashville, TN
Abstract: Isolation-induced ultrasonic vocalizations are considered an early indicator of behavioral dysregulation in many mouse models of Autism spectrum disorder (ASD). Previous studies in both our lab and others utilizing the Fmr1 mutant model have demonstrated several quantitative and qualitative changes in vocalization production during early postnatal periods, ranging from postnatal days (PD) 8 - 12. The present study sought to determine the impact of prenatal high fat dietary manipulations on isolation-induced ultrasonic vocalization production in both male and female Fmr1 mutants on PD9 using high-throughput analysis methods. For the dietary manipulation breeding pairs were assigned to one of three diet conditions prior to breeding and remained on the diet throughout gestation: standard lab chow, omega-3 fatty acid enriched chow, and a diet controlling for the fat increase. Resulting offspring (male WT, male KO, female WT, female HET and female KO pups) were maintained on this diet and genotyped prior to vocalization recording on PD9. On PD9 we used the maternal separation paradigm to elicit vocalizations from pups. The vocalizations were recorded using an Avisoft recording system. Results indicated that prenatal exposure to high omega-3’s increased average fundamental frequency of calls in both male WT and KO mice. Exposure to omega-3 fatty acids restored the number of calls produced by Fmr1 female HETs to levels similar to female wildtype mice. Moreover, diminished spectral purity in the female Fmr1 homozygous mouse was rescued by exposure to high fat diets, though these effects were not seen in the male Fmr1 knockout. These results are in line with data in adult Fmr1 knockout males, showing that omega-3 fatty acids administered prenatally, attenuate a broad host of behavioral impairments in adulthood, such as deficits in prepulse inhibition and fear learning and memory. These data support future development of this dietary therapeutic for neurodevelopmental disorders.


Poster

280. Genetic and Environmental Factors for Autism Spectrum Disorders

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 280.15/B16

Topic: A.07. Developmental Disorders

Support: NIH Grant NS088776

Title: A single early-life seizure results in long-term behavioral changes in the adult Fmr1 knockout mouse

Authors: *S. L. Hodges¹, C. D. Reynolds², S. O. Nolan¹, J. L. Huebschman³, J. T. Okoh⁴, M. S. Binder¹, J. N. Lugo¹;
¹Baylor Univ., Waco, TX; ²Texas Col. of Osteo. Med., Univ. of North Texas Hlth. Sci. Ctr.,
Abstract: Fragile X syndrome (FXS) is the leading cause of inherited intellectual disability and a significant genetic contributor of Autism spectrum disorder. In addition to autistic-like phenotypes, individuals with FXS are subject to developing numerous comorbidities, one of the most prevalent being seizures. In the present study, we investigated how a single early-life seizure superimposed on a genetic condition impacts the autistic-like behavioral phenotype of the mouse. We induced status epilepticus (SE) on postnatal day (PD) 10 in Fmr1 wildtype (WT) and knockout (KO) mice. We then tested the mice in a battery of behavioral tests during adulthood (PD90) to examine the long-term impact of an early-life seizure. Our findings replicated prior work that reported a single instance of SE results in behavioral deficits, including increases in repetitive behavior, enhanced hippocampal-dependent learning, and reduced sociability and prepulse inhibition. We also observed genotypic differences characteristic of the FXS phenotype in Fmr1 KO mice, such as enhanced prepulse inhibition and repetitive behavior, hyperactivity, and reduced startle responses. Superimposing a seizure on deletion of Fmr1 significantly impacted repetitive behavior in a nose poke task. Specifically, a single early-life seizure increased consecutive nose poking behavior in the task in WT mice, yet seizures did not exacerbate the elevated stereotypy observed in Fmr1 KO mice. Overall, these findings help to elucidate how seizures in a critical period of development can impact long-term behavioral manifestations caused by underlying gene mutations in Fmr1. Utilizing double-hit models, such as superimposing seizures on the Fmr1 mutation, can help to enhance our understanding of comorbidities in disease models.


Poster

280. Genetic and Environmental Factors for Autism Spectrum Disorders

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 280.16/B17

Topic: A.07. Developmental Disorders

Support: Lou Lou Foundation
Hope4Harper Foundation

Title: Cannabidiol attenuates behavioral and epileptiform deficits in a mouse model of CDKL5 developmental disorder

Authors: A. WIEST1, M. YENNAWAR1, *R. S. WHITE2, J. WACKER2, F. E. JENSEN2; 1Pharmacol., 2Neurol., Univ. of Pennsylvania Perelman Sch. of Med., Philadelphia, PA
**Abstract:** Cyclin-dependent-kinase-like 5 (CDKL5) mutation and loss of function result in a range of autistic-like behaviors, neurodevelopmental deficits, and often refractory seizures. Recent clinical studies with cannabidiol have shown efficacy in suppressing seizures in this population as well as other neurodevelopmental syndromes characterized by epilepsy, autistic-like symptoms, and intellectual disability. We used a CDKL5 mouse model where the protein is functionally knock (R59X). We have previously demonstrated behavioral and cognitive deficits and increased seizure susceptibility within this model (Yennawar, et al., J. Neurosci, 2019). Adult (P60-80) R59X mice and littermate WT mice were treated with vehicle (saline) or cannabidiol (CBD:100mg/kg) 1 hour prior to behavioral testing or chemoconvulsant (pentylenetetrazol (PTZ) 50 mg/kg) seizure induction. Cannabidiol treatment attenuated deficits in social choice with an average increase of 18.4±5.5sec spent at the social chamber (R59X veh: 21±1.5s vs R59X CBD: 39.4±4.3s; p<0.01). Treatment also helps with learning and memory, improving successful trials on the Y-maze test (R59X veh: 39.3±1.1s vs R59X CBD: 56.5±2.8.3s; p<0.0001) and long-term fear conditioning (R59X veh: 20.6±3s vs R59X CBD: 37.8±5.9s; p<0.05). In addition, CBD reversed the increase in seizure susceptibility to PTZ seizures in the R59X mice (R59X veh: 86.4±4.7s vs R59X CBD: 150.3±24.2s; p<0.001). In contrast, CBD treatment did not affect these parameters in WT mice. To begin to understand the potential therapeutic effects of CBD, we evaluated the developmental trajectory of protein expression of different known endocannabinoid pathway targets. We showed that CB1 expression is significantly elevated in R59X mice at P50 in the hippocampus when compared to WT littermate controls (30.9±11% diff, p<0.05). We have also shown that CB2 expression is significantly elevated in the cortex of R59X mice at P50 (60.9±16% diff., p<0.01). We are investigating other known targets of the endocannabinoid system (A1, TRPV1, TRPV2, and GPR55) to understand how protein expression is regulated during development in R59X mice to elucidate a mechanism of CBD in our mouse model. Taken together, these data suggest that cannabidiol may have therapeutic potential in rescuing not only seizure susceptibility but also the behavioral deficits in CDKL5 disorder and justify further study of the role of the endocannabinoid system as a target for treatment.

**Disclosures:** A. Wiest: None. M. Yennawar: None. R.S. White: None. J. Wacker: None. F.E. Jensen: None.

**Poster**

**280. Genetic and Environmental Factors for Autism Spectrum Disorders**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 280.17/B18

**Topic:** A.07. Developmental Disorders

**Support:** NIH Grant AG061288
Title: IGF2 dependent paracrine signaling in pericyte and neuronal crosstalk

Authors: *Z. Zhao1, X. W. Xie1, J. Zeng2, Y. Wu3, B. Pluimer3;  
1Physiol. and Neurosci., 2Physiol. & Neurosci., 3USC, Los Angeles, CA

Abstract: Neuronal functions and brain connectivity require a highly coordinated neurovascular unit (NVU). Neurons and vascular cells are not just adjacently located; they communicate with each other vigorously via different signaling modules. Pericytes are vascular mural cells of the endothelium and vital integrators of NVU functions, including maintaining the blood-brain barrier (BBB) and vascular integrity, regulating blood flow and tissue oxygenation, modulating neuroinflammation and supporting neuronal health. Pericyte injury and loss occur commonly in CNS diseases including Alzheimer’s disease and dementia. Our current knowledge implicates a critical role of pericytes for neuronal functions, which calls for investigation of pericyte-neuronal communication for different neuronal functions in health and particularly in Alzheimer’s disease. Using new 3D co-culture systems and novel transgenic models, we found that pericytes can directly regulate neurogenesis and neuronal functions, which can be attributed to pericyte-derived insulin-like growth factor 2. IGF2 is a peptide hormone with multiple roles in regulating metabolic functions and developmental processes. Patients carry IGF2 mutation and mice lacking IGF2 exhibited strong growth defects with abnormal neural development. IGF2 is produced locally in the brain; however, the roles of brain IGF2 in neurogenesis and neuronal dysfunction in CNS diseases are poorly understood. Our preliminary studies additionally indicated that IGF2 mediates pericyte-neuronal communication by activating a noncanonical IGF2R-Gai-PLC pathway to enhance neuronal functions, as well as stimulating a canonical PI3K/Akt pathway to promote neurogenesis or suppressing Tau-phosphorylation. We hope to generate first evidence of functional pericyte-neuron crosstalk for brain function in health and diseases, and pinpoint the mechanism of this signaling at the molecular level for IGF2-mediated pericyte-neuron crosstalk. The outcomes may provide new insights to the IGF system and neurovascular interaction in the brain, and close an important gap between metabolic diseases and CNS neurodegenerative diseases such as AD.

Disclosures: Z. Zhao: None. X.W. Xie: None. J. Zeng: None. Y. Wu: None. B. Pluimer: None.

Poster

280. Genetic and Environmental Factors for Autism Spectrum Disorders

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 280.18/B19

Topic: A.07. Developmental Disorders

Support: T32GM099608
5TL1TR001861
**Title:** In-vivo tracking and biological function of engineered mesenchymal stem cell-secreted artificial transcription factors

**Authors:** *P. Deng*¹, D. Cameron², J. J. Waldo², J. A. Halmai², U. BeitnerE¹, J. Nolta³, D. J. Segal¹, K. Fink²;
¹Genome Ctr., UC Davis, Davis, CA; ²Neurol., ³Stem Cell Program, UC Davis Med. Ctr., Sacramento, CA

**Abstract:** The advent of nuclease-deficient DNA binding domains (DBDs) such as zinc fingers and CRISPR/dCas9 for tunable gene expression have revolutionized approaches towards treating rare neurologic disorders. An effective delivery system that can traffic these proteins to target tissue remains a key barrier for translating gene modifying DBDs into the clinic. Cell-based delivery methods, such as mesenchymal stem cells (MSC), are an attractive delivery vehicle for DBDs due to their transient nature and ability to create favorable microenvironments through the release of trophic factors. Along with their strong clinical safety profile, MSCs can be readily reprogrammed to secrete large proteins and are not limited in their transgene packaging size like adeno-associated viral approaches. To evaluate this system, we have engineered bone-marrow derived MSCs to act as in-vivo biofactories that secrete DBDs into the extracellular space whereby the DBDs are uptaken by neighboring cells, and shuttled into the nucleus to alter gene expression.

Presently, we demonstrate the feasibility of this platform through in-vivo tracking of MSC-DBDs engineered to secrete a PET-reporter following multiple routes of cell administration: intranasal, intracerebral, and cisterna magna. Robust distribution of PET-reporter protein was observed along the spinal cord and in the brain of cisterna magna-transplanted mice. Additionally, we evaluated the biological activity of secreted DBD following transplantation of MSC-DBD in a YFP-fused Ube3a reporter model of Angelman Syndrome. Significant activation of the YFP-fused Ube3a gene was observed 3- to 6- weeks following both cisterna magna and intracerebral transplantation as assessed by Western Blot and qPCR. Together, this is suggestive that an MSC-secretory system for DNA-binding domains can alter gene transcription in a durable fashion in mammalian organisms. Current ongoing experiments are evaluating the translational approach of this platform in non-human primates.


**Poster**

280. Genetic and Environmental Factors for Autism Spectrum Disorders

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 280.19/B20
**Topic:** A.07. Developmental Disorders

**Title:** Neural crest loss caused by Pak1ip1 mutation can be rescued by Tp53 inhibition

**Authors:** *A. A. PANOUSTOPOULOS*\(^1,2\), A. LEE\(^2,1\), A. DE CRESCENZO\(^2,1\), E. HELMKE\(^2\), R. MARCUCIO\(^3\), P. TRAINOR\(^4\), K. ZARBALIS\(^2,1\);
\(^1\)UC Davis, Sacramento, CA; \(^2\)IPRM - Shriners Children Hosp., Sacramento, CA; \(^3\)Univ. of California, San Francisco, CA; \(^4\)The Univ. of Kansas, Kansas City, MO

**Abstract:** Pak1ip1 has been characterized as a nucleolar protein required for ribosome biogenesis. Loss of Pak1ip1 stabilizes Tp53 by increasing the levels of freely circulating ribosomal proteins Rpl5 and Rpl11, which inhibit the Tp53 inhibitor Mdm2. In previous work, we have shown specific defects in cranial NC-derived structures in Pak1ip1 mutant embryos, key amongst them midline facial clefting. These defects are apparently caused by increased neuroepithelial apoptosis and/or diminished proliferation during the formation and migration of cranial NC cells. Here, we show that pharmacological and/or genetic interference with Tp53 signaling can ameliorate or even prevent the mutant phenotype. Intriguingly, while pharmacological inhibition by Pifithrin-α (PFTα) can diminish phenotypic features and rescue NC cells, genetic Tp53 inactivation has the capacity to completely prevent midline clefting. Rescue effectiveness is based on gene dosage with only homozygous Tp53 loss leading to complete prevention of the median cleft. Morphological outcomes are associated with improvements in cell viability and proliferation within the neural plate and increased numbers of migrating NC cells in of Pak1ip1; Tp53 compound mutants compared to Pak1ip1 mutant embryos.

**Disclosures:** A.A. Panoutsopoulos: None. A. Lee: None. A. De Crescenzo: None. E. Helmke: None. R. Marcucio: None. P. Trainor: None. K. Zarbalis: None.

**Poster**

**280. Genetic and Environmental Factors for Autism Spectrum Disorders**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 280.20/B21

**Topic:** A.07. Developmental Disorders

**Title:** Assessment of spine density and brain morphological phenotype in three rodent models of neurodevelopmental psychiatric disorders, poly(I:C): two-hit poly(I:C); and prenatal MAM rat model

**Authors:** *D. RADL*\(^1\), J.-C. BIZOT\(^2\), S. DAVID\(^2\), J. COMBEAU\(^2\), F. MASSE\(^2\), Y. OBAYASHI\(^1\), N. CHEN\(^1\), P. O’DONNELL\(^2\), R. PETROSKI\(^4\), O. TOURY\(^4\), B. BUISSON\(^4\), R. HODGSON\(^1\),
Abstract: Prenatal infection and postnatal exposure to traumatizing experience are involved in the pathogenic processes of various neurodevelopmental psychiatric disorders such as schizophrenia. Dendritic spine number undergoes substantial changes during development. Spine density loss and cortical thinning is observed in patients with schizophrenia during adolescence. Excessive spine pruning is thought to underlie the reduced cortical gray matter volume in schizophrenia. The aim of the present study was to compare the morphological phenotype in young adult offspring of: 1. C57BL/6J mouse dams infected with polyriboinosinic-polyribocytidylic acid (poly I:C; 5 mg/kg ip on gestational day 15), 2. C57BL/6J mouse dams infected with a subthreshold exposure of polyriboinosinic-polyribocytidylic acid (1 mg/kg iv gestational day 9) that are exposed to stress, 3. Sprague-Dawley rat dams infected with methylazoxymethanol acetate (MAM). Cohort 2 was exposed to a sub-chronic unpredictable stress between post-natal day (PND) 35 and PND 43, a period which corresponds to the peripubertal period. Stress consisted of: Day 1 Electric foot shock (12 min 3 shocks), day 3 Restraint stress (45 min), day 5 Food deprivation (20 h: 12:00-08:00), day 7 Forced swimming test (18°C water, two 1-min FST 2 min apart), day 9 Repeated changing of home cage (5 changes irregular interval). A third cohort of pregnant rats received an IP injection of MAM (22 mg/kg) or saline on GD17. Rats of cohort 3 and mice of the cohort 1 were sacrificed at 9 weeks of age; mice of the cohort 2 were sacrificed at 6 weeks of age. The brains were harvested and used for several analyses: immunohistochemical analyses of inflammatory markers (IL1b) and glia morphology (CD11b, GFAP), spine analysis by Golgi staining and RNA extraction to analyze markers of inflammation (TNF-alfa, Il1b, IL6, C3, C4). These data provide a way to differentiate these three prenatal perturbation models of schizophrenia in terms of their impact on synaptic density and cortical thickness. These models will be further explored with behavioral and electrophysiological investigations for schizophrenia drug discovery programs.


Poster

280. Genetic and Environmental Factors for Autism Spectrum Disorders

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 280.21/B22

Topic: A.07. Developmental Disorders

Title: Longitudinal characterization of the Cln8^md/- mouse model of CLN8 Batten Disease - Fine motor performance, brain pathology and metabolic changes
Authors: K. LEHTIMÄKI¹, T. BRAGGE¹, *J. T. PUOLIVÄLI¹, J. RYTKÖNEN¹, P. POUTIÄEN², T. B. JOHNSON³, J. T. CAIN³, S. DAVIS³, A. J. NURMI¹, J. WEIMER³; ¹Charles River Discovery, Kuopio, Finland; ²Radiopharmacy, Kuopio Univ. Hosp., Kuopio, Finland; ³Sanford Res., Sioux Falls, SD

Abstract: The naturally occurring Cln8<sup>mnd</sup>-/− mouse model of CLN8 Batten disease (B6.KB2-Cln8<sup>mnd</sup>/MsrJ, known also as Northern epilepsy) has been shown to exhibit progressive retinopathy, accumulation of autofluorescent lipopigment (stain positive for subunit c of mitochondrial ATPase), atrophy of neocortex and loss of cortical and hippocampal interneurons (Cooper JD et al. 1999, J Neurosci). However, relatively little is known on in vivo phenotype on neurometabolic profile in relation to brain atrophy and fine motor deficits. Here, we characterize the Cln8<sup>mnd</sup>-/− model from 2 to 8 months of age with a multimodal and longitudinal approach, combining advanced technologies such as kinematic gait analysis, MR based imaging techniques (T2 volumetry, DTI for white matter defects), spectroscopy (1H-MRS), and metabolic profiling (FDG PET). We have shown in our previous work in Cln6<sup>nclf</sup> mice (bioRxiv, doi.org/10.1101/522011) that phenotypic fingerprint derived from longitudinal imaging and fine motor deficits can be identified and reduced to highly descriptive and significant metrics utilizing the contrastive PCA approach.

We used imaging, spectroscopic, and behavioral data collected from the same Cln8<sup>mnd</sup>-/− and wild type littermate controls (pooled and genders separated; 6 female and 6 male mice/genotype,) at 2, 4, 6 and 8 months of age. The data reduction and fusion were performed in two phases: first, PCA was applied separately to normalized parameter data from each modality. Then, the first phase PC scores were transformed into final scores using contrastive PCA (cPCA).

Data showed progressive nature of NCL resembling phenotype, with brain atrophy and neurometabolic disturbances obtained utilizing MR spectroscopy and PET-imaging. Abnormal motor performance was observed by fine motor kinematic analysis, and while declining motor performance is typical to Batten disease, the built-in motor neuron degeneration (mnd) origin in the mouse model is acknowledged. Contrastive PCA was capable of identifying the fingerprint typical to the model. Like we have shown in the previous works, this approach demonstrates how structural and functional readouts are connected using unbiased modeling and allows interventions with novel treatments with higher statistical window.


Poster

280. Genetic and Environmental Factors for Autism Spectrum Disorders

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 280.22/B23
**Title:** Mesenchymal stromal cell-derived exosomes mitigate neural progenitor cell impairment in bronchopulmonary dysplasia

**Authors:** *M. A. LITHOPOULOS*¹,², L. STRUEBY³, S. ZHONG¹, A. VADIVEL¹, R. SLACK²,⁴, J. WANG¹,², D. C. LAGACE²,⁴, B. THÉBAUD¹,²,⁵;

¹Ottawa Hosp. Res. Inst., Ottawa, ON, Canada; ²Cell. and Mol. Med., Univ. of Ottawa, Ottawa, ON, Canada; ³Univ. of Saskatchewan, Saskatoon, SK, Canada; ⁴Univ. of Ottawa Brain and Mind Res. Inst., Ottawa, ON, Canada; ⁵Children's Hosp. of Eastern Ontario Res. Inst., Ottawa, ON, Canada

**Abstract:** **Rationale:** Bronchopulmonary dysplasia (BPD), a chronic lung disease, is the most common complication of prematurity. BPD is characterized by an arrest in lung growth and is an independent risk factor for adverse neurodevelopment. The potential implications of neural progenitor cells (NPCs), cells crucial for proper brain development, are unknown. Previously, we showed that intratracheal administration of umbilical cord-mesenchymal stromal cells (UC-MSCs), protects the lungs in a BPD rodent model. We hypothesized that NPCs are functionally impaired in experimental BPD, contributing to adverse neurodevelopment and further, that UC-MSC-derived exosomes can mitigate injury to NPCs in the brain. **Objective:** To assess NPC function in experimental BPD before and after UC-MSC administration. **Methods:** Two models of BPD were investigated. Neonatal C57/Black 6 mice were exposed to: i) 85% oxygen from postnatal day (P)0-P14 or ii) lipopolysaccharide at P7/8 and 8 hours of mechanical ventilation at P9/10, to mimic the conditions contributing to BPD in preterm infants. For treatment, mice (P9/10) were administered PBS or UC-MSC-derived exosomes intratracheally before ventilation. Exosomes were tracked using PKH26 staining. Age-matched control mice were housed in room air. NPC function was examined using sequential neurosphere assays. Mice were further assessed at 1 year for neurodevelopmental outcomes using a selection of motor, anxiety, and cognitive tests. **Results:** NPCs isolated from hyperoxia-exposed (n=5) and ventilated mice (n=7) formed significantly fewer secondary neurospheres compared to those isolated from control mice (P9/10, n=8; P14, n=5), indicating self-renewal impairments. One-year-old hyperoxia-exposed mice showed significant motor deficits on the rotarod test, a hypoanxiety phenotype on the light dark test, and learning and memory deficits during fear conditioning (controls, n=19; hyperoxia, n=12). NPCs from ventilated, exosome treated mice, formed significantly more secondary neurospheres compared to those isolated from PBS treated mice, indicating a rescue in self-renewal function (exosome, n=11; PBS, n=8). Labelled exosomes were identified in the lungs and NPC niche regions in the brain. No significant differences between sexes were observed. **Conclusions:** This study demonstrates that NPC function is impaired in BPD and that UC-MSC-
derived exosomes administered to target the lungs, results in remote organ protection, mitigating injury to NPCs in the brain. This critical research will strengthen the rationale for implementing UC-MSC therapy in the clinic, to reduce the life-threatening complications of preterm birth.


Poster

280. Genetic and Environmental Factors for Autism Spectrum Disorders

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 280.23/B24

Topic: A.07. Developmental Disorders

Support: NRF-2017R1D1A1B03032212
NRF-2016R1D1A1B03933283
NRF-2016M3C7A1904149
NRF-2017M3A9C6027009
NRF-2017M3A9C4092979

Title: 14-3-3gamma haploinsufficient mice display hyperactive and stress-sensitive behaviors

Authors: *C.-H. CHO¹, D. KIM², K. SIM¹, O. KWON¹, E. HWANG³, H.-W. KIM², J.-Y. PARK¹;
¹Korea Univ., Seoul, Korea, Republic of; ²Sejong Univ., Seoul, Korea, Republic of; ³Ctr. for Functional Connectomics, Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: 14-3-3γ plays diverse roles in different aspects of cellular processes. Especially in the brain where 14-3-3γ is enriched, it has been reported to be involved in neurological and psychiatric diseases (e.g. Williams-Beuren syndrome and Creutzfeldt-Jakob disease). However, behavioral abnormalities related to 14-3-3γ deficiency are largely unknown. Here, by using 14-3-3γ deficient mice, we found that homozygous knockout mice were prenatally lethal, and heterozygous mice showed developmental delay relative to wild-type littermate mice. In addition, in behavioral analyses, we found that 14-3-3γ heterozygote mice display hyperactive and depressive-like behavior along with more sensitive responses to acute stress than littermate control mice. These results suggest that 14-3-3γ levels may be involved in the developmental manifestation of related neuropsychiatric diseases. In addition, 14-3-3γ heterozygote mice may be a potential model to study the molecular pathophysiology of neuropsychiatric symptoms.

**Poster**

**281. Monoamine Transport and Signaling**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 281.01/B25

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

**Support:**

2R01DA035263

5F31MH114316

**Title:** Phosphatidylinositol (4, 5)-biphosphate coordinates functional interactions in the dopamine transporter to promote amphetamine preference

**Authors:** *J. I. AGUILAR*¹,², S. J. MABRY², H. MATTHIES², A. GALLI²;¹Vanderbilt Univ., Nashville, TN; ²Dept. Surgery, Univ. of Alabama at Birmingham, Birmingham, AL

**Abstract:** The psychostimulant amphetamine (AMPH) mainly mediates its pharmacological and behavioral effects by increasing extracellular dopamine (DA) availability. DA homeostasis is maintained by the dopamine transporter (DAT), a presynaptic membrane protein that mediates the high-affinity reuptake of released DA from the synaptic cleft. We have previously demonstrated that AMPH induces N-term phosphorylation of the DAT, which supports transport-mediated efflux of DA. Furthermore, we have shown that phosphatidylinositol (4, 5)-bisphosphate (PIP₂) directly interacts with the DAT and facilitates AMPH-induced DA efflux, but is not required for DA uptake. Specially, PIP₂ binds DAT through electrostatic interactions with positively charged DAT N-terminal residues. Disrupting the interactions between DAT and PIP₂ or depleting PIP₂ diminishes reverse transport (efflux) of DA. Previous studies on the human serotonin transporter show that non-N-terminal PIP₂ binding sites (within the fourth intracellular loop) modulate transporter function. Here, we show that a neutralizing substitution of residue R443 to Ala, which resides in the fourth intracellular loop of DAT, decreases DAT/PIP₂ interactions and AMPH-induced DA efflux, despite normal DA uptake. As such, this DAT variant dissociates forward transport (uptake) from reverse transport (efflux), allowing specific transport functions of the DAT to be studied independently. Using a coordinated genetic and pharmacological approach in *Drosophila melanogaster*, we translate our molecular discoveries *in vivo* to probe the physiological role for DAT efflux in motivated behavior. We uncover that residue R443 in the DAT regulates both AMPH-induced DA efflux and psychomotor behaviors, including preference.

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Poster

281. Monoamine Transport and Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 281.02/B26

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

Support: NIH Grant DA014204

Title: Subcellular localization of the dopamine transporter in nigrostriatal axons of the mouse medial forebrain bundle

Authors: K. BUCCHIN¹, J. Balcita-Pedicino¹, A. Sorkin², *S. R. SESACK¹; ¹Neurosci., ²Cell Biol., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The structure and function of the nigrostriatal pathway has been extensively examined at the level of midbrain dopamine (DA) cell bodies and their axons within the striatal complex. To date, no ultrastructural studies have visualized the axons that interconnect these regions within the medial forebrain bundle (MFB). Furthermore, we have previously reported the subcellular localization of the DA transporter (DAT) in the dorsolateral striatum (DLStr), showing that DAT is dominantly expressed on the plasma membrane of axons and minimally observed at internal sites: synaptic vesicles, small endosomes, and multivesicular bodies (Block et al., 2015 J Neurosci 35:12845). Here, in addition to investigating the morphological features of DA axons in the MFB, we sought to determine whether the transit of DAT through this region involves primarily diffusion in the plasma membrane or transport through intracellular organelles. Transgenic mice with a hemagglutinin (HA) tag incorporated into the DAT protein were used for these studies. Confocal immunofluorescence microscopy imaging of HA-DAT in the MFB showed axons of greater thickness than reported for the DLStr. Vesicular structures labeled for HA-DAT were also observed in MFB axons. The subcellular localization of HA-DAT in the MFB was further examined by immunogold-silver labeling and electron microscopy in 4 mice from the 2015 study. 166 axons labeled for HA-DAT were analyzed and had a mean diameter of 0.58 µm (± 0.02 µm, SEM), substantially larger than intervaricose axons in the DLStr (average of 0.24 µm; Descarries et al., 1996 J Comp Neurol 375:167) and larger as well than axon initial segments of nigral DA cells (average of 0.37 µm; González-Cabrera et al., 2017 J Comp Neurol 525:3529). Although the majority of immunogold-silver particles for HA-DAT were observed on the plasma membrane of these axons, the proportion of membrane gold was noticeably lower for the MFB than previously reported for the DLStr: 64% versus 84%, respectively. Of the 36% of HA-DAT detected intracellularly, roughly 37% was found in association with vesicles or slightly larger endosomes. The results indicate that nigrostriatal axons increase diameter as they travel through the MFB and that DAT is transported via both membrane diffusion and internal organelles. The findings have important implications for
understanding action potential conduction and trafficking of DAT within the nigrostriatal bundle in both the healthy state and in neurological disorders.


Poster

281. Monoamine Transport and Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 281.03/B27

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

Support: “Bioinformatics for Brain Sciences” performed under the SRPBS from MEXT and AMED, “Brain/MINDS” from AMED
JSPS KAKENHI Grant 17J10615
JSPS KAKENHI Grant 17H01380

Title: Dopamine and adenosine signaling pathways are analyzed in striatal medium spiny neurons using kinase-associated neural phospho-signaling (KANPHOS) database

Authors: *X. ZHANG1, T. NAGAI2, K. KURODA1, K. KAIJUCHI1;
1Dept. of Cell Pharmacol., 2Dept. of Neuropsychopharm. and Hosp. Pharm., Nagoya Univ. Grad. Sch. of Med., Nagoya, Japan

Abstract: Protein phosphorylation is one of the most ubiquitous and essential mechanisms mediating intracellular signal transduction in various cellular processes. However, a large number of substrates that account for various kinases’ function remain to be elucidated. To identify the specific substrates for the specific kinases, we have developed an in vitro approach termed the kinase-interacting substrate screening (KISS) method and an in vivo approach termed the kinase-oriented substrate screening (KIOSS) method. An online database system named KANPHOS (Kinase-Associated Neural Phospho-Signaling) were built, providing the phosphorylation signals identified by our methods. In the midbrain, dopaminergic neurons mainly project toward the striatum. Medium spiny neurons (MSNs) expressing dopamine D1 receptor (D1R) or D2 receptor (D2R) are major components of the striatum. Stimulation of D1R activates PKA through Gs to increase neuronal activity in D1R-MSN, while D2R stimulation inhibits PKA through Gi in D2R-MSN. By using KIOSS method, we found more than 100 PKA substrate candidates downstream of D1R including Rasgrp2 and Rap1gap, which are positive and negative regulators of the small G protein Rap1, respectively. We demonstrated that dopamine activates PKA to increase Rap1 activity through Rasgrp2 and Rap1gap phosphorylation in D1R-MSN. Rap1 activation increases D1R-MSN’s neuronal excitability to enhance rewarding behavior (Nagai et al. Neuron, 2016). In D2R-MSN, adenosine A2A receptor...
(A2AR) coupled to Gs is highly expressed. However, how dopamine and adenosine cooperatively regulate PKA activity remains largely unknown. Here, we measured Rap1gap serine 563 phosphorylation to monitor PKA activity and examined dopamine and adenosine signals in MSNs. We found that A2AR agonist CGS21680 increased Rap1gap phosphorylation, and pretreatment with the D2R agonist quinpirole blocked this effect in striatal slices. D2R antagonist eticlopride increased Rap1gap phosphorylation in D2R-MSN in vivo, and the effect of eticlopride was blocked by the pretreatment with the A2AR antagonist SCH58261. These results indicate that adenosine positively regulates PKA in D2R-MSN through A2AR, while this effect is blocked by basal dopamine in vivo. We propose that the shift from D1R-MSN to D2R-MSN or vice versa appears to depend predominantly on a change in dopamine concentration. In addition to Rap1 pathway, we also found some regulators of small G protein RhoA among PKA substrate candidates. We here discuss phospho-proteomic approach and our findings based on our database.


Poster

281. Monoamine Transport and Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 281.04/B28

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

Title: Striatal dopamine in time and space: Effects of methylphenidate

Authors: M.-T. HSU¹, J. A. CHOUINARD¹, N. KITAMURA¹, K. LIYANAGAMA¹, L. TIAN², *J. R. WICKENS¹;
¹Okinawa Inst. of Sci. and Technol., Onna, Japan; ²Biochem. and Mol. Med., Univ. of California at Davis, Davis, CA

Abstract: Methylphenidate - a widely used and effective medication for attention-deficit hyperactivity disorder - is known to be a dopamine reuptake inhibitor at molecular level. However, its macroscopic effects are incompletely understood. We investigated the effects of methylphenidate on the spatiotemporal distribution of dopamine in the striatum. Using fast-scan cyclic voltammetry we measured the time-course of concentration changes at single locations after electrical stimulation (20 pulses at 10 Hz, biphasic, 2 ms per phase, 2-5V). When clearance of dopamine is reduced by bath application of methylphenidate (30 µM), the peak concentration of dopamine measured at a single distant point is delayed several tens of milliseconds after the end of the stimulation. This cannot be explained by a simple time delay due to diffusion time because there is no corresponding delay in onset of dopamine increase at the distant recording site. We hypothesized that the immediacy of the initial increase in dopamine can be explained by electrical current spread causing release at distant sites, while the delayed peak is due to
diffusion delays. A two-compartment model of dopamine concentration was implemented, in which electrically-stimulated release, reuptake and diffusion occurred in both compartments. Predictions of the model were compared to the experimental data and produced good quantitative fit. These findings partially support the two-compartment model. To test whether the model assumptions were correct, we obtained measurements of the spatiotemporal distribution of dopamine in two dimensions, using variants of the genetically encoded dopamine sensor, dLight, and epifluorescence imaging. These measurement showed that increases in dopamine concentration occurred simultaneously over the imaged area, consistent with an effect of current spread. The effect of methylphenidate on apparent diffusion distance of dopamine may be important for understanding its effects on processing of reward signals and its mechanisms of action in the treatment of attention-deficit hyperactivity disorder.


Poster

281. Monoamine Transport and Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 281.05/B29

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

Title: High time resolution analyses in online microdialysis experiments for dopamine and serotonin

Authors: *H.-J. BROUWER¹, M. EYSBERG², L. VAN HEERWAARDEN¹, N. REINHOUD¹; ¹Antec Scientific, Zoeterwoude, Netherlands; ²Antec Scientific (USA), Boston, MA

Abstract: Microdialysis in vivo is an important sampling technique for continuous monitoring of neurotransmitter concentrations in the living brain. Extracellular fluid of the brain is sampled through a semipermeable membrane in a microdialysis probe, followed by HPLC analysis for quantification. However, HPLC analysis requires fractionation of the sample stream, and the size of the fractions will affect time resolution. Typical flow rates in microdialysis are 1 - 2 µL/min. In case of conventional HPLC, which require typical injection volumes of 10 µL - 20 µL, such samples represent a time resolution of 5 to 20 min. Such time resolution is not enough to accurately describe fast neural responses that take place within a few minutes. Therefore, decreasing the fraction size to a few microliters is necessary to enable measurements with a temporal resolution of 1 to 2 minutes. We developed a robust commercially available on-line solution for the simultaneous analysis of DA and 5-HT with high time resolution in microdialysis experiments. The solution is based on a UHPLC system equipped with a dual loop sampling valve in combination with the new DECADE Elite electrochemical detector and SenCell. Small samples with a volume of 1.5 µL were collected online into the dual loop sampling valve and
simultaneously analyzed (see figure 1). With this approach a temporal resolution of less than 2 minutes can be reached, with a detection limit of 100 pmol/L for both DA and 5-HT.


Poster

281. Monoamine Transport and Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 281.06/B30

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

Support: E.J. Van Liere Endowed Medicine Professorship

Title: Regulator of G protein signaling-12 modulates the expression and function of the serotonin transporter (SERT)

Authors: *A. N. WHITE\textsuperscript{1}, J. D. GROSS\textsuperscript{2}, S. W. KASKI\textsuperscript{3}, K. R. TREXLER\textsuperscript{4}, R. M. RODRIGUIZ\textsuperscript{5}, A. B. SCHROER\textsuperscript{5}, K. A. WIX\textsuperscript{2}, W. C. WETSEL\textsuperscript{8}, S. G. KINSEY\textsuperscript{6}, D. P. SIDEROVSKI\textsuperscript{2}, V. SETOLA\textsuperscript{7};
\textsuperscript{1}Dept. of Physiol. and Pharmacol. and Dept. of Neurosci., \textsuperscript{2}Dept. of Physiol. and Pharmacol., \textsuperscript{3}Dept. of Physiol. and Pharmacol. and Dept. of Behavioral Med. & Psychiatry, West Virginia Univ. Sch. of Med., Morgantown, WV; \textsuperscript{4}Dept. of Psychology, \textsuperscript{5}Dept. of Physiol. and Pharmacol., \textsuperscript{6}Dept. of Psychology and Dept. of Neurosci., \textsuperscript{7}Dept. of Neuroscience, Dept. of Behavioral Med. & Psychiatry, West Virginia Univ., Morgantown, WV; \textsuperscript{8}Dept Psychiat & Behav Sci., Duke Univ. Med. Ctr., Durham, NC
Abstract: Regulators of G protein signaling (RGS) proteins inhibit G protein signaling by acting as GTPase accelerating proteins for the G-alpha subunits of G protein heterotrimers. G protein coupled receptors (GPCRs) constitute a significant portion of the druggable proteome and mediate responsiveness to multiple neurotransmitters; thus, a deeper understanding of the mechanisms by which particular, CNS-resident RGS proteins regulate G protein signaling could lead to the development of novel neuropsychopharmacologic agents. Our labs have previously shown that mice lacking RGS12 exhibit reduced hyperlocomotion in response to the dopamine system-dependent psychostimulant amphetamine (Gross, Kaski, et al., 2018, J Psychopharm.). This prior finding led us to investigate whether RGS12 loss also attenuates behavioral responses to the serotonin system-dependent psychostimulant 3,4-methylene-dioxymethamphetamine (MDMA). Here, we report that these mice display a bimodal locomotor response to MDMA: hyperlocomotion to 10 mg/kg MDMA is markedly reduced in RGS12-null mice; however, RGS12-null mice exhibit augmented hyperlocomotion to a 30 mg/kg dose compared to wildtype littermate controls. To evaluate whether SERT expression and/or function of the serotonin transporter (SERT) is disrupted upon RGS12 loss, [3H]citalopram binding and [3H]serotonin (5-HT) uptake assays were performed. Increased numbers of [3H]citalopram binding sites are found in the cortex and midbrain of RGS12-null mice, as well as increased citalopram-sensitive [3H]5-HT uptake in the cortex, midbrain, and ventral striatum. In contrast, we observed no differences in [3H]citalopram binding nor [3H]5-HT uptake in the hippocampus or dorsal striatum, indicating that RGS12 loss engenders SERT dysfunction in a region-specific manner. These biochemical observations support the notion that basal serotonergic neurotransmission is disrupted upon RGS12 loss. To further elucidate this hypothesis, we are now testing RGS12-null mice and wildtype littermate controls in behavioral paradigms typically associated with/affected by SERT-acting drugs. Taken together, our data will demonstrate that RGS12 is a previously unidentified modulator of serotonergic neurotransmission.


Poster

281. Monoamine Transport and Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 281.07/B31

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

Support: NIH Grant DA035499
PhRMA Predoctoral Fellowship

Title: A mechanism of serotonin transporter regulation by cholesterol biosynthetic intermediates
Abstract: The serotonin transporter (SERT) is necessary for the regulation of serotonin in the brain through transport of extracellular serotonin (5HT) into neuron terminals. The activity of SERT is thought to be modulated in part by cholesterol and lipid rich microdomains within the plasma membrane where SERT localizes. These microdomains aid in SERT protein-protein interactions and post-translational modifications that modify SERT and modulate the amount and rate at which 5HT is removed from the synapse. However, experiments related to the mechanism of membrane cholesterol on SERT function in the brain has yielded conflicting results and no studies have examined the contribution of cholesterol biosynthetic intermediates in regulating SERT function. To investigate the role of cholesterol and its biosynthetic intermediates on neuronal SERT function, serotonergic RN46A-B14 cells were stably overexpressed with myc-tagged SERT. Simvastatin was used to block synthesis of cholesterol and cholesterol biosynthetic intermediates by blocking the rate limiting enzyme HMGCoA reductase. 5HT transport was assayed by measuring 5HT taken up by cells after incubation with exogenous 5HT. As expected, simvastatin treatment decreased total cell cholesterol. Unexpectedly, simvastatin increased 5HT uptake in a manner blocked by fluoxetine. Kinetic analyses showed a shift in the Km and Ki, but not the Vmax for 5HT. However, biotinylation studies demonstrated enhanced SERT and higher molecular weight SERT at the plasma membrane, suggesting the formation of higher order oligomeric SERT complexes induced by simvastatin. To demonstrate a role for cholesterol in simvastatin-mediated 5HT uptake, cholesterol was repleted during simvastatin treatment. Cholesterol repletion did not block simvastatin-enhanced 5HT uptake, but the addition of cholesterol biosynthetic intermediates farnesyl and geranylgeranyl pyrophosphate blocked the enhanced uptake. Lastly, our results are supported by in vivo studies showing enhanced 5HT uptake in the prefrontal cortex of rats administered 10 mg/kg/day simvastatin intraperitoneal for 7 days. These results indicate that simvastatin enhances 5HT uptake via SERT in a manner dependent on cholesterol biosynthetic intermediates, but independent of cholesterol per se.

Disclosures: C.M. Mitchell: None. B.K. Yamamoto: None.

Poster

281. Monoamine Transport and Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 281.08/B32

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

Support: R01 MH114017
R01 MH107390

Title: Humanizing and tagging the Drosophila serotonin transporter
**Authors:** *R. C. M. ARNOLD*, N. DALILI, M. M. SAMPSON, S. L. BONANNO, A. EAMANI, K. MYERS GSCHWENG, D. E. KRANTZ;
1Neurosci., 2Mol. Toxicology, 3UCLA, Los Angeles, CA; 4Hatos Ctr. for Neuropharm., Univ. of California, Los Angeles, Los Angeles, CA; 5Univ. California Los Angeles, Los Angeles, CA

**Abstract:** The neurotransmitter serotonin is important for modulating complex behaviors including appetite, mating, and sleep. The duration and intensity of serotonin signaling are mediated by the plasma membrane serotonin reuptake transporter (SerT). We used molecular techniques to 1) tag dSerT for subcellular localization mapping and 2) mutate dSerT to increase sensitivity to serotonergic drugs in the fruit fly *Drosophila melanogaster*. To map dSerT expression, we engineered a transgene containing dSerT cDNA with an N-terminal epitope tag hemagglutinin (HA). HA-SerT was expressed in vitro (insect cells) and in vivo (flies) to examine subcellular localization. SerT is found in both *Drosophila* and humans, however, dSerT is less sensitive to pharmacological inhibition. To bypass this, we have “humanized” dSerT by introducing a point mutation (M167I) into the *Drosophila* gene. The effect of this mutation on drug response was examined in both insect cell lines and in vivo. These tools allow us to pharmacologically control serotonin signaling and examine the molecular mechanisms of serotonergic neuromodulation.


**Poster**

281. Monoamine Transport and Signaling

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 281.09/B33

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

**Support:** NSF Grant 1822517
NIMH Grant MH117488
California NanoSystems Institute Challenge Grant

**Title:** *Ex-vivo* dynamics of serotonergic fibers

**Authors:** *M. T. HINGORANI*, A. M. VIVIANI, J. E. SANFILIPPO, S. N. SIU, J. C. CHIU, J. L. SELF, C. M. BATES, S. JANUSONIS;
1Psychological & Brain Sci., 2Materials, Univ. of California Santa Barbara, Santa Barbara, CA

**Abstract:** Experimental evidence and computer simulations strongly suggest that brain serotonergic axons (fibers) perform random walks in their terminal fields. Since these walks are likely to underlie the self-organization of regional serotonergic densities, they have important
implications for fundamental and applied neuroscience. Pathological changes in the dynamics of fibers may lead to alterations in serotonergic signaling in a number of mental disorders, including major Depressive Disorder and Autism Spectrum Disorder. Recently we have introduced several mathematical models that can reproduce the properties of serotonergic fiber trajectories in fixed brain tissue (Janusonis and Detering (2019) *Biochimie*, in press; Janusonis, Mays, Hingorani (2019) *ACS Chemical Neuroscience*, in press). However, the selection among these models is complicated by two problems: (i) little information is available about the spatial geometry of micro-obstacles serotonergic fibers have to navigate and (ii) no temporal information can be extracted from fixed preparations. This lack of experimental data significantly impacts theoretical analyses, especially those based on continuous-time processes (step-wise random walks are simpler but less realistic biologically). In the study, we co-cultured mouse serotonergic neurons and rat glia to study the dynamics of serotonergic axons. We used standard culture conditions and also investigated the possibility of growing axons in artificial, structured spatial environments, such as hydrogels. These experimental approaches can provide key data for stochastic modeling of individual serotonergic fibers (e.g., using Fractional Brownian Motion), as they evolve in time, and can support predictive approaches to the self-organization of the brain serotonergic system.


**Poster**

**281. Monoamine Transport and Signaling**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 281.10/B34

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

**Support:** NIH Grant 1F31NS108406-01

**Title:** Transcriptomically polarized subtypes of median raphe serotonergic neurons have divergent bouton neurochemical phenotypes targeting distinct brain regions

**Authors:** *R. A. SENFT, M. E. FRERET, S. M. DYMECKI;* Harvard Med. Sch., Boston, MA

**Abstract:** The median raphe (MR), a brainstem nucleus enriched in neurons producing serotonin (5-hydroxytryptamine, 5-HT), is important in a variety of emotional and cognitive processes and behaviors. Serotonin neurons of the MR, while populating a single anatomical nucleus, arise developmentally from three distinct embryonic progenitor cell compartments - rhombomeres (r) - each transcriptomically unique and each generating 5-HT neurons transcriptomically distinct
from one another. Moreover, the MR 5-HT neuronal lineage arising from r2 can be further subdivided transcriptomically, as revealed by our recent single-cell RNAseq findings [Okaty et al., 2015; Okaty and Dymecki, 2019]. One sub-sublineage is classically 5HTergic and referred to as Pet1-Tph2\textsuperscript{high}, while the other sub-sublineage appears more glutamatergic and is referred to as Pet1-Tph2\textsuperscript{low}. Unknown is how these mRNA profiles relate to the neurochemical phenotypes of their highly collateralized axons and boutons; equally lacking is an understanding of how neurotransmission may be differentially regulated across their numerous target regions. We performed immunohistochemistry and \textit{in situ} hybridization in brain sections from mice expressing synaptophysin-GFP to mark boutons from r2-derived 5-HT neurons (referred to as r2\textit{Hoxa2-Pet1} cells). We found region-specific neurochemical bouton distributions consistent with three types of innervation: primarily 5-HT-only, Vglut3-only, or a mixture of Vglut3-only and 5-HT/Vglut3 dual positive boutons, the latter suggestive of co-transmission. Dual positive boutons in the septum preferentially associated with pericellular baskets, a structure which coordinates dense axonal targeting to the cell soma, suggesting these boutons may be strategically located for temporally precise and highly effective control of target cell activity. Finally, we queried the cell identity of basket targets, finding different interneuron populations depending on bouton type and target region. These results suggest a model wherein 5-HTergic r2\textit{Hoxa2-Pet1} cells innervate thalamic and hypothalamic regions and the olfactory bulb while putative glutamatergic cells innervate hippocampus, cortex, and the septum, with each subtype poised to modulate different populations of interneurons. Our results demonstrate the neurochemical heterogeneity and segregation of axonal boutons within a developmentally-defined 5-HT neuron subtype. This work enhances our understanding of Pet1 neuron neurochemical diversity and circuit interactions in the adult forebrain.

**Disclosures:** R.A. Senft: None. M.E. Freret: None. S.M. Dymecki: None.

**Poster**

281. Monoamine Transport and Signaling

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 281.11/B35

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

**Support:** NARSAD Young Investigator  
NIDA Grant R01DA034022

**Title:** Molecular diversity of 5-HT neurons of the mouse dorsal raphe nucleus

**Authors:** *B. W. OKATY, N. STURROCK, O. V. ALEKSEYENKO, K. A. LYON, S. M. DYMECKI;*  
Harvard Med. Sch., Boston, MA
Abstract: Serotonin (5-HT) is a monamine neurotransmitter released throughout the nervous systems of all organisms, where it regulates numerous biological processes crucial to health and survival. In vertebrates, brainstem neurons that synthesize and release 5-HT are often viewed as a single neuron type based on neurotransmitter identity, however 5-HT neurons display wide-ranging phenotypic diversity, including heterogeneous anatomy, morphology, hodology, electrophysiology, neurochemistry, and gene expression. These diverse molecular and cellular properties are likely critical determinants of the higher-order physiological, behavioral, cognitive, and affective functions supported by the 5-HT neuronal system as a whole. Recent findings by our group and others promote the hypothesis that phenotypically distinct 5-HT neuron subtypes modulate distinct functions. While 5-HT neuronal diversity is increasingly recognized by the field, a consensus framework for rigorously defining 5-HT neuron subtypes, linking these subtypes to specific functions, and understanding how micro-, meso-, and macro-scale properties of the 5-HT neuronal system interact is presently lacking. Here we present the results of our latest efforts towards these ends, focused specifically on mouse 5-HT neurons with cell somata located in the dorsal raphe nucleus (DR). We performed single cell RNA-seq of intersectionally labeled and sorted 5-HT neuron subgroups from microdissected subdomains of the DR followed by unbiased clustering and differential expression analyses to identify 5-HT neuron molecular subtypes. Specifically, we generated triple transgenic mice by crossing Pet1-Flpe mice (a pan 5-HT driver) with a number of Cre driver mice, including Npy2r-Cre, Crh-Cre, Slc17a8-Cre, Sert-Cre, and P2ry1-Cre, in combination with dual recombinase responsive fluorescent reporter lines. We found a relationship between molecularly and anatomically defined subtypes, with significant gene expression differences between 5-HT neurons located in dorsolateral, dorsomedial, ventromedial, or caudal subcompartments. We also found diverse molecular subtypes intermingled within a given anatomical subdomain, arguing against a simple anatomical organization of 5-HT neuronal diversity. We are currently working towards functional and hodological characterization of these molecularly and anatomically defined subtypes by intersectionally combining different marker and effector mouse lines and performing behavioral and physiological assays.

Disclosures: B.W. Okaty: None. N. Sturrock: None. O.V. Alekseyenko: None. K.A. Lyon: None. S.M. Dymecki: None.

Poster

281. Monoamine Transport and Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 281.12/B36

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

Support: VA Grant BX002085
VA Grant IO1 BX001804
Title: Serotonin and histamine modulation in inflammation: Implications in depression

Authors: *M. HERSEY¹, S. N. BERGER⁴, J. L. WOODRUFF², P. HASHEMI⁴, L. P. REAGAN³;
²Pharmacology, Physiol. and Neurosci., ³Pharmacology, Physiol. & Neurosci., ¹Univ. of South Carolina Sch. of Med., Columbia, SC; ⁴Univ. of South Carolina, Columbia, SC

Abstract: Neuroinflammation is associated with changes to the central nervous system (CNS) and plays a role in the pathology of depression and many other psychiatric diseases. By targeting the neurotransmitter systems of serotonin and histamine we aim to understand the neurochemical underpinnings of depression. Serotonin has long been hypothesized to play a role in depression as serotonin signaling is often impaired during depression. Histamine has a well-established role in peripheral inflammation and novel data showing that histamine release inhibits serotonin signaling justifies the need to consider the two neurotransmitters in tandem. In this work, fast-scan cyclic voltammetry (FSCV) is used to simultaneously measure the release and reuptake of histamine and serotonin, in the posterior hypothalamus and fast-scan controlled adsorption voltammetry (FSCAV) is used to measure ambient serotonin in the hippocampus of rodents following acute (peripheral injection of lipopolysaccharide) or chronic (high fat diet (45 kcal % fat) and chronic mild stress) neuroinflammation. Biochemical (for inflammation) and behavioral (for depression) analysis are correlated with neurochemical measurements. Our preliminary results allow us to hypothesize that inflammation corresponds to increased histamine release, thus increasing the inhibition of serotonin. We also find that the capacity of selective serotonin reuptake inhibitors (SSRIs), like escitalopram, to increase extracellular serotonin by inhibiting serotonin transporters (SERTs) is reduced in both inflammation models. However, a targeted decrease in histamine synthesis was able to increase SSRI efficacy. These results suggest that histamine plays a fundamental role in modulating serotonin during inflammation, thereby providing a novel therapeutic target as well as providing insights into the neurochemical basis for depressive illness.

Title: Inhibition of presynaptic monoamine transporters by the HIV-1 viral protein Tat in the prefrontal cortex: Evidence from a transgenic mouse model

Authors: *M. J. STRAUSS*¹, B. O'DONOVAN², P. I. ORTINSKI³, J. P. MCLAUGHLIN⁴, J. ZHU⁵;
¹Univ. of South Carolina, Columbia, SD; ²Pharmacology, Physiol. and Neurosci.,
³Pharmacology, Physiology, and Neurosci., Univ. of South Carolina, Columbia, SC; ⁴Univ. of Florida, Gainesville, FL; ⁵Drug Discovery and Biomed. Sci., Col. of Pharmacy, Univ. of South Carolina, Columbia, SC

Abstract: Abnormal dopaminergic transmission has been implicated as a mediating factor of HIV-1 associated neurocognitive disorders (HAND). Our laboratory has demonstrated that in vitro HIV-1 Tat protein reduces dopamine transporter (DAT) function via a direct allosteric interaction. This study determined whether the inhibitory effects of Tat on DAT function could be replicated in an inducible Tat transgenic (iTat) mouse model. Additionally, as the norepinephrine transporter (NET) is also responsible for the reuptake of dopamine in the prefrontal cortex (PFC), an area which has been linked to cognitive functioning, and shares a high level of homology with the DAT, effects of Tat on this transporter were also investigated. Following 7-day administration of doxycycline (Dox) or saline, the maximal velocity ($V_{max}$) of $[^3]H$dopamine uptake via the DAT and the NET in PFC of Dox-treated iTat mice was decreased by 27% and 34%, respectively, compared to their saline controls. 14-day Dox treatment demonstrated similar inhibition, as the $V_{max}$ of $[^3]H$dopamine uptake via DAT and NET in the PFC was reduced by 34% and 22%, respectively. No differences in the $V_{max}$ were found between Dox and saline treated control C57BL/6J mice. The reduction in DAT and NET function in the PFC was accompanied by a similar decrease in available substrate binding sites, with 7-day Dox treated mice displaying a 28% decrease in $[^3]H$WIN 35,428 binding to the DAT and a 36% decrease in $[^3]H$Nisoxetine binding to the NET. These decreases in function and substrate binding were not due to changes in total protein expression. Furthermore, whole-cell patch clamp recordings in layer V pyramidal neurons of the prelimbic cortex, which primarily project to subcortical dopaminergic structures such as the striatum and VTA, revealed a decreased action potential firing frequency from these neurons. Our findings demonstrate the extent to which
HIV-1 Tat may disrupt the presynaptic monoamine transport system and induce dysregulation of dopamine homeostasis, which ultimately may underlie the neurocognitive impairment evident in HAND patients.


Poster

281. Monoamine Transport and Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:/Poster #: 281.14/B38

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

Support: NIH 1 R15 NS48916-01
Rush Elliott Award

Title: Examining monoamine oxidase inhibitor targets using Caenorhabditis elegans

Authors: M. J. VINCE¹, *J. S. DUERR²;
¹Dept. of Biol. Sci., ²Ohio Univ., Athens, OH

Abstract: Monoamines (MA) are neurotransmitters and hormones in humans and Caenorhabditis elegans that are responsible for ionotropic and metabotropic signaling. MAs in humans include serotonin, dopamine, epinephrine and norepinephrine; C. elegans use serotonin, dopamine, octopamine, and tyramine. Changes in MAs are important in several psychological neurological disorders such as depression, Parkinson’s disease, anxiety, and schizophrenia. Monoamine oxidases (MAO) are enzymes that degrade MA in presynaptic cells. Monoamine oxidase inhibitors (MAOIs) inhibit MAOs and generally increase MA signaling.

In our research, we are using the model organism C. elegans to examine the genetic targets of two prescribed MAOIs: tranylcypromine (prescribed for depression and anxiety) and phenelzine (prescribed for Parkinson’s disease). In C. elegans, monoamines alter egg laying, metabolism, habituation, feeding, and movement. Mutations in the single identified MAO (amx-2) decrease, but do not eliminate responses to these MAOIs. Since these MAOIs are structurally similar to MAs, other possible targets for the drugs are MA receptors. We have examined the responses to these drugs in mutants lacking MA synthesis and/or receptors. Our current work focuses on four genes. The gene cat-2 encodes tyrosine hydroxylase, an enzyme required for synthesis of dopamine. tdc-1 encodes tyrosine decarboxylase, an enzyme essential for synthesis of octopamine and tyramine. We have observed that cat-2 and tdc-1 mutants are behaviorally hypersensitive to both tranylcypromine and phenelzine. This may be due to the remaining MAs. On the other hand, mutants in dop-3, which encodes a dopamine type DRD2 receptor, are hypersensitive to tranylcypromine, but not phenelzine while mutants in lgc-
which encodes a tyramine gated chloride channel receptor, are hypersensitive to phenelzine, but not tranylcypromine. To test whether these receptors are likely to be direct targets of MAOIs, we have constructed mutants that knockout both monoamine synthesis and monoamine receptors and are examining their behavioral sensitivity to specific MAOIs.

Disclosures: M.J. Vince: None. J.S. Duerr: None.

Poster

281. Monoamine Transport and Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 281.15/B39

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

Support: Finska Läkaresällskapet
Jane and Aatos Erkko Foundation
Sigrid Juselius Foundation

Title: Behavioral and molecular analysis of a monoamine oxidase zebrafish mutant

Authors: *D. BARONIO, Y. C. CHEN, P. A. PANULA;
Univ. of Helsinki, Helsinki, Finland

Abstract: The monoamine oxidases (MAO) degrade biogenic and dietary monoamines and are key regulators of the monoaminergic systems. Disrupted functioning of these regulators plays a role in the pathophysiology of some brain disorders, such as schizophrenia and autism spectrum disorder (ASD). Zebrafish has become a valuable tool in neuroscience because it shares key genes with humans and possesses all main neurotransmitter systems. Differently than mammals, zebrafish possesses only one mao gene which shares some properties of both mammalian MAO genes. Surprisingly, mao zebrafish mutants remain uncharacterized and their relevance to study the pathophysiology of major disorders is uncertain. Thus, we characterized behavioral and molecular phenotype of mao zebrafish mutants. Neurotransmitter systems including catecholaminergic, serotonergic and histaminergic ones were analyzed by immunohistochemistry. qPCR was utilized to study genes relevant for ASD and neuropsychiatric disorders. Locomotor activity, sociability and anxiety-like behavior were evaluated. Surprisingly, mao knockout (KO) fish died within 2 weeks post-fertilization. At 10 days post-fertilization (dpf) they showed a hypoactive phenotype during a 24 hours locomotor activity evaluation, whereas heterozygous (HET) animals behaved similarly with the wild-type (WT) fish. KO animals presented strong intra- and extracellular brain serotonin immunoreactivity, which probably had a toxic effect towards other adjacent cell populations. This was indicated by the reduced number of histamine and tyrosine hydroxylase 1 (TH1)-positive cells. We also found strong GFAP immunoreactivity in the brains of KO animals that was confirmed by qPCR. The qPCR also
showed reduced levels of Mecp2 mRNA in HET fish at 10 dpf. Mecp2 expression is attenuated in different models of ASD. In a social preference test adult mao HET fish navigated more distantly from the social cue and showed a hyperactive phenotype during part of the trial. They also showed an anxious-like behavior in the novel tank test. These results indicate that the phenotype of mao zebrafish mutants resembles some of the molecular and behavioral features of brain disorders that have MAO deficiency as a feature. An in-depth analysis of this model is necessary to reveal in detail mechanisms behind the premature death of KO animals and the impaired behavior displayed by mao HET fish.

Disclosures:  D. Baronio: None. Y.C. Chen: None. P.A. Panula: None.

Poster

281. Monoamine Transport and Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 281.16/B40

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

Support: Independent Research Fund Denmark – Medical Sciences

Title: New insights into amphetamine-induced efflux by employment of genetically encoded biosensors in dopaminergic neurons

Authors: *J. F. STØIER, S. H. JØRGENSEN, M. A. C. GREGOREK, A. T. SØRENSEN, F. HERBORG, U. GETHER;
Dept. of Neurosci., Univ. of Copenhagen, Copenhagen N, Denmark

Abstract: Amphetamines are widely abused as well as commonly prescribed for treating children with ADHD. The compounds are substrates for the dopamine transporter (DAT) and once inside the cell, amphetamines can indirectly reverse the transport of DAT through activation of intracellular signaling pathways. However, the current insights into how amphetamines activate such intracellular signaling in dopaminergic neurons is baffling with a majority of studies being performed in heterologous cells. Here, we employ genetically encoded sensors expressed in cultured midbrain neurons to achieve a better understanding of how amphetamines induce reversal of DAT function. To ensure selective expression in dopaminergic neurons, we used an adeno-associated virus to mediate tyrosine hydroxylase promotor driven Cre expression. This enabled the expression of different genetically encoded biosensors in the dopaminergic neurons using Cre-dependent constructs of the sensors. By expressing the recently developed green dopamine sensor dLight1.1 in the dopaminergic neurons, it was possible to directly visualize amphetamine-induced efflux. Addition of amphetamine to the culture caused a detectable increase in dLight1.1 fluorescence that was strongly amplified by preloading of the culture with dopamine. To assess in parallel putative changes in intracellular calcium levels in
response to amphetamine, the red calcium sensor JrGECO1a was co-expressed with dLight1.1. Amphetamine has previously been reported to elicit a calcium response and activate Ca\(^{2+}\)/calmodulin-dependent protein kinase II. However, we did not find any evidence for such an increase in intracellular calcium upon addition of amphetamine to the culture. In contrast, we observed large increases in intracellular calcium levels when stimulating NMDA-type ionotropic glutamate receptors and by activating G\(_q\)-coupled muscarinic receptors in the dopaminergic neurons. In conclusion, we have established an experimental set-up ideally suited for studying the cellular basis for amphetamine-induced efflux. Our current results strongly challenge the importance of intracellular calcium for amphetamine-induced efflux, underlining that further studies are required to unravel the complex actions of amphetamine in dopaminergic neurons.


**Poster**

281. Monoamine Transport and Signaling

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 281.17/B41

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

**Support:** The Lundbeck Foundation R303-2018-3540  
The Lundbeck Foundation R181-2014-3090  
Danish Research Council: 4183-00571A

**Title:** Rare genetic variants in monoamine transportes as a risk factor for neuropsychiatric disease? - Insights from a population based case-cohort sample

**Authors:** *F. HERBORG\(^1\), V. APPADURAI\(^2\), A. B. DEMUR\(^2\), T. WERGE\(^2\), U. GETHER\(^3\);  
\(^1\)Dept. of Neurosci., Univ. of Copenhagen, Copenhagen, Denmark; \(^2\)The Lundbeck Fndn. Initiative for Integrative Psychiatric Res., Copenhagen Univ. Hosp., Copenhagen, Denmark; \(^3\)Fac. of Hlth. and Med. Sciences, Univ. Copen, Copenhagen N, Denmark

**Abstract:** The monoaminergic circuits of the brain have long been implicated in the aetiology and symptomatology of most common neuropsychiatric diseases. The monoamine transporters (MAT) are key regulators of monoaminergic neurotransmission and serve as principal targets for neuropsychiatric therapeutics. Nevertheless, it is unclear if MAT dysfunction can directly influence the risk of developing neuropsychiatric diseases. Using exome sequencing data from a cohort of ~20,000 controls and cases diagnosed with attention deficit hyperactivity disorder, (ADHD), autism spectrum disorder (ASD), schizophrenia, single or recurrent depression, or bipolar disorder, we investigated if rare genetic variants of monoamine transporters may contribute to the genetic architecture of neuropsychiatric diseases at a populational level. By
comparing the occurrence and allelic diversity of rare variants in genes encoding monoamine transporters among cases and controls we found new evidence supporting the monoamine hypothesis of neuropsychiatric disease by directly linking inherited changes to MAT function to an increased the risk of neuropsychiatric disease.

**Disclosures:** F. Herborg: None. V. Appadurai: None. A.B. Demur: None. T. Werge: None. U. Gether: None.

**Poster**

281. Monoamine Transport and Signaling

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 281.18/B42

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

**Title:** Nanoscale distribution of the dopamine transporter assessed by super-resolution microscopy: Regulation by membrane potential

**Authors:** *M. D. LYCAS¹, J. F. STØIER¹, S. H. JØRGENSEN¹, A. T. SØRENSEN¹, M. SAUER², F. H. HANSEN¹, U. GETHER¹;
¹Univ. of Copenhagen, Copenhagen, Denmark; ²Univ. of Würzburg, Würzburg, Germany

**Abstract:** The dopamine transporter (DAT), a membrane protein present on dopaminergic neurons and responsible for the uptake of dopamine from the extracellular space, resides in discrete nanodomains on the plasma membrane of neuronal projections. Through direct stochastic optical reconstruction microscopy (dSTORM) on primary dopaminergic cultures and mouse brain slices, changes in the clustered, nanoscale distribution of DAT were observed upon manipulations affecting the neuronal membrane potential. The dynamic character of the clustered architecture was supported by dispersing of clusters following both NMDA receptor activation and nicotinic acetylcholine receptor activation. However, nanoclusters were not affected by the presence of the DAT inhibitor cocaine, nor was the NMDA induced declustering influenced by the inhibition of nitric oxide production. The declustering of DAT following NMDA receptor activation was muted through the sequestering of calcium by BAPTA-AM, and it was blocked by inhibition of voltage gated calcium channels, indicating the change in clustering is dependent on calcium influx. Interestingly, the changes in clustering architecture were also induced following viral transduction of ion channels into the dopaminergic neurons that caused the neuronal membrane to either hyperpolarize or depolarize. The data suggest that the dynamic changes in DAT clustering is a generalized phenomenon and is a response to membrane potential or neuronal activity in dopaminergic neurons. We also assessed the context for dopaminergic clusters by dual color dSTORM imaging of DAT with other presynaptic proteins, revealing concomitant declustering of some but increased clustering of other proteins. In order to observe if this phenomena takes place also in the developed brain, live mouse brain slices were treated
with NMDA, and the changes in DAT clustering were observed following dSTORM imaging of DAT in the striatum. In summary, DAT is organized into nanoscale clusters that are dynamic, and these clusters change in response to stimuli that influence membrane potential, indicating that the clustered distribution of DAT serves a functional role in dopaminergic neurotransmission.


**Poster**

281. Monoamine Transport and Signaling

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 281.19/B43

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

**Support:** 2 T32 EY 7001-41 A1

**Title:** Calcium spike-dependent modulation of locus coeruleus excitability

**Authors:** *A. M. MCKINNEY, X. JIANG; Neurosci., Baylor Col. of Med., Houston, TX

**Abstract:** Noradrenaline-producing neurons in the locus coeruleus (LC), the largest noradrenergic nucleus in the central nervous system (CNS), project to all major CNS structures and play a crucial role in an array of behaviors and cognitive processes crucial to survival, including attention, arousal, and the stress response. The excitability of these densely-packed, large neurons is believed to be negatively controlled by noradrenaline release via α2 adrenoceptors (α2-mediated inhibition). However, using simultaneous recording of up to 12 LC neurons in pontine brain slices to examine the interactions within a group of recorded LC neurons, we found no evidence of lateral inhibition between LC neurons or self-inhibition via α2 adrenoceptors in neither juvenile (&lt;P25) nor adult (&gt;P60) mice, suggesting that this previously reported α2-mediated inhibition may only be active when a large population of LC neurons are activated. At the single-cell level, the main mechanism regulating LC excitability, instead, is a novel prolonged hyperpolarization that follows brief depolarization or phasic firing, which is mostly absent in neurons of the neocortex and hippocampus. This LC-specific self-inhibition persists in the presence of α2 adrenoceptor and galanin receptor antagonists, as well as sodium channel blockers which uncovered fast, prominent calcium spikes mediated by voltage-gated calcium channels (Cav). Further experiments with Cav channel blockers showed that it was not depolarization itself, but calcium influx from these spikes that drove this strong hyperpolarization of LC neurons. The hyperpolarization reversed at the potassium reversal potential and could be blocked or significantly reduced by KCa-specific blockers, suggesting that
the increase in intracellular calcium activated calcium-activated potassium channels ($K_{Ca}$) to hyperpolarize the neuron. To identify the $CaV$ and $K_{Ca}$ channels that mediate the novel hyperpolarization in LC, we are performing single-cell RNA-sequencing (scRNA-seq) to derive the single-cell transcriptome of LC neurons. Analysis is underway to determine which $CaV$ and $K_{Ca}$ channels are specifically expressed in the LC to mediate the novel hyperpolarization in LC neurons and how molecular variability underlies the organization of the locus coeruleus.

**Disclosures:** A.M. McKinney: None. X. Jiang: None.

**Poster**

282. Metabotropic Glutamate and GABAB Receptors

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 282.01/B44

**Topic:** B.03. G-Protein Coupled Receptors

**Title:** Modulation of beta and gamma frequency oscillations in the rat anterior cingulate cortex (ACC) *in vitro* by group II metabotropic glutamate (mGlu) receptors (mGlu2/3)

**Authors:** B. H. DENNIS$^1$, *S. A. NEALE$^{2,1}$, F. E. LEBEAU$^1$, T. E. SALT$^{2,1,3}$; $^1$Inst. of Neurosci., Newcastle Univ., Newcastle upon Tyne, United Kingdom; $^2$Neurexpert Limited, Newcastle upon Tyne, United Kingdom; $^3$Inst. of Ophthalmology, Univ. Col. London, London, United Kingdom

**Abstract:** The anterior cingulate cortex (ACC) plays a role in remote spatial memory, attention and executive functions. These cognitive functions are associated with network oscillations in the beta (20-30 Hz) and gamma (30-80 Hz) range. We have recently demonstrated that both beta and/or gamma oscillations can be recorded *in vitro* either alone, or in combination, in the deep and superficial layers of ACC (Adams et al 2017 DOI: https://doi.org/10.1523/ENEURO.0313-16.2017). Changes in cortical beta and gamma frequency activity occur in patients in a range of neuropsychiatric and neurodegenerative diseases, and Group II mGlu receptors have been suggested as targets for diseases of both types. Therefore it is important to determine how these receptors participate in cortical network oscillations.

Following transcardial sucrose perfusion in anaesthetised rats ACC slices 450 µM thick were prepared. Slices were transferred to an interface chamber for recording. Network oscillations were evoked using bath application of kainate (800 nM) for 1-3 hours until oscillation magnitude stabilised. Following application of kainate, beta activity (assessed over the 20-32.99 Hz frequency band) and gamma activity (assessed over the 33-80 Hz frequency band) were observed in ACC slices. Both beta and gamma activity were attenuated in the presence of the Group II mGlu receptor agonist LY354740. Following 60 mins application, LY354740 (3 µM) reduced the area under the curve (AUC) of gamma activity to 22.37 ± 6.59% (n = 4; p = 0.0013) of baseline levels and beta
was reduced to 49.25 ± 4.40% (n = 6; p < 0.0001). LY354740 (100 nM) reduced gamma to 61.3 ± 7.746% (n = 5; p= 0.0154) of baseline levels and AUC of beta activity was reduced to 94.77 ± 7.523% (n = 5). The effects of the LY354740 were reversed by bath application of the antagonist LY341495 (300 nM).

Gamma oscillations have been proposed to play a role in cortical local processing, while beta frequency activity is implicated in long range communication. Gamma activity showed a somewhat greater sensitivity compared to beta activity in response to mGlu2/3 activation. This could suggest differential roles for these receptors in modulating cortical function, and this may reflect localisation at certain synapses. Furthermore these results underline the importance of Group II receptors as potential targets for the treatment of neuropsychiatric and neurodegenerative diseases.

**Disclosures:** B.H. Dennis: None. S.A. Neale: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurexpert Limited. F.E. LeBeau: None. T.E. Salt: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurexpert Limited.

**Poster**

**282. Metabotropic Glutamate and GABAB Receptors**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 282.02/B45

**Topic:** B.03. G-Protein Coupled Receptors

**Support:** NIAAA Division of Intramural Clinical and Biological Research
NIAAA K99 AA025403

**Title:** Operant self stimulation of thalamic terminals in the dorsomedial striatum is constrained by metabotropic glutamate receptor 2

**Authors:** *K. A. JOHNSON*¹, L. VOYVODIC², Y. MATEO³, D. M. LOVINGER⁴;
¹Uniformed Services Univ., Bethesda, MD; ²Natl. Inst. on Alcohol Abuse and Alcoholism, Rockville, MD; ³Lab. for Integrative Neurosci., NIAAA, Bethesda, MD; ⁴Chief, Lab. Integrative Neurosci, Natl. Inst. on Alcohol Abuse and Alcoholism Rockville Office, Rockville, MD

**Abstract:** The striatum plays a central role in the learning and performance of motivated behaviors. Cortical and thalamic glutamatergic inputs to the striatum are critical determinants of striatal neuron activity. In addition, both corticostral and thalamostriatal activation have been shown to elicit dopamine release, and thalamostriatal stimulation has recently been shown to support reinforcement of operant behavior. However, surprisingly little is known about how thalamostriatal transmission is regulated. Presynaptic G protein-coupled receptors such as group
II metabotropic glutamate (mGlu$_{2/3}$) receptors can robustly modulate the strength of transmission at many synapses. We previously demonstrated that activation of mGlu$_2$ dramatically decreases the strength of thalamically-driven glutamate and dopamine release in the dorsal striatum. Because both glutamate and dopamine could play roles in the reinforcing properties of thalamo-striatal stimulation, we predicted that mGlu$_2$ manipulation would modulate this form of reinforcement. To test this, we established a thalamo-striatal optical self-stimulation paradigm. We expressed channelrhodopsin-2 (ChR2) in striatum-projecting thalamic neurons in the intralaminar nuclei of the thalamus and bilaterally implanted adult male and female mice with optical fibers in the dorsomedial striatum. We trained the mice to press a lever for a brief (1 second, 5, 10, or 20 Hz) train of optical stimulation on an FR1 reinforcement schedule. Mice rapidly acquired lever-pressing behavior, and responding was readily extinguished. We then evaluated the effects of pharmacological manipulation of mGlu$_{2/3}$ receptors. Consistent with its ability to dampen thalamically-driven glutamate and dopamine release, the mGlu$_{2/3}$ agonist LY379268 (3 mg/kg, i.p.) robustly reduced the rate of pressing for thalamo-striatal ICSS, and this effect was mimicked by the mGlu$_2$-selective positive allosteric modulator BINA (20 mg/kg, i.p.). Conversely, the mGlu$_{2/3}$-preferring antagonist LY341495 (3 mg/kg, i.p.) increased response rates. In contrast to the effects on thalamo-striatal ICSS, LY379268 caused a very modest decrease in responding for a food reinforcer, whereas LY341495 robustly decreased rates of responding for food. Our findings suggest that group II mGlu receptors differentially influence operant behavior depending on the type of reinforcement.

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**Poster**

282. Metabotropic Glutamate and GABAB Receptors

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 282.03/B46

**Topic:** B.03. G-Protein Coupled Receptors

**Support:** NRG-USF

**Title:** Identification of glutamatergic bidirectional synapses in the Portuguese man-of-war (*Physalia physalis*, hydrozoa)

**Authors:** *C. BOUCHARD*¹, B. BOSERUP³, N. BILL¹, R. H. JIANG²;
¹Col. of Sci. & Mathematics, Univ. of South Florida, Sarasota, FL; ²Global and Planetary Health, Col. of Publ. Health, USF Genomics Program, Univ. of South Florida, Tampa, FL; ³Dr. Kiran C. Patel Col. of Allopathic Med., Nova Southeastern Univ., Fort Lauderdale, FL
Abstract: The Portuguese man-of-war is a floating colony made up of polymorphic polyps. The animal belongs to the phylum Cnidaria, which includes animals such as hydra, sea anemone, coral and jellyfish. Modern day cnidarians are descendants of ancient metazoan lineages that were among the first animals to possess a nervous system. Understanding the function of cnidarian neurons that make up those nervous systems involves the identification of their transmitters as well as characterizing integral membrane proteins that bind to those neurotransmitters. One important taxonomic characteristic of cnidarians is the presence of stinging cells (cnidocytes). That a jellyfish must 'hear' a prey swim by its tentacles in order to catch it involves a sensory ability also present in humans and other vertebrate species who use hearing to communicate. When the cnidocyte detects a stimulus, the transduced vibrational stimulus is sent to pathways that control the release of a filament encapsulated within the stinging cell. This filament works like a harpoon to snare or perforate the prey’s teguments. Because the discharge of the stinging cell is a one-time event, it is important that the discharge of the stinging cell occur when the chances of catching prey are high. Prior studies have identified glutamate as a player in the modulation of the cnidocyte discharge. However, the identity of neurotransmitters in cnidarians remains enigmatic. The goal of our study was to identify glutamatergic synapses associated with the cnidocytes. A transcript coding for a metabotropic glutamate receptor was isolated and a specific antibody was generated. Ultrastructural studies were not able to demonstrate the presence of synapses directly associated with cnidocytes, however bidirectional synapses in proximity to cnidocytes were shown to express the glutamate receptor.


Poster

282. Metabotropic Glutamate and GABAB Receptors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 282.04/B47

Topic: B.03. G-Protein Coupled Receptors

Support: NRF-2016R1D1A1B03930951
NRF-2017M3C7A1029611
NRF-2018R1A2B604759
Korea Health Industry Development Institute (KHIDI) grant (HI18C0789)
SNUH Research Fund (0320150260)
Cooperative Research Program from SNUCM (800-20180195)
Brain Korea 21 PLUS program

Title: Nedd4 E3 ligase and beta-arrestins regulate ubiquitination, trafficking, and stability of the mGlu7 receptor
**Authors:** *S. LEE, S. PARK, H. LEE, S. HAN, J.-M. SONG, Y. SUH; Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of

**Abstract:** The metabotropic glutamate receptor 7 (mGlu7) is a class C G protein-coupled receptor (GPCR) that modulates excitatory neurotransmitter release at the presynaptic active zone. Although post-translational modification of cellular proteins with ubiquitin is a key molecular mechanism governing protein degradation and function, mGlu7 ubiquitination and its functional consequences have not been elucidated yet. Here, we report that Nedd4 ubiquitin E3 ligase and β-arrestins regulate ubiquitination of mGlu7 in heterologous cells and neurons. Upon agonist-stimulation, β-arrestins recruit Nedd4 to mGlu7 and facilitate Nedd4-mediated ubiquitination of mGlu7. Nedd4 and β-arrestins regulate constitutive and agonist-induced endocytosis of mGlu7 and are required for mGlu7-dependent MAPK signaling in neurons. In addition, Nedd4-mediated ubiquitination results in the degradation of mGlu7 by both the lysosomal and proteasomal degradation pathways. These findings provide a model in which Nedd4 and β-arrestin act together as a complex to regulate mGlu7 surface expression and function at the presynaptic terminals.

**Disclosures:** S. Lee: None. S. Park: None. H. Lee: None. S. Han: None. J. Song: None. Y. Suh: None.

**Poster**

282. Metabotropic Glutamate and GABAB Receptors

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 282.05/B48

**Topic:** B.03. G-Protein Coupled Receptors

**Support:** NRF Grant 2017M3C7A1029611
NRF Grant 2018R1A2B6004759
KHIDI grant HI18C0789
Brain Korea 21 PLUS program

**Title:** Neddylation of metabotropic glutamate receptor 7

**Authors:** *M. KANG, Y. SUH;

**Abstract:** Metabotropic glutamate receptor 7 (mGlu7) is a G protein-coupled receptor (GPCR) that modulates synaptic transmission and synaptic plasticity by inhibiting glutamate release in the presynaptic terminals. It has been considered that posttranslational modification (PTM) is a major regulator of many GPCRs by triggering receptor internalization, recycling, or degradation. Previous studies have showed that SUMOylation and ubiquitination regulate mGlu7 trafficking
and stability in heterologous cells and neurons. However, Neddylation of mGlu7 in the C-terminal tail remains unknown. Neddylation is a PTM that covalently conjugates the ubiquitin-like protein Nedd8 (neural precursor cell expressed developmentally downregulated protein 8) to specific lysine residues on targeted proteins. This modification plays important roles in regulating several biological processes such as cell cycle progression, signaling cascades, and apoptosis. In addition, neddylation is shown to control axonal outgrowth and spine maturation in the mammalian CNS. Like ubiquitination, Nedd8 conjugation requires a series of enzymatic reactions catalysed by one Nedd8-activating enzyme complex (NAE1/UBA3 heterodimer), two E2 Nedd8-conjugating enzymes (UBE2F and UBE2M), and several E3 ligases. In this study, using cell biology and confocal imaging technology, we show that mGlu7 is a target of neddylation in HEK293T cells and cultured neurons. In addition, we identify neddylated lysine residues among the eight lysine residues at the C-terminal tail of mGlu7. We also explore the possibility that neddylation involves mGlu7 trafficking and agonist-induced signaling of mGlu7. These data will provide new insights into the function of mGlu7 in neurons.

Disclosures: M. Kang: None. Y. Suh: None.

Poster

282. Metabotropic Glutamate and GABAB Receptors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 282.06/B49

Topic: B.03. G-Protein Coupled Receptors

Support: NRF-2018R1A2B6004759
NRF-2017M3C7A1029611
KHID1 grant HI18C0789
Brain Korea 21 PLUS program

Title: Characterization of N-linked glycosylation of metabotropic glutamate receptor 7

Authors: *D.-H. PARK¹, Y. SUH²;
¹Dept. of Biomed. Sci., ²Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Metabotropic glutamate receptor 7 (mGlu7) regulates neurotransmitter release at the presynaptic active zone in the mammalian brain. Regulation of mGlu7 trafficking in and out of the plasma membrane by binding proteins within the C-terminal tail of mGlu7 governs bidirectional synaptic plasticity. However, the functional importance of the extracellular domain (ECD) of mGlu7 has not been characterized. N-linked glycosylation is an abundant post-translational modification that plays crucial roles in proper protein folding in the endoplasmic reticulum (ER) and in forward trafficking to the plasma membrane. In addition to controlling trafficking, N-linked glycans can deliver intracellular signaling or modulate cell-cell
communications complexed with glycan-binding proteins. In this study, we report that mGlu7 is N-glycosylated at the asparagine (Asn) residues in the ECD in heterologous cells and in neurons. We found that deglycosylated mutations at the N-glycosylation site of mGlu7 caused a marked reduction in the surface expression of mGlu7, which resulted in retention of the receptor in the ER. In addition, the deglycosylated mGlu7 is degraded through the autophagy-lysosome degradation pathway. Furthermore, we evaluate whether N-glycosylation of mGlu7 could alter its pharmacological properties and downstream signaling. Taken together, these findings support the evidence that N-glycosylation contributes to surface expression and presynaptic function of mGlu7.

Disclosures: D. Park: None. Y. Suh: None.

Poster

282. Metabotropic Glutamate and GABAB Receptors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 282.07/B50

Topic: B.03. G-Protein Coupled Receptors

Support: Italian Ministry of Health Grant RF-2011-02352582

Title: Analgesic activity of N-acetylcysteine in the streptozotocin model of diabetic neuropathy

Authors: *S. NOTARTOMASO*1, G. MASCIO1, P. SCARSELLI1, F. LIBERATORE2, E. MAZZON4, A. GUGLIANDOLO4, D. PIERAGOSTINO5, G. CRUCCU3, F. NICOLETTI1,2, V. BRUNO1,2, G. BATTAGLIA1,2;

1Mol. Pathology, I.R.C.C.S. Neuromed, Pozzilli, Italy; 2Physiol. and Pharmacol., 3Human Neurosci., Univ. Sapienza, Rome, Italy; 4Exptl. Neurol., I.R.C.C.S. Ctr. Neurolesi "Bonino-Pulejo", Messina, Italy; 5Univ. G. D'Annunzio, Chieti-Pescara, Italy

Abstract: A growing body of evidence indicates that type-2 metabotropic glutamate (mGlu2) receptors regulate pain transmission acting at multiple levels within the pain neuraxis, and are candidate drug targets for therapeutic intervention in inflammatory and neuropathic pain. N-Acetylcysteine (NAC) is an old drug marketed as a mucolytic agent and indicated for the treatment of acetaminophen poisoning and contrast-induced nephropathy. NAC activates the cystine/glutamate membrane antiporter (Xc system), thereby enhancing the synthesis of intracellular glutathione. However, this mechanism also enhances the export of glutamate, thereby facilitating the endogenous activation of presynaptic mGlu2 receptors. We have shown previously that NAC treatment causes analgesia in mouse models of inflammatory pain and in the chronic constriction injury model of neuropathic pain (Bernabucci et al., Mol. Pain, 2012). In addition, we were able to demonstrate that NAC treatment inhibits pain transmission in healthy human volunteers (Truni et al., Mol. Pain, 2015). We have now extended the study to a mouse
model of painful diabetic neuropathy, a condition which is often resistant to analgesic medication. Mice were injected i.p. with 200 mg/kg of streptozotocin (STZ), a nitrosourea that kills pancreatic β-cells causing diabetes. STZ-treated mice developed hyperglycemia and painful neuropathy, as shown by large reductions in mechanical pain thresholds. A single administration of NAC (100 mg/kg, i.p.) 14 day after STZ injection caused analgesia to the same extent as pregabalin (30 mg/kg, i.p.), a first line drug in the treatment of painful diabetic neuropathy in humans. Analgesia was also observed after repeated injections of NAC, indicating the lack of tolerance. NAC-induced analgesia was abrogated by a single injection of sulfasalazine or LY341495, which inhibit Xc– and mGlu2 receptors, respectively. These findings suggest that NAC-induced analgesia in the STZ model was mediated by the sequential activation of the cystine/glutamate antiporter and the mGlu2 receptor. Finally, NAC treatment in diabetic mice normalized some biochemical correlates of nociceptive sensitization, e.g., MAP kinase activation in the dorsal horns of the spinal cord, and induced substantial changes in spinal cord protein levels, as assessed by proteomic analysis. Taken together, these data suggest that NAC caters the potential as an add-on drug in the treatment of painful diabetic neuropathy. The excellent profile of safety and tolerability of NAC encourages the use of the drug in clinical studies.


Poster

282. Metabotropic Glutamate and GABAB Receptors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 282.08/B51

Topic: B.03. G-Protein Coupled Receptors

Title: Type-3 metabotropic glutamate receptors and Parkinson’s disease: Preclinical studies and human genetics

Authors: L. DI MENNA1, M. ALBORGHETTI2, A. TRAFICANTE1, J. A. MONN1, M. SIMMACO2, G. SPALLETTA4, F. NICOLETTI1,3, *G. BATTAGLIA1,3, V. BRUNO1,3, F. E. PONTIERI1, M. BORRO2; 1Mol. Pathology, I.R.C.C.S. Neuromed, Pozzilli, Italy; 2Neuroscience, Mental Hlth. and Sensory Organs (NESMOS), 3Physiol. and Pharmacol., Univ. Sapienza, Rome, Italy; 4I.R.C.C.S. Fondazione S. Lucia, Rome, Italy

Abstract: Type-3 metabotropic glutamate (mGlu3) receptors exert pleiotropic functions in the CNS depending on their anatomical localization. Presynaptic mGlu3 receptors inhibit glutamate release, whereas postsynaptic mGlu3 receptors boost mGlu5 receptor signaling. In addition, activation of mGlu3 receptors in astrocytes stimulates the production of GDNF and TGF-β and
drives microglia towards an anti-inflammatory phenotype. We used the MPTP model of toxicological parkinsonism in mice to examine whether endogenous or pharmacological activation of mGlu3 receptors is neuroprotective. Chronic administration of MPTP (20 mg/kg, s.c., every other day) caused a substantial drop in dopamine (DA), DOPAC and HVA levels in the striatum observed at 15 and 30 days. At 15 days, MPTP induced DA depletion was slightly but significantly attenuated in mGlu2<sup>−/−</sup> mice, whereas an opposite trend was seen in mGlu3<sup>−/−</sup> mice. At 30 days, the DA loss was significantly greater in mGlu3<sup>−/−</sup> mice as compared to their wild-type counterparts (94% vs. 84%). These findings support the hypothesis that activation of mGlu3 receptors is neuroprotective in models of parkinsonism whereas activation of mGlu2 receptors might be neurotoxic. We are currently performing studies with the selective mGlu3 receptor agonist, LY2794193, to further explore this hypothesis. We are also examining the association between polymorphic variants of GRM3 (rs12704290, rs13242038, rs1468412, rs1527768, rs187993, rs1989796, rs2228595, rs2237562, rs2282966, rs2299225, rs274622, rs6465084, rs724226, rs802457, rs906415, and rs917071) or GRM5 (rs60954128 and rs3824927) and Parkinson’s disease (PD) and L-DOPA-induced dyskinesias in a large cohort of patients. Ad interim analysis suggests an association between the GRM3 variant, rs2228595, and PD. This polymorphism (exon 3; Ala293Ala) has been linked to schizophrenia (Marti et al., Am. J. Med. Genet., 2002; Egan et al., Proc. Natl. Acad. Sci., USA, 2004) and to a greater expression of a truncated form of the mGlu3 receptor (mGlu3Δ4) (Sartorius et al., Neuropsychopharmacology, 2008). These findings suggest that mGlu3 receptors might shape the balance between neurodegeneration and neuroprotection in PD and might be targeted by therapeutic intervention.


Poster

282. Metabotropic Glutamate and GABAB Receptors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 282.09/B52

Topic: B.03. G-Protein Coupled Receptors

Title: Type-5 metabotropic glutamate receptors regulate the formation of perineuronal nets in the mouse cerebral cortex and cerebellum during postnatal development

Authors: G. Mascio<sup>1</sup>, S. Notartomaso<sup>1</sup>, P. Scarselli<sup>1</sup>, D. Bucci<sup>1</sup>, M. Cannella<sup>1</sup>, T. Imbriglio<sup>2</sup>, A. Traficante<sup>1</sup>, G. Battaglia<sup>1,2</sup>, V. Bruno<sup>1,2</sup>, *R. Gradini<sup>3</sup>, F. Nicoletti<sup>1,2</sup>
Abstract: Type-5 metabotropic glutamate (mGlu5) receptors are highly expressed and functional in the early postnatal development, when glutamate and other receptor agonists stimulate polyphosphoinositide hydrolysis to a large extent (Nicoletti et al., Proc. Natl. Acad. Sci. USA, 1986). In the first two weeks after birth, mGlu5 receptors are also found in cell types (e.g., cerebellar Purkinje cells), in which the receptor is absent in the adult life (Notartomaso et al., Sci. Rep., 2018). The precise role played by mGlu5 receptors in postnatal development is unknown. Here, we examined whether genetic deletion of mGlu5 receptors affects the formation of perineuronal nets (PNNs) in the CNS. PNNs represent a specialized form of the extracellular matrix surrounding cell somata and proximal dendrites of parvalbumin-positive (PV+) interneurons and other neuronal types in different CNS regions. Chondroitin sulfate proteoglycans (CSPGs), hyaluronan, tenasin-R, and link proteins are major constituents of PNN. PNNs have been implicated in mechanisms of developmental plasticity, and PNN development and maintenance are altered in CNS disorders (Wen et al., Front. Mol. Neurosci. 2018). We could detect PNNs in the mouse cerebral cortex and cerebellum by fluorescent staining with WFA (Wistaria Fluoribunda Agglutinin). Interestingly, the number of neurons surrounded by PNNs was higher in the cortex of mice lacking mGlu5 receptors (mGlu5−/− mice) at postnatal day 16 (PND16), when PNNs begin to be fully expressed in the mouse cortex. Double fluorescent staining showed that a large proportion of neurons surrounded by PNNs were PV+. The activity of type-9 matrix metalloproteinase, an enzyme involved in PNN degradation, was reduced in the cortex of mGlu5−/− mice suggesting that a reduced PNN turnover rate could at least in part account for the greater number of neurons decorated by PNNs in these mice. We extended the analysis to the cerebellum, finding that PNNs were increased in the cerebellar cortex (particularly those surrounding Purkinje neurons), but were reduced in deep cerebellar nuclei of mGlu5−/− mice at PND16. The two phenomena might be causally linked because Purkinje neurons project to deep cerebellar nuclei. We hypothesize that endogenous activation of mGlu5 receptors regulates formation and/or degradation of PNNs in the CNS during postnatal development. Experiments in which mGlu5 receptors are pharmacologically blocked or activated at different postnatal time windows are ongoing to examine this hypothesis in further detail.


Poster

282. Metabotropic Glutamate and GABAB Receptors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 282.10/B53
Title: Activation of mGlu5 metabotropic glutamate receptors protects WaG/Rij rats against spontaneous absence seizures by enhancing GABA re-uptake and restraining tonic inhibition in the thalamus


1 Mol. Pathology, I.R.C.C.S. Neuromed, Pozzilli, Italy; 2 Univ. of Warwick, Coventry, United Kingdom; 3 Pharmacol., Univ. of Genova, Pozzilli, Italy; 4 Biol. Psychology, Donders Ctr. for Cognition, Radboud Univ., Nijmegen, Netherlands; 5 Biol. Psychology, Donders Ctr. for Cognition, Univ. of Lincoln, Lincoln, United Kingdom; 6 Physiol. and Pharmacol., Univ. Sapienza, Rome, Italy

Abstract: A growing body of evidence suggests that mGlu5 receptors are candidate targets for drug treatment of absence epilepsy. Pharmacological activation of mGlu5 receptors with the positive allosteric modulator, VU0360172, reduces spike-and-wave discharges (SWDs) in WAG/Rij rats, which show spontaneous absence seizures after 2-3 months of age. However, intrathalamic injection of VU0360172 causes a paradoxical enhancement of SWD frequency when combined with subthreshold doses of the GABA transporter (GAT-1) inhibitor, tiagabine (D’Amore et al, 2015). Here we explored the possibility that activation of mGlu5 receptors could protect against absence seizures by enhancing synaptic clearance of GABA in the thalamus. Ex vivo measurements of $\[^{3}\text{H}\]$-GABA uptake were performed in thalamic and cortical synaptosomes prepared from symptomatic WAG/Rij rats receiving single or repeated s.c. injections of vehicle or VU0360172 (3 mg/kg). Both VU0360172 treatments significantly enhanced $\[^{3}\text{H}\]$-GABA uptake only in thalamic synaptosomes. Western blot analysis showed an up-regulation of GAT-1 in the thalamus of pre-symptomatic and symptomatic WAG/Rij rats, as well as in age-matched non-epileptic control rats, one hour after a single s.c. injection of VU0360172. In contrast GAT-1 mRNA levels were unchanged in rats challenged with VU0360172. Confocal microscopy analysis showed that the increase of GAT-1 expression in response to mGlu5 receptor activation occurred in neurons and there was no co-localization between GAT-1 and GFAP immunostaining. An up-regulation of GAT-1 could also be observed in thalamic slices challenged with the orthosteric mGlu1/5 receptor agonist DHPG or VU0360172, and this effect was abrogated by pharmacological inhibition of phospholipase-C. This suggests that activation of mGlu5 receptors enhances thalamic GABA re-uptake by increasing the stability of GAT-1 without changes in gene expression. Finally, we examined whether this effect has any impact on extrasynaptic GABA$_\lambda$ receptor-mediated tonic inhibition in the thalamus by recording synaptic inhibition in the absence or presence of the extrasynaptic GABA$_\lambda$ receptor blocker, gabazine. We found that addition of VU0360172 to thalamic slices caused a large reduction in tonic inhibition. Similar data were obtained in ex vivo experiments, in which tonic inhibition was largely reduced in thalamic slices of WaG/Rij rats treated systemically with VU0360172. Taken together, these findings demonstrate that activation of mGlu5 receptors enhances GABA
clearance and restrain tonic inhibition in the thalamus, thereby disclosing a novel potential strategy for the treatment of absence epilepsy.


**Poster**

**282. Metabotropic Glutamate and GABAB Receptors**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 282.11/B54

**Topic:** B.03. G-Protein Coupled Receptors

**Title:** Changes in interneuron related genes in the prefrontal cortex and hippocampus of mice lacking mGlu5 receptors during early postnatal development

**Authors:** T. IMBRIGLIO¹, R. VERHAEGHE¹,³, *M. CANNELLA⁴, A. TRAFICANTE⁴, G. MASCIO⁴, S. MACCARI¹,³, G. BATTAGLIA¹,⁴, F. NICOLETTI¹,⁴;
¹Physiol. and Pharmacol., ²Sci. and Med. - Surgical Biotechno., Univ. Sapienza, Rome, Italy; ³CNRS, UMR 8576, UGSF, Unité de Glycobiologie Structurale et Functionnelle, Univ. of Lille, Lille, France; ⁴IRCCS Neuromed, Pozzilli, Italy

**Abstract:** Type-5 metabotropic glutamate (mGlu5) receptors are highly expressed and functional in the early postnatal development (Catania et al., Neurosci. 1994 ; Nicoletti et al., Proc. Natl. Acad. Sci. USA, 1986). The function of mGlu5 receptors in developmental processes occurring after birth is unknown. Here, we examined the expression of genes related to GABAergic interneurons in the prefrontal cortex (PFC) and hippocampus of mGlu5⁻/⁻ mice and their wild-type counterparts at postnatal day (PND) 9 and 21. PFC and hippocampal interneurons are dysfunctional in schizophrenia, and mGlu5 receptors are candidate drug targets for antipsychotic medication. At PND9, we were surprised to find an increase in parvalbumin (PV) mRNA levels in the PFC and hippocampus of mGlu5⁻/⁻ mice, whereas PFC levels of the transcripts encoding for CB1 receptors and reelin were decreased. The transcript encoding for the NMDA receptor subunits and somatostatin were unchanged. These findings were divergent from those seen in mice lacking mGlu3 receptors in which a reduction in PV mRNA levels was consistently seen in both regions at PND9. In mGlu5⁻/⁻ mice, the increase in PV mRNA levels persisted in the PFC but not in the hippocampus. Unexpectedly, we found large increases in the expression of the GluN1 subunit of NMDA receptors (both mRNA and protein levels) in the PFC of mGlu5⁻/⁻ mice at PND21. This was associated with a significant increase in GluN2A mRNA levels, whereas the transcripts of the GluN2C and GluN2D subunits were unchanged. GluN1 protein levels were also substantially increased in the hippocampus of mGlu5⁻/⁻ mice at PND21, and this was associated with an increase in the transcript of the GluN2C subunit. NMDA and mGlu5 receptors
are both expressed in GABAergic interneurons and they are physically linked by a chain of anchoring proteins. In addition, activation of mGlu5 receptors facilitates NMDA receptor function and vice versa. Thus, we hypothesize that the up-regulation of NMDA receptor subunits found in mGlu5−/− mice during development may represent a compensatory mechanism aimed at supporting synaptic activation of GABAergic interneurons in the absence of mGlu5 receptors. This hypothesis warrants further investigation. We expect that, at least during early postnatal development, mice are more sensitive to drugs that interact with NMDA receptors. We are currently examining this possibility at behavioral level.


Poster

282. Metabotropic Glutamate and GABAB Receptors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 282.12/B55

Topic: B.03. G-Protein Coupled Receptors

Title: TRPC3 channel is important for mGluR1-dependent Ca²⁺ and firing regulation in nigral dopamine neurons

Authors: *K. U. UM, M. PARK;
Dept. of Physiol., Sch. of Medicine. Sungkyunkwan Univ., Suwon-si, Chunchun-dong, Korea, Republic of

Abstract: Pacemaker dopamine neurons in the substantia nigra pars compacta (SNc) exhibit low-frequency spontaneous firing without any input stimuli and often produce high-frequency burst firing in response to strong glutamatergic afferent inputs. Since the tonic firing determines ambient dopamine levels, regulation of tonic firing rate is very important. Although activation of mGluR1 is reported to increase cell excitability via activation of some type of TRP channels, it is still unclear how mGluR1 regulates tonic firing activities in SNc dopamine neurons. In this study, we present that mGluR1 increases Ca²⁺ levels and tonic firing rate via TRPC3 channels in SNc dopamine neurons.

In SNc dopamine neurons, activation of mGluR1 inhibited spontaneous firing transiently but subsequently led to a slow increase in tonic firing rate. DHPG, a mGluR1 agonist, evoked two clear phases of Ca²⁺ elevations: the fast Ca²⁺ surge released from the endoplasmic reticulum (ER) Ca²⁺ store and then the following sustained Ca²⁺ influxes. When the intracellular Ca²⁺ store was emptied by caffeine or thapsigargin, we could not observe any Ca²⁺ influxes but DHPG induced a sustained Ca²⁺ influx, suggesting that DHPG induces Ca²⁺ influx regardless of store operated Ca²⁺ channels in dopamine neurons. Using the several TRP channel blockers, we found that DHPG-induced Ca²⁺ influx was dramatically reduced by pre-treatment of TRPC3 channel blocker.
blockers. In addition, we observed that DHPG-induced Ca\textsuperscript{2+} influx was inhibited by L-type Ca\textsuperscript{2+} channel blockers, but not by P/Q- or T- type channel blockers. Taken together, these data suggest that mGluR1 activate TRPC3 channels which induces Ca\textsuperscript{2+} influx by increased tonic firing rate and consequential activation of L-type Ca\textsuperscript{2+} channels. Therefore, we conclude that TRPC3 channels are a key player in mGluR1-induced cytosolic Ca\textsuperscript{2+} and tonic firing changes in SNc dopamine neurons.

**Disclosures:**  K.U. Um: None. M. Park: None.

**Poster**

**282. Metabotropic Glutamate and GABAB Receptors**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 282.13/B56

**Topic:** B.03. G-Protein Coupled Receptors

**Title:** Analgesic activity of cinnabarinic acid in models of inflammatory and neuropathic pain

**Authors:** *F. FAZIO\textsuperscript{1}, S. NOTARTOMASO\textsuperscript{2}, S. BOCCELLA\textsuperscript{3}, M. ULIVIERI\textsuperscript{2}, N. ANTENUCCI\textsuperscript{4}, F. LIBERATORE\textsuperscript{3}, P. SCARSELLI\textsuperscript{1}, V. BRUNO\textsuperscript{1,4}, G. BATTAGLIA\textsuperscript{1,4}, L. LUONGO\textsuperscript{3}, F. NICOLETTI\textsuperscript{1,4}, S. MAIONE\textsuperscript{3};

\textsuperscript{1}Mol. Pathology, I.R.C.C.S. Neuromed, Pozzilli, Italy; \textsuperscript{2}Mol. Pathology, Neuromed I.R.C.C.S., Pozzilli, Italy; \textsuperscript{3}Exptl. Med., Univ. of Campania L. Vanvitelli, Napoli, Italy; \textsuperscript{4}Physiol. and Pharmacol., Univ. Sapienza, Rome, Italy

**Abstract:** L-Tryptophan (Try) has been involved in the regulation of pain, and tryptophan depletion in humans reduces morphine-induced analgesia (Abbott et al., Psychoph.,1992). It is generally believed that the action of Try on pain is mediated by serotonin. However, Try is the metabolic precursor of the kynurenine pathway, which generates a number of neuroactive compounds, such as kynurenic, quinolinic, and xanthurenic acids (Schwarcz et al., Nat. Rev. Neurosci., 2012). Metabolites of the kynurenine pathway have been implicated in the regulation of pain transmission (Godefroy et al., Brain Res.,1990; Heyliger et al., Pharmacol. Res., 1998). In the last few years, we have focused on cinnabarinic acid, a trace kynurenine metabolite which is formed by two joined molecules of 3-hydroxyanthranilic acid. We have found that cinnabarinic acid acts as an orthosteric agonist of mGlu4 metabotropic glutamate receptors (Fazio et al., Mol. Pharmacol., 2012), and this stimulated our interest for the study of cinnabarinic acid in pain models because drugs that activate mGlu4 receptors are known to cause analgesia (Vilar et al., J. Neurosci., 2013). We first examine the effect of cinnabarinic acid in mice injected s.c. with formalin in the hind paw. Low doses of cinnabarinic acid (0.125 and 0.25 mg/kg, i.p.) significantly reduced nocifensive behaviour in the second phase of the formalin test, which reflects the development of central nociceptive sensitization. In contrast, higher doses of cinnabarinic acid (0.5 and 3 mg/kg, i.p.) were inactive. We extended the analysis to the chronic
constriction injury (CCI) model of neuropathic pain, in which mechanical pain thresholds were assessed by von Frey filaments. Acute injection of low doses of cinnabarinic acid (0.25 mg/kg, i.p.) caused substantial analgesia two weeks after CCI induction. Analgesia was also observed after seven days of repeated administrations of cinnabarinic acid (0.25 mg/kg, i.p., q.d.), indicating the lack of tolerance. Taken together, these findings suggest that trace amounts of cinnabarinic acid are able to modulate pain transmission and induce analgesia in different models of pain. We are currently examining whether the analgesic effect of cinnabarinic acid is mediated by the activation of mGlu4 receptors or involves other mechanisms (e.g., the activation of aryl hydrocarbon receptors).


**Poster**

282. Metabotropic Glutamate and GABAB Receptors

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 282.14/B57

**Topic:** B.03. G-Protein Coupled Receptors

**Title:** Regulation of GABA\(_B\) receptor trafficking and localization by APP, AJAP1 and PIANP

**Authors:** *P. REM, S. FRÜH, M. CHOO, M. GASSMANN, B. BETTLER;
Dept. of Biomedicine, Univ. of Basel, Basel, Switzerland

**Abstract:** GABA\(_B\) receptors (GBRs) are key regulators of synaptic release, but little is known about mechanisms that localize GBRs at axon terminals. Presynaptic GBRs comprise the GB1a subunit that contains a pair of protein-protein interaction motifs termed ‘sushi domains’ at their extracellular N-terminus. One of these sushi domains interacts in a mutually exclusive manner with the three transmembrane proteins β-amyloid precursor protein (APP), AJAP1 and PIANP (Schwenk et al., Nature Neuroscience 19(2), 2016). Using heterologous assay systems we now show that complex formation of GBRs with APP, AJAP1 or PIANP does not allosterically influence G-protein signaling by the receptor. Bimolecular fluorescence complementation (BiFC) assays in neurons reveal that APP, AJAP1 and PIANP all interact with GBRs in cis. Using a hemi-synapse assay we show that AJAP1 also binds to GBRs in trans. We found that specifically APP transports GBRs to axon terminals and that lack of APP results in a significant deficit in presynaptic GBR-mediated inhibition. In line with this, axonal expression of GBRs is reduced in APP\(^{-/-}\) but not in AJAP1\(^{-/-}\) or PIANP\(^{-/-}\) mice. However, expression of PIANP is essential for normal GBR-mediated inhibition of neurotransmitter release. We hypothesize that after delivery to the axon terminal, APP transfers GBRs to the higher-affinity binding sites of PIANP and AJAP1 that localize GBRs to presynaptic sites.
**Abstract:**

The rostral ventrolateral medulla (RVLM), the so-called brainstem ‘pressor area’, is a key site for the regulation of sympathetic tone and blood pressure. The major source of excitatory sympathetic drive from the RVLM are spinally projecting glutamatergic neurons which receive tonic glutamatergic excitatory signals from the paraventricular nucleus of the hypothalamus (PVN), and gamma-aminobutyric acid (GABA)ergic inhibitory signals from the caudal ventrolateral medulla (CVLM). Brain angiotensin II (Ang II) increases mean arterial pressure (MAP) and sympathetic nerve activity through stimulation of AT1Rs on spinally projecting glutamatergic neurons located in the RVLM.

There is increasing evidence that glutamate and GABA interact within the RVLM to modulate the central regulation of blood pressure and sympathetic tone.

**DESIGN and METHOD**

The present study was designed to investigate whether the well established hypertensive response to local AT1R stimulation by Ang II is associated with changes in glutamate and GABA levels within the RVLM. *In vivo* microdialysis, for measurement of extracellular glutamate and GABA levels, was carried out in the RVLM of conscious normotensive Wistar rats while locally infusing Ang II (3 µg/µl/h). Glutamate and GABA levels were quantified by liquid chromatography.

**RESULTS**

Infusion of Ang II into the RVLM significantly increased glutamate levels (P < 0.05) and significantly decreased GABA levels (P < 0.05).

**CONCLUSION**

The results suggest that the well established hypertensive response to AT1R stimulation in the RVLM by Ang II is associated with enhanced glutamate release from glutamatergic projections originating in the PVN, possibly through stimulation of presynaptic AT1R on these nerve...
endings. In addition, stimulation of AT1R located on inhibitory GABAergic nerve endings originating in the CVLM appears to reduce GABA release and subsequent disinhibition of the excitatory neurons driving the sympathetic tone within the RVLM. Further experiments with selective AT1R blockade are currently ongoing to assess the AT1R selectively of these responses.

**Disclosures:** L. Legat: None. A. G. Dupont: None. I. Smolders: None.

**Poster**

283. Potassium Channels II

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 283.01/B59

**Topic:** B.04. Ion Channels

**Support:** Telethon Foundation Grant GGP15113 to MT
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**Title:** Beyond retigabine: Pharmacological characterization of novel neuronal Kv7 channel activators

**Authors:** *F. MICELI*¹, P. NAPPI¹, L. CAROTENUTO¹, C. OSTACOLO², P. CAMPIGLIA³, M. TAGLIALATELA¹;
¹Dept. Neurosci., ²Dept. of Pharm., Univ. of Naples "Federico II", Naples, Italy; ³Dept. of Pharm., Univ. of Salerno, Salerno, Italy

**Abstract:** Retigabine is the only antiepileptic drugs approved for human use that acts by activating voltage-gated potassium channels belonging to Kv7 subfamily. Among this family, Kv7.2 and Kv7.3 subunits are mainly expressed in the neurons where they underlie a K⁺ current (I_{Km}), which plays a fundamental role in controlling neuronal excitability. Because of poor selectivity for Kv7 subtypes, short half-life, poor brain penetration and chemical instability, leading to blue-gray skin discoloration, retigabine is no longer available on the market. In this work, we have tested a small library of retigabine derivatives by means of fluorescence-based assay and manual patch-clamp technique with the aim to indentify novel Kv7.2/3 channel modulators.

Stable CHO cell lines co-expressing Kv7.2 and Kv7.3 channel subunits were used for a fluorescence-based assay that uses Thallium (Tl⁺) as a surrogate of K⁺ ions and a fluorescent Tl⁺-sensitive dye (FluxOR).
In Kv7.2/3 stable cell lines retigabine (0.1-100 µM) dose-dependently increased the maximal fluorescence and the initial slope of the fluorescent signal; both effects were abolished by the pan-Kv7 blocker XE991 (10 µM). The calculated EC_{50} for retigabine was 4.6±0.7 µM, a value similar to that calculated by electrophysiological techniques (EC_{50}= 1.9±0.2 µM). Some of the newly-synthesized retigabine analogues increased the maximal fluorescence and the initial slope of the fluorescent signal significantly more than retigabine. In particular, the compound CPRET-46 was at least 2000 times more potent than retigabine, showing an EC_{50} of 2.2±0.1 nM. Electrophysiological experiments corroborated such conclusion. In fact, in CHO cells expressing Kv7.2/3 channels, 100 nM retigabine failed to modify Kv7.2/3 channel gating; whereas, the same concentration of CPRET-46 negatively shifted Kv7.2/3 currents activation V_{1/2} by about 23 mV. When compared to retigabine, CPRET-46 also showed a higher maximal efficacy; indeed, at the maximal concentration of 10 µM, the V_{1/2} of Kv7.2/3 currents was shifted by 40 and 80 mV for retigabine and CPRET-46, respectively. In conclusion, we have identified a novel retigabine analogous showing higher potency over existing compounds.


Poster

283. Potassium Channels II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 283.02/B60

Topic: B.04. Ion Channels

Support: NIH R01 NS083402
NIH R01 NS097610

Title: A novel role for Kv7/KCNQ potassium channels in synaptic strength

Authors: *G. C. TRACY^[1], H. CHUNG^[1,2];

Abstract: Neuronal Kv7/KCNQ channels are voltage-gated, slow-activating, and non-inactivating potassium channels. They are critical in the regulation of neuronal excitability by limiting the repetitive firing of action potentials (APs) and modulating the resting membrane potential. Kv7 channels are composed of tetramers which, in the brain, are mostly heteromers of Kv7.2/KCNQ2 and Kv7.3/KCNQ3. In the excitatory pyramidal neurons of the hippocampus and cortex, Kv7 channels are enriched at the axon initial segment (AIS) and prevent intrinsic burst firing of APs. Ankyrins provide cytoskeletal support and organize membrane proteins to a macromolecular functional hub. AnkyrinG, a mammalian ankyrin protein, has multiple splice
isoforms which share ankyrin repeats but display different localization: the 480kD and 270kD AnkyrinG proteins are concentrated at the AIS whereas the 190kD AnkyrinG isoform is found in dendritic spines that harbors excitatory synapses. The AnkyrinG-480kD binding to Kv7.2 and Kv7.3 C-termini is required for their AIS localization. However, whether the AnkyrinG-190kD binds to Kv7 channels and regulates their expression remains unknown. In this study, we discover that Kv7.2, Kv7.3, and AnkyrinG-190kD in the postsynaptic density fractions of the rat hippocampus. In the rat primary dissociated culture of hippocampal neurons, Kv7.2, Kv7.3, and AnkyrinG-190kD are localized to the dendritic spines. Interestingly, acute inhibition of Kv7 channels using their blocker XE991 increases surface density of GluA2-containing AMPA receptors in the dendrites and dendritic spines. Activation of NMDA receptors upon a brief bath application of NMDA potentiated this effect by 1.36-fold. These findings suggest an exciting possibility that AnkyrinG-190kD may target Kv7 channels to the dendritic spines where they regulate excitatory synaptic strength. We are currently using biochemical, cellular, and electrophysiology approach to investigate the mechanism and physiological implication for Kv7 targeting to the dendritic spines.

Disclosures: G.C. Tracy: None. H. Chung: None.

Poster

283. Potassium Channels II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 283.03/B61

Topic: B.04. Ion Channels

Support: NIH Grant NS032387
        NIH Grant NS108874
        Dravet Foundation
        Northwestern University Dixon Translational Award

Title: Impaired M-current in KCNQ2 epileptic encephalopathy evokes dyshomeostatic modulation of excitability in patient-derived neurons

Authors: *D. SIMKIN¹, T. J. SEARL¹, B. PIYEVSky², K. A. MARSHALL², M. FORREST³, M. SCHWAKE², G. L. ROBERTSON², P. PENZES³, J. J. MILLICHAP⁴, A. L. GEORGE, Jr¹, E. KISKINIS²;
¹Pharmacol., ²Neurol., ³Physiol., Northwestern University, Feinberg Sch. of Med., Chicago, IL; ⁴Pediatrics and Neurol., Ann & Robert H. Lurie Children's Hosp. of Chicago, Feinberg Sch. of Medicine, Northwestern Univ., Chicago, IL

Abstract: Mutations in KCNQ2, which encodes a pore-forming K⁺ channel subunit responsible for neuronal M-current, have been associated with neonatal epileptic encephalopathy (NEE).
This complex disorder manifests as severe early-onset seizures and impaired neurodevelopment due to an imbalance in neuronal circuit activity in the brain. While the effects of KCNQ2 mutations have been studied extensively in heterologous expression systems, their effects on the inherent properties of human neurons have not. Specifically, what remains unclear is how the likely defects in M-current affect the electrophysiological properties of human neurons during a critical period of neuronal maturation. Here we use KCNQ2-NEE patient-specific and isogenic control iPSC-derived excitatory neurons to elucidate the dynamic functional effects of a KCNQ2 mutation.

We find that while patient-derived excitatory neurons exhibit reduced M-current early, they develop intrinsic and network hyperexcitability progressively over time in culture (using multi-electrode arrays and patch-clamp electrophysiology, respectively). This hyperexcitability is associated with faster action potential repolarization, larger afterhyperpolarization, and a functional enhancement of Ca²⁺-dependent K⁺ channels (BK and SK). These properties facilitate a burst-suppression firing pattern that is reminiscent of the interictal electroencephalography pattern in patients. Importantly, we were able to phenocopy these excitability features in control neurons only by chronic but not acute pharmacological inhibition of M-current. Our findings suggest that dyshomeostatic mechanisms compound KCNQ2 loss-of-function and lead to alterations in the neurodevelopmental trajectory of patient-derived neurons. Our work has therapeutic implications in explaining why KCNQ2 agonists may not be beneficial unless started at an early disease stage.

Abstract: The contribution of the inwardly rectifying K⁺ channel subtype Kir4.1 has been focused mainly on astrocytes, where they play important roles in the maintenance of resting membrane potential, extracellular K⁺ uptake and facilitation of glutamate uptake in the central nervous system. Here, we report the role of Kir4.1 channels in NG2-glia during brain development, potassium signaling and in an ischemic stroke disease model. Kir4.1 channels are widely expressed in NG2-glia during brain development. In the adult mouse hippocampus, Kir4.1 channels in NG2-glia constitute more than 80% of K⁺ channels inward currents. This large portion of Kir4.1 channel currents exhibits a deficit in NG2-glia as an initial response in a transient ischemic mouse model (tMCAO). Further evidence indicates that Kir4.1 deficits in NG2-glia potentially cause axonal myelin loss in ischemia through the association with oligodendrocyte-specific protein (OSP / Claudin-11), which unravels a potential therapeutic target in the treatment of ischemic stroke.

Disclosures: X. Tong: None. F. Song: None. X. Hong: None. J. Cao: None. J. Wan: None.

Poster

283. Potassium Channels II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 283.05/B63

Topic: B.04. Ion Channels

Support: NRF-2016M3C7A1904149
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KIST grant 2E29180

Title: Exon2-deleted TWIK-1 KO mice are not an appropriate model for TWIK-1 deficiency

Authors: H.-G. JUNG¹, A. KIM¹, Y. BAE², J.-Y. PARK², *E. HWANG¹;
¹Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; ²Korea Univ., Seoul, Korea, Republic of

Abstract: TWIK-1 is a member of the two-pore domain K⁺ (K2P) channel family that plays an essential part in the regulation of resting membrane potential and cellular excitability. We recently reported that TWIK-1/TREK-1 heterodimeric channel mediates astrocytic passive conductance with gene silencing tools. However, this work was challenged by a report showing no alteration of astrocytic passive conductance in Exon2-deleted TWIK-1 mice (TWIK-1 ΔEX2 KO mice). Here we developed an antibody against C-terminus of TWIK-1 and we found that strong signals of immunohistochemistry was detected in hippocampal astrocytes of TWIK-1 ΔEX2 KO mice using this antibody. Molecular biological and biochemical experiments demonstrate TWIK-1 ΔEX2 KO mice expressed unsuspected TWIK-1 proteins showing an inflamed connection between Exon1 and Exon3 of TWIK-1. Targeting of gene silencing on
Exon1 or Exon3 of TWIK-1 showed clear reduction of astrocytic passive conductance in TWIK-1 ΔEX2 KO mice. In addition, heterologous co-expression of TWIK-1 ΔEX2 and TREK-1 in COS-7 cells shows a linear-like I-V relationship. Taken together, our data strongly suggested that TWIK-1 ΔEX2 KO mice is not a proper KO model for the study of TWIK-1 functions and reconfirmed the role of TWIK-1 as a molecular identity of astrocytic passive conductance.


Poster

283. Potassium Channels II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 283.06/B64

Topic: B.04. Ion Channels

Support: The Gerald Kerkut Charitable Trust Studentship

Title: A 5’ UTR GGN repeat sequence forms a stable G-quadruplex that controls translation initiation and neurite targeting of the potassium leak channel Task3 mRNA

Authors: *C. J. MALTBY1, J. P. R. SCHOFIELD1, I. M. O’KELLY2, K. DEINHARDT1, M. J. COLDWELL1;

1Biol. Sci., 2Sch. of Med., Univ. of Southampton, Southampton, United Kingdom

Abstract: Neurons are structurally complex, highly polar cells that exhibit spatial compartmentalisation of function. Activity-driven local translation of pools of translationally-repressed mRNAs underpins this functional compartmentalisation, allowing for distinct local proteomes to facilitate the dynamic synaptic remodelling attributed to normal cellular function. There has been growing evidence to suggest that cation-stabilised non-canonical secondary RNA structures, known as G-quadruplexes, within untranslated regions of mRNAs are key to regulating this translational control and distal transport of particular neuronal mRNAs, a process implicated in several neurodevelopmental and degenerative disorders.

2-pore potassium leak (K2P) membrane channels maintain the resting membrane potential of neurons. Task3 is a hippocampal and cerebellar-enriched K2P channel thought to play a role in maintaining neuronal excitability during long-term potentiation attributed to learning and memory. Minor aberrations in the levels of Task3 expression leads to neuronal dysfunction and mental retardation, though the mechanisms controlling the narrow physiological window of Task3 expression are largely unknown.

Through 5’ Rapid Amplification of cDNA Ends (RACE), we revealed a 5’ UTR cap-proximal (GGN)12 repeat in human brain Task3 mRNA that is conserved across species. GGN repeat expansions have been implicated in Fragile-X mental retardation syndrome due to G-quadruplex formation in vivo, and so could be controlling physiological Task3 mRNA delivery and...
expression in neurons. To investigate the nature of this GGN repeat, we employed circular dichroism (CD) and native PAGE assays, showing that under physiological K⁺ conditions the (GGN)₁₂ repeat sequence forms a highly thermo-stable and K⁺ dependent parallel G-quadruplex structure.

Through mutagenesis of this (GGN)₁₂ repeat sequence, we show in vitro that this G-quadruplex forming sequence inhibits translation of Task3 peptides, which is relieved upon overexpression of the brain-enriched G-quadruplex specific helicase, DHX36. We also find that endogenous Task3 mRNA is upregulated rapidly during neuronal activity and is present extensively throughout distal neurite projections. Using mutant 5’ UTR reporter constructs, we find a strong correlation between the presence of this 5’ cap-proximal G-quadruplex and distal mRNA localisation within axons and dendrites.

We therefore suggest that these G-quadruplex structures are central to regulating the delivery of Task3 mRNA to distal sites of translation, as well as the narrow window of Task3 expression levels attributed to normal cellular function.

**Disclosures:** C.J. Maltby: None. J.P.R. Schofield: None. I.M. O'Kelly: None. K. Deinhardt: None. M.J. Coldwell: None.

**Poster**

**283. Potassium Channels II**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 283.07/B65

**Topic:** B.04. Ion Channels

**Support:** National Natural Science Foundation of China Grant 81872801
National Natural Science Foundation of China Grant 81202607

**Title:** Structural molecular sites for local anesthetic modulation of TASK channels

**Authors:** *G. DU¹, Y. HOU¹, Y. HUANG²;¹Translational Neurosci. Center, Dept. of Anesthesiol., West China Hosp. of Sichuan Univ., Chengdu, China; ²Dept. of Anesthesiol., Affiliated Hosp. of Guizhou Med. Univ., Guiyang, China

**Abstract: Introduction.** TASK-1 (K2P3.1) and TASK-3 (K2P9.1), members of the K2P family, are widely expressed leak potassium channels responsible for maintenance of cell membrane potential and input resistance. TASK channels generate pH-sensitive, background K⁺ currents. They are sites of action for a variety of modulatory agents and local anesthetics show inhibition of the channels. Our previous findings show TASK channels are important molecular targets for the CNS toxicity by local anesthetics. Inhibition of the TASK channels causes membrane depolarization and increases neuronal excitability, contributing to central local anesthetic
toxicity. However, the structural mechanism underlying local anesthetic acting on TASK channels remain elusive. **Methods.** Based on the crystal structures of human K2P channel subtype TREK1, homology models of TASK channels were created. We predicted candidate sites using the docking conformations for local anesthetic and TASK channels. Site-directed mutagenesis was constructed using mouse TASK-1-pcDNA3 and TASK-3-pcDNA3 wild type templates and Quikchange site-Directed mutagenesis kit (KAPA). Human embryonic kidney 293t cells were transfected with different mutations tagged with green fluorescent protein. Voltage commands were applied and currents recorded and analyzed with pClamp software. **Results.** Docking conformations for a local anesthetic within TASK channels were created. We predicted 15 different candidate sites involved in the local anesthetic modulation of TASK channels. Among the mutations, local anesthetics (bupivacaine, ropivacaine and lidocaine) showed more inhibition of the mutations T93A and F125R and less inhibition of the double mutation L122A-L239A, when compared with wild type TASK channels. **Conclusions.** T93, F125 and L122-L239 are potent and highly selective sites for local anesthetic modulation of TASK channels. Such structural molecular sites might be targets for development of new local anesthetic compounds with reduced toxicity in the future.

**Disclosures:**  **G. Du:** None. **Y. Hou:** None. **Y. Huang:** None.

**Poster**

**283. Potassium Channels II**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 283.08/B66

**Topic:** B.04. Ion Channels

**Support:** NIH Grant RO0AG044444

**Title:** Progressive myoclonic epilepsy-associated gene Kctd7 regulates retinal neurovascular patterning

**Authors:** *J. ALEVY, C. A. BURGER, N. E. ALBRECHT, D. JIANG, M. A. SAMUEL; Dept. of Neuroscience, Huffington Ctr. on Aging, Baylor Col. of Med., Houston, TX

**Abstract:** Homozygous mutations in the human potassium channel tetramerization domain-containing protein 7 (KCTD7) are associated with progressive myoclonic epilepsy. However, the molecular and neurological basis of this disease remains poorly understood. Using a high-throughput screening pipeline in murine retina, we recently identified Kctd7 as a potential neural regulator. Here we show that mice deficient in Kctd7 develop epileptic seizures and display neural activity defects in both the brain and the retina. Kctd7 is enriched in hippocampal and Purkinje neurons in the brain as well as in excitatory bipolar cells in the retina, and the absence
of Kctd7 results in altered bipolar cell development. These defects are accompanied by specific alterations to vascular patterning that results in delayed vessel development and altered vascular structure. Together, these data identify a disease-inducing role for Kctd7 in the nervous system and establish a model for KCTD7-related progressive myoclonic epilepsy.


Poster

283. Potassium Channels II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 283.09/B67

Topic: B.04. Ion Channels

Support: BBSRC grant RM56G0083
Medical Research Council, UK
Biomedical Services, Preclinical Research Facility, University of Leicester

Title: Kv3.1 and Kv3.3 differentially contribute to action potential repolarization in principal neurons of the auditory brainstem

Authors: *N. CHAUDHURY1, A. RICHARDSON1, D. M. LINLEY2, S. ROBINSON3, M. ANDERSON1, V. MARRA1, C. KOPP-Scheinpflug4, J. R. STEINERT1, I. D. FORSYTHE1;
1Univ. of Leicester, Leicester, United Kingdom; 2Univ. of Leeds, Leeds, United Kingdom; 3MRC Toxicology Unit, Leicester, United Kingdom; 4LMU Munich, Planegg-Martinsried, Germany

Abstract: The ‘delayed rectifier’ potassium current is mediated by Kv3 channels which open at voltages during action potential (AP)-mediated depolarisation and thus provide the repolarization drive to support high-frequency firing in neurons. Multiple Kv3 genes are co-expressed in several parts of the brain, either as hetero- or homo-tetramers but subunit specific roles are largely unknown. Neurons of the medial nucleus of the trapezoid body (MNTB) and the lateral superior olive (LSO), in the superior olivary complex (SOC) of the auditory brainstem, compute sound source localisation through integration of binaural stimuli. Both MNTB and LSO neurons express Kv3.1 and Kv3.3 subunits. We have used this neuronal network to investigate role of these subunits in generating functional Kv3 channels and during repolarization of the postsynaptic AP. The study was conducted on CBA/Ca mice, and on Kv3.1 knockout (KO), and Kv3.3KO mice backcrossed onto the same strain. Expression of Kv3 channels in the MNTB and LSO principal neurons was investigated by qRTPCR, western blot and immunohistochemistry. For ex vivo whole cell patch-clamp electrophysiology, transverse sections of SOC were used for
recording currents and voltages in the neurons under different biophysical and pharmacological conditions. Intracellular Ca\(^{2+}\) transients were studied by Fura-2 ratiometric fluorescence measurement. Statistical significance was determined using Student’s t-test and one-/two-way ANOVA and expressed as mean ± SD.

The results obtained reveal that MNTB neurons effectively employed either Kv3.1 or Kv3.3 subunits in Kv3 channels, with similar whole-cell current amplitude; however, the fastest APs were achieved when both subunits were expressed together (AP halfwidth-WT: 0.29 ± 0.07ms, n=14; Kv3.1KO: 0.41 ± 0.11ms, n=11, p=0.0207; Kv3.3KO: 0.48 ± 0.06ms, n=11, p<0.0001). In the LSO, Kv3.3 subunits were essential; Kv3.1 mRNA was present, but somatic Kv3 channels must contain at least one Kv3.3 subunit, since Kv3.3KO mice had little or no TEA-sensitive Kv3 current (Kv3.3KO: 6.0 ± 2.5nA, n=11; with TEA: 5.0 ± 1.8nA, n=7), and AP halfwidths were increased compared to WT (WT: 0.28 ± 0.04ms, n=10; Kv3.3KO: 0.69 ± 0.15ms, n=7, p<0.0001). Measurement of whole-cell voltage-gated Ba\(^{2+}\) currents showed no change in the MNTB or LSO, but the longer AP duration in the KOs increased [Ca\(^{2+}\)] in an activity-dependent manner. We conclude that Kv3 channels make major contributions to AP repolarization in both the MNTB and LSO. Kv3.1 and Kv3.3 subunits each contribute to Kv3 currents in the MNTB but Kv3.3 is crucially dominant for Kv3 channels in the LSO, while both of the subunits influence Ca\(^{2+}\) influx in these neurons.


**Poster**

**283. Potassium Channels II**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 283.10/B68

**Topic:** B.04. Ion Channels

**Support:** NIH grants DE018661
NIH grants DE023090

**Title:** Characterizing electrophysiological properties of the nodes of Ranvier at motor never fibers using the *in situ* pressure-patch-clamp recording technique

**Authors:** *S. TONOMURA, K. HIROSATO, J. LING, J. G. GU;* 
Univ. of Alabama at Birmingham, Birmingham, AL

**Abstract:** The nodes of Ranvier (NRs) are key sites for saltatory conduction, the rapid action potential conduction along myelinated nerve fibers. Saltatory conduction through NRs is often impaired under pathological conditions such as Multiple sclerosis and Guillain-Barre syndrome,
leading to sensory and movement dysfunctions. However, ion channel mechanisms underlying the saltatory conduction of action potentials at NRs are incompletely understood. In the present study, we investigated electrophysiological properties of NRs in spinal ventral root motor fibers using our newly developed in situ pressure-patch-clamp recording technique. We found that NRs of motor fibers displayed large non-inactivating outward currents that were not highly sensitive to the voltage-gated K$^+$ channel inhibitors TEA (20 mM) and 4-AP (1 mM). However, the outward currents were significantly inhibited by the two-pore domain potassium (K2P) channel blockers norfluoxetine. These results suggest that K2P channels rather than voltage-gated K$^+$ channels are principal type of K$^+$ channels present at NRs of rat motor nerve fibers. We also used the in situ pressure-patch-clamp recording technique to characterize other membrane and action potential properties at NRs. This is the first study to directly make whole-cell patch-clamp recordings from intact NRs of mammalian motor nerve fibers. This new study may help to understand ion channels and their functions at NRs in saltatory conduction along motor nerve fibers. The study was supported by NIH grants DE018661 and DE023090 to J.G.G.


Poster

283. Potassium Channels II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 283.11/B69

Topic: B.04. Ion Channels

Support:  NIH grant DE018661
          NIH grant DE023090

Title: K2P channels are molecular machineries at nodes of Ranvier for saltatory conduction

Authors: *H. KANDA$^{1,2,3}$, S. TONOMURA$^3$, J. LING$^3$, K. NOGUCHI$^2$, S. MATALON$^3$, Y. DAI$^{1,2}$, J. G. GU$^3$;
$^1$Pharmacol., Hyogo Univ. of Hlth. Siences, Kobe, Japan; $^2$Hyogo Col. of Med., Nishinomiya, Japan; $^3$Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Timely sensory and motor responses are critical in life and largely rely on saltatory conduction of action potentials through nodes of Ranvier (NRs) along myelinated nerves. Impaired saltatory conduction occurs in many diseases causing sensory and motor dysfunctions. However, molecular machineries at NRs for saltatory conduction remain largely unknown at mammalian NRs. Here we show that two-pore domain potassium (K2P) channels are highly clustered at NRs. We demonstrate that 2P channels, but not voltage-gated K$^+$ channels, are required for action potential formation at NRs. We show that K2P channels control the speed and frequency of nerve impulses conducted through NRs. Furthermore, genetic knockdown of these
channels at NRs retards saltatory conduction and impair *in vivo* sensory behavioral responses. Collectively, K2P channels are molecular machineries at NRs for saltatory conduction in mammals, which may provide new insights into multiple neurological disorders.


**Poster**

*283. Potassium Channels II*

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 283.12/B70

**Topic:** B.04. Ion Channels

**Support:** EC REA 722098 LISTEN

**Title:** Distinguishing subunit-specific roles of Kv3 channels in action potential repolarization

**Authors:** *K. BONDARENKO*¹, J. P. SMALLEY², M. S. ANDERSON¹, P. M. CULLIS², I. D. FORSYTHE¹;

¹Dept. of Neuroscience, Psychology & Behaviour, ²Dept. of Chem., Univ. of Leicester, Leicester, United Kingdom

**Abstract:** Voltage-gated potassium channels (Kv) of the Kv3 subfamily play key roles in generating short duration APs, which also permit firing at high frequency in many brain regions, including the auditory brainstem. We have shown that two subunits are of particular importance: Kv3.1 and Kv3.3. Histological studies show that both Kv3.1 and Kv3.3 subunits are expressed in principal neurons of the Medial Nucleus of Trapezoid Body (MNTB) and other nuclei of the superior olivary complex.

The aim of this study was to determine the role of each subunit in generating functional Kv3 channels. Four investigations have been performed: 1. Subcellular immunofluorescent labelling and localization of the Kv3 subunits; 2. Electrophysiological characterization of the outward potassium currents mediated by Kv3.1 or Kv3.3 subunits in MNTB neurons; 3. Examination of homozygous channels mediated by these subunits in cell lines. 4. Use of photo-activated pharmacology to identify the actions of different Kv3 channel subunits.

We show that Kv3.1 and Kv3.3 are localized in MNTB soma membrane axon initial segments. Whole-cell patch clamp was used to characterize outward potassium currents. MNTB neurons possessed Kv3 currents which were blocked by tetraethylammonium (TEA, 1mM), and comparable to Kv3 currents expressed in cell lines. The results show that Kv3.1 and Kv3.3 immunostaining were indistinguishable suggesting similar localization of both subunits in MNTB neurons. To test if these Kv3 channels were heteromers or homomers, we employed light-activated pharmacology based on the MAQ blocker (Fortin et al., 2011).
Single amino acid substitution using CRISPR/Cas9 was used to introduce a mutation at N484C of the Kv3.3 subunit in CBA mice. Preliminary results showed WT and mutant MNTB currents were similar. Whole-cell voltage clamp of N484C homozygous MNTB neurons showed moderate current reduction under 500 nm light following 90 min incubation in 300 uM MAQ (7-8%, n=3); currents were further blocked by 1 mM TEA. Histology and electrophysiological studies show that MNTB neurons maintained Kv3.3 expression after genetic modification. Partial block of MNTB Kv3 channels by light-activated MAQ photo-switching was observed.

Disclosures:  
K. Bondarenko: None. J.P. Smalley: None. M.S. Anderson: None. P.M. Cullis: None. I.D. Forsythe: None.

Poster

283. Potassium Channels II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

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Topic: B.04. Ion Channels

Support:  
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NSF CAREER 1750199  
NIH/NIGMS 5P20GM113132

Title: Regulation of presynaptic Ca\textsuperscript{2+} microdomains and synaptic transmission by K\textsuperscript{+} channel variants

Authors:  
*I. CHO\textsuperscript{1}, S. ALPIZAR\textsuperscript{2}, M. CHIN\textsuperscript{1}, L. PANZERA\textsuperscript{3}, M. B. HOPPA\textsuperscript{2};  
\textsuperscript{2}Biol. Sci., \textsuperscript{3}Biol., \textsuperscript{1}Dartmouth Col., Hanover, NH

Abstract: Presynaptic terminals are fundamental computational units in the brain, and their dysfunction is associated with several neurological diseases. They mediate the transduction of incoming electrical signals (action potentials) into chemical signals (neurotransmitter release), and the efficiency of conversion determines the strength of circuits underlying memory and behavior. Many synapses in the hippocampus display frequency-dependent changes in transduction efficiency. The shape of the presynaptic action potential is of fundamental importance in determining the timing and magnitude of neurotransmitter release. However, the plasticity of action potential waveform shape during frequency dependent stimulation and a role in transduction efficiency is unknown. This is largely due to the fact that the \textit{en passant} synapses that are prevalent in the hippocampus are difficult to measure with classic electrophysiology owing to their small size. To overcome these limitations in our current study we combine a genetically encoded far-red voltage indicator named QuasAr with quantitative measurements of presynaptic calcium and vesicle fusion by GCaMP and vGlut-pHluorin imaging, respectively.
We found significant frequency-dependent changes in presynaptic action potential shape even from paired pulse stimulation in primary cultured rat hippocampal neurons. Namely, a significant broadening of action potentials at excitatory synapses and narrowing at inhibitory synapses were shown. We determined that these changes were due to unique molecular identities and functional role of K⁺ channels that can modulate the electrogenic properties of the presynaptic membrane at excitatory and inhibitory terminals. Our results indicate that Kvβ1-induced inactivation of Kv1.1/1.2 channels underlies the broadening, while calcium-gated potassium channels underlie narrowing of the action potential for excitatory and inhibitory neurons respectively. Furthermore, while the changes in AP shape are strongly correlated with vesicle fusion probability, however they are independent of net calcium influx as classically predicted, suggesting a role for calcium-microdomain signaling. Taken together, these results suggest that variability in presynaptic K⁺ channels may play a fundamental role in controlling frequency-dependent changes in synaptic strength.

**Disclosures:** I. Cho: None. S. Alpizar: None. M. Chin: None. L. Panzera: None. M.B. Hoppa: None.

**Poster**

**283. Potassium Channels II**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

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**Topic:** B.04. Ion Channels

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NIH/NINDS R01#NS073832-01A1 (to AZ)
NIH/NINDS U54 3U54NS083932-05S1 (to MSM/Neuroscience Institute)
R01NS104349 (to ZX)

**Title:** Regulation of neuronal ion channel activity by Polycomb and Trithorax group proteins in unexpected manners

**Authors:** *S. DAVE, T. LENG, Z.-G. XIONG, A. ZHOU;
Neurosci. Inst., Morehouse Sch. of Med., Atlanta, GA

**Abstract:** Polycomb Group (PcG) and Trithorax Group (TrxG) proteins are epigenetic regulators installing either repressive or activating marks, respectively, on histones. PcG proteins may potentially target nearly half of the known K⁺ channel genes in addition to genes involved in cellular stress responses and cell cycle control. Previously we have shown that changes in cellular levels of selected PcG proteins, via gene silencing or over expression, can increase or decrease, respectively, K⁺ currents in neuronal cultures. This led us to consider a potentially novel, previously undescribed role of PcG proteins in regulating neurophysiological properties of
neuronal cells, and whether TrxG proteins may act antagonistically in such regulation. The objective of this study was to determine: 1) Effects of pharmacologic inhibition of PcG proteins on K⁺ channel activity; 2) Specific channel subtypes that may contribute to PcG-mediated changes in K⁺ channel activity; and 3) Effects of inhibition of TrxG proteins on K⁺ channel activity. Patch-clamp analyses were performed on differentiated, mouse brain-derived NS20Y neuroblastoma cells that were treated with one of the following inhibitors: 50 μM PRT 4165, or 100 μM MM-102, or 10 μM PFCBP-1 (antagonists of PcG protein BMI-1, TrxG protein MLL-1 and Creb Binding Protein(CBP, required for TrxG’s activating transcription regulation), respectively). K⁺ currents were recorded with or without appropriate channel blockers. Results to date showed that, inhibition of BMI1 resulted in an increase in whole cell K⁺ currents, and an increase in negativity of the resting membrane potential (RMP). The afore-noted effects were masked by 100 nM alpha-guaniitoxin 1E, suggesting the involvement of Kv2.1, 2.2 and 4.3. Inhibition of TrxG protein MLL-1, unexpectedly, also resulted in an increased K⁺ currents but showed little effects on resting RMP, whereas inhibition of CBP resulted in a decrease in K⁺ currents. Taken together, we conclude that the activity of neuronal K⁺ channels are subject to repressive regulation by PcG proteins that can be achieved through changes in either the expression level or the biochemical activity of PcG proteins. The on-going study focuses on molecular mechanisms that underlie PcG protein-mediated regulation of K⁺ channels, and characterization of the effects of PcG and TrxG proteins (MLL-1 and additional TrxG proteins) on other ion channels and neuronal excitability. Our ultimate goal is to investigate regulatory roles of PcG/TrxG proteins in neuronal stress response via regulation of ion channels.

Disclosures:  S. Dave: None. T. Leng: None. Z. Xiong: None. A. Zhou: None.

Poster

284. Synaptic Transmission: Modulation and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 284.01/B73

Topic: B.06. Synaptic Transmission

Support:  MDA Grant 603852
         NS Grant 090644

Title: Mechanism of action of clinically relevant concentrations of 3,4-diaminopyridine at the neuromuscular junction

Authors: *K. S. OJALA¹, S. P. GINEBAUGH¹, M. WU¹, B. VUOCOLO¹, E. W. MILLER², S. D. MERINEY¹;
¹Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; ²Chem. & Mol. and Cell Biol., Univ. of California, Berkeley, Berkeley, CA
Abstract: The most common treatment for the neuromuscular disease Lambert-Eaton Myasthenic Syndrome (LEMS) is the low dose administration of a potassium channel blocker, 3,4-diaminopyridine (3,4-DAP), which increases the magnitude of transmitter release from the neuromuscular junction (NMJ). The mechanism of 3,4-DAP action is canonically thought to be block of voltage-gated potassium channels, which is hypothesized to prolong the motoneuron action potential. However, a recent debate has centered on other potential effects of 3,4-DAP; namely, direct effects on voltage-gated calcium channels. In particular, it has been proposed that the action of 3,4-DAP on nerve terminal Cav1 type calcium channels may contribute to 3,4-DAP-induced increases in transmitter release; however, these previously published experiments were performed with substantially higher doses of 3,4-DAP than is clinically relevant. To address this debate, we performed a series of experiments. We expressed the most predominant presynaptic potassium channel present at the mammalian NMJ, Kv3.3 (human isoform), in HEK293 cells and used patch-clamp electrophysiology to characterize the concentration-dependent block of potassium current after application of clinically relevant doses of 3,4-DAP (0.5, 1.0, 1.5 uM). In addition, we showed that there was no effect on Cav2.1 or Cav1.2 calcium currents after application of 1.5 uM 3,4-DAP. Furthermore, the presence of the Cav1.2 calcium channel antagonist nitrendipine in ex vivo mouse and frog NMJs did not significantly alter the increase in transmitter release caused by application of 1.5 uM 3,4-DAP, measured via current clamp electrophysiology. Finally, we used a voltage-sensitive dye to measure the motor nerve terminal action potential and showed that ex vivo application of 0.5-1.5 uM 3,4-DAP significantly increased the duration of the presynaptic action potential in a dose-dependent manner in both mouse and frog NMJs. These results strongly support the hypothesis that improvements in neuromuscular function experienced by LEMS patients taking clinically relevant doses of 3,4-DAP are restricted to its mechanism of action on voltage-gated potassium channels and are not due to off-target effects on calcium channels.


Poster

284. Synaptic Transmission: Modulation and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 284.02/B74

Topic: B.06. Synaptic Transmission

Title: 3,4-diaminopyridine enhances muscle contraction strength by increasing asynchronous synaptic vesicle fusion to generate multiple muscle action potentials

Abstract: Aminopyridines are a family of selective potassium channel antagonists that have been investigated as potential treatments for a variety of neurological diseases. Their therapeutic effects are believed to result from inhibition of Kv1 delayed rectifier channels and subsequent broadening of neuronal action potentials. In motor neurons, the extended period of presynaptic depolarization increases activation of voltage-gated calcium channels (VGCCs), resulting in larger Ca\(^{2+}\) currents, enhanced acetylcholine release, and greater depolarization of the muscle membrane. Firdapse, a form of 3,4-diaminopyridine (DAP), has received FDA approval for the treatment of Lambert-Eaton myasthenic syndrome (LEMs) and is in clinical trials for treatment of congenital myasthenic syndromes, myasthenia gravis and spinal muscular atrophy, and has been proposed as a treatment for botulism. In each of these diseases, muscle function is inhibited by reducing end-plate potential (EPP) amplitude below the threshold required to trigger muscle action potentials (mAPs) and muscle contractions. Aminopyridines are believed to therapeutically function by generating EPPs that exceed the muscle fiber threshold potential and thus generate mAPs and muscle contraction. However, aminopyridines enhance the contraction strength of undiseased neuromuscular preparations, suggesting that they promote muscle function through another mechanism. To better understand the mechanisms by which aminopyridines affect muscle function, we analyzed the effects of DAP on presynaptic release, postsynaptic mAP formation and postsynaptic mAP propagation. By inhibiting muscle contraction while maintaining mAPs using the myosin ATPase inhibitor 3-(N-butylethanimidoyl)-4-hydroxy-2H-chromen-2-one (BHC), we find that DAP enhances muscle contraction by generating multiple mAPs. The extra action potentials are not triggered by prolonged EPPs resulting from the synchronous fusion of increased numbers of synaptic vesicles, but instead are a consequence of large numbers of asynchronous vesicle fusions that induce stochastic depolarization of the membrane above the threshold potential required for mAP generation.

Disclosures: J.B. Machamer: None. K.T. Pagarigan: None. P.M. McNutt: None.

Poster

284. Synaptic Transmission: Modulation and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program#/Poster #: 284.03/B75

Topic: B.06. Synaptic Transmission

Title: The role of peptidoglycans receptors in the response to bacterial endotoxin LPS on synaptic transmission: NMJ and CNS

Authors: *C. R. BALLINGER BOONE\(^1\), D. HARRISON\(^1\), R. L. COOPER\(^2\);
\(^1\)Biol., Univ. of Kentucky, Lexington, KY; \(^2\)Dept Biol, Univ. of Kentucky Dept. of Biol., Lexington, KY
Abstract: Gram-negative bacteria produce and release endotoxins in the form of lipopolysaccharides (LPS). The different forms of LPS produce varying secondary immune responses. The direct effect of LPS itself, which occurs in seconds, has not been well studied; however, the receptors which bind LPS were first identified in Drosophila which led to their discovery in mammals. We continue to use Drosophila as a model in these studies. Exposing the heart of larval Drosophila to LPS (500 µg/ml) from Serratia marcescens causes the heart rate to initially increase and then slow down. Whereas exposing the body wall muscle, while stimulating the motor nerve, results in hyperpolarization. Evoked as well as spontaneous excitatory junction potentials become depressed with the presences of LPS in high concentrations. Low concentrations can enhance evoked transmission. The decrease in synaptic transmission is likely due to the postsynaptic glutamate receptors being blocked by LPS. We set out to determine if there was an alteration in the rapid effects upon exposure to LPS in RNAi expressing lines for the peptidoglycan recognition proteins (PGRPs) PGRP-LC and PGRP-LE in body wall muscle, motor neurons and sensory neurons as well as cardiac muscle. Knocking down the receptor expression for PGRP-LC and PGRP-LE did not alter the acute effects of LPS exposure to the body wall muscle and effects on synaptic transmission or heart rate in larval Drosophila. The responses of LPS are not due to NOS, TEA sensitive K+ channels or K(ATP) channels as profiled by agonist and antagonist to these possibilities. We are still investigating CNS effects. Thus, it has yet to be determined the mechanism by which LPS and associated peptidoglycans are causing these rapid cellular changes. This is significant to address potential effects in human and other animals exposed to gram negative bacterial infections.

Disclosures: C.R. Ballinger Boone: None. D. Harrison: None. R.L. Cooper: None.

Poster

284. Synaptic Transmission: Modulation and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 284.04/B76

Topic: B.07. Synaptic Plasticity

Title: Investigating the role of a synaptically targeted intronic IncRNA in memory

Authors: *E. GRINMAN, I. ESPADAS, Y. AVCHALUMOV, S. SWARNKAR, S. V.
PUTHANVEETTIL;
Neurosci., The Scripps Res. Inst., Jupiter, FL

Abstract: Advances in next-generation sequencing have prompted increased attention to non-coding transcriptional elements in neurobiology, with long non-coding RNAs (IncRNAs) representing a significant portion within the central nervous system. However, despite their significance as regulators of transcription and translation, the functions of IncRNAs in long-term memory storage are poorly understood. Through next-generation RNA sequencing and qRT-
PCR, we have uncovered multiple lncRNAs that are enriched in mouse hippocampal synaptic fractions in a signaling pathway that mediates learning in the hippocampus: cAMP/PKA signaling. One such transcript was confirmed by fluorescence in situ hybridization to be transcribed, and targeted to distal processes in a microtubule and actin-dependent manner, and colocalizes with dendritic and synaptic proteins. This lncRNA, termed ADEPTR (Activity DEPendent Transpo

lectronic RNA), is intronic in its genomic origin but is expressed and transported independently of its protein-coding host gene. Functional and mechanistic experiments have demonstrated that this lncRNA 1) is rapidly and robustly expressed and dendritically targeted in a cAMP-dependent manner, 2) mediates cAMP-dependent changes in excitatory synaptic transmission and spine density, 3) is dendritically transported by Kif2a and binds proteins required for cytoskeletal reorganization 4) is expressed in the hippocampal CA1 subregion during contextual fear conditioning (CFC). Together, these results suggest that this lncRNA has a critical functional role in mediating hippocampus-dependent learning. This study is the first to demonstrate that an intronic lncRNA holds such potential to shape neurobiology; future work will elucidate its role in mediating learning in vivo. This may ultimately shape future study of lncRNAs in learning and memory and present intronic lncRNAs as worthwhile targets of memory-related disorders.


Poster

284. Synaptic Transmission: Modulation and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 284.05/B77

Topic: B.06. Synaptic Transmission

Title: Synaptic microRNAs and their role in major depressive disorder pathogenesis

Authors: *Y. YOSHINO*, Y. DWIVEDI;

*Psychiatry and Behavioral Neurobio., Birmingham, AL; Dept of Psychiatry, Univ. of Alabama At Birmingham, Birmingham, AL
Abstract: Major Depressive Disorder (MDD) is a debilitating disease affecting a large population all over the world. Several antidepressants that target monoamines such as serotonin, norepinephrine, and dopamine are available. These antidepressants work by adjusting levels of monoamines specifically in the synapse. The change of monoamine levels in synapse leads to alleviation of depression in patients. At the epigenetic level, microRNAs (miRNAs) are well studied and have emerged as a regulator of neural plasticity and higher brain functioning. Indeed, miRNAs changes have been reported in human blood and postmortem brain. To our knowledge, there is no report that focuses on miRNAs change in synaptosomes. This study aims to reveal the miRNAs changes in synaptosome of human postmortem brain related to MDD. The study was conducted in dorsolateral prefrontal cortex (dIPFC) of 15 non-psychiatric controls and 15 MDD subjects. The synaptic fraction was isolated by sucrose gradient and ultra-centrifugation methods. miRNAs were examined in both total and synaptosome fractions using small RNA sequencing. We found 13 miRNAs to be upregulated and 7 miRNAs were downregulated in the synaptic fraction from MDD subjects. From the pathway analysis, it appears that synaptic functions are significantly altered which occurs via changes in miRNAs. In addition, neuroinflammatory pathways were also detected. These pathways may provide new insight into how miRNAs may play a role in altering synaptic functions and their involvement in MDD pathogenesis.

Disclosures: Y. Yoshino: None. Y. Dwivedi: None.

Poster

284. Synaptic Transmission: Modulation and Mechanisms I

Location: Hall A

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Program #/Poster #: 284.06/B78

Topic: B.06. Synaptic Transmission

Support: DFG (ZI 1224/4-1)
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Title: A new mechanism of synaptic activity regulation through DNA methylation

Authors: *D. PENSOLD¹, C. BAYER¹, K. VAN LOO³, N. CIGANOK², A. HAHN⁵, J. REICHARD¹, L. LIEBMANN⁵, J. GROß⁵, T. LINGNER⁶, G. SALINAS-RIESTER⁶, C. HALFMANN², T. PIETER⁷, C. HUEBNER⁵, H. VATTER⁴, B. KAMPA², A. BECKER³, G. ZIMMER-BENSCH¹;
Abstract: Deciphering the cellular and molecular mechanisms underlying learning and memory, in which inhibitory interneurons play a crucial role, is a fundamental goal in neuroscience. Epigenetic signatures execute transcriptional control and were shown to be dynamically remodeled in the adult nervous system. The emerging evidence for activity-dependent DNA methylation executed by DNA methyltransferases (DNMTs) and TET-mediated DNA demethylation in synaptic function irrevocably raises the question for the targeted subcellular processes and mechanisms. Indeed, we found improved inhibitory synaptic transmission in electrophysiological recordings using a conditional DNMT1 knockout mice model specific for parvalbuminergic interneurons. Transcriptome and methylome analysis on FACS-enriched interneurons of wild-type and DNMT1 deficient mice as well as in vitro approaches provide evidence for differential modulation of processes involved in synaptic vesicle replenishment. Further, the analysis of human brain samples suggests that this mechanism of synaptic function regulation by DNA methylation could potentially contribute to the pathophysiology of temporal lobe epilepsy. In summary, we here provide evidence for DNA methylation-dependent modulation of synaptic transmission in cortical interneurons by acting on synaptic vesicle replenishment.


Poster 284. Synaptic Transmission: Modulation and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 284.07/B79

Topic: B.07. Synaptic Plasticity

Support: Boehringer Ingelheim PhD Fonds

Title: Cell type-specific action of the alternative splicing factor SLM2 for specifying synaptic properties

Authors: *L. TRAUNMÜLLER, E. FURLANIS, G. FUCILE, P. SCHEIFFELE; Univ. of Basel, Basel, Switzerland
Abstract: Neuronal circuits consist of hierarchical assemblies of highly specialized neuronal cell types. The intrinsic properties of neurons and the functional specification of their synapses are fundamental for how circuits process information. However, how different classes of neurons orchestrate their synaptic and functional properties remains largely unclear. We are testing the hypothesis that post-transcriptional mechanisms, like alternative splicing, play a central role in the regulation of cell type-specific properties. We systematically mapped ribosome-associated transcript isoforms in genetically defined cell populations in the mouse neocortex and hippocampus. This approach revealed complex and highly distinct alternative splicing programs for the control of synaptic proteins and intrinsic neuronal properties— even within closely related classes of pyramidal cells and inhibitory neurons. Since alternative splicing is regulated by RNA-binding proteins, we further sought to identify cell type-specific RBPs that might regulate these distinct splicing programs. Interestingly, we found that different cell populations exhibit highly selective gene expression patterns for RBPs. This includes the alternative splicing factor SLM2 which is selectively expressed in subpopulations of excitatory and inhibitory neurons in the mouse brain. Cell type-specific loss-of-function studies for SLM2 combined with cell type-specific transcript isoform profiling, indicate that SLM2 regulates alternative splicing of different transcripts in hippocampal somatostatin positive interneurons and CA1 pyramidal cells. Loss of SLM2 does not affect general neuronal properties but rather results in modulation of the functional specification of synapses. Thus, our work reveals a major role for cell type-specific alternative splicing programs in the genetic determination of neuronal circuit specificity and function.


Poster

284. Synaptic Transmission: Modulation and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 284.08/B80

Topic: B.06. Synaptic Transmission

Support: Consejo Nacional de Ciencia y Tecnología (CONACyT) Grant #221568
Bowes Laboratories, Inc. (BOWE) Grant #123456
Consejo Nacional de Ciencia y Tecnología (CONACyT) Grant #220448
Consejo Nacional de Ciencia y Tecnología (CONACyT) Grant #299057

Title: Adhesion GPCR Latrophilin-1 simultaneous coupling to both Gαs and Gα12/13 proteins pathways is dictated by an intracellular alternatively splice insert

Authors: *J. C. OVANDO-ZAMBRANO¹, J. A. ARIAS-MONTAÑO¹, A. A. BOUCARD²; ¹Dept. de Fisiología, Biofísica y Neurociencias, ²Dept. de Biología Celular, Ctr. de Investigación
Abstract: ADGRL1/latrophilin-1 (Lphn1) is a member of the autoproteolytically-cleaved subfamily of adhesion G protein-coupled receptors (aGPCR) that participate in the formation of synapses through heterophilic interactions with its endogenous ligands such as neurexin-1β (nrx-1β) or fibronectin-leucine-rich transmembrane protein 3 (FLRT3), which allow the Lphn1-ligand complex to mediate intercellular adhesion. The gene encoding Lphn1 undergoes alternative splicing which results in receptor variants bearing a modification between two N-terminally located extracellular adhesion motifs (SSA) and within the C-terminal tail (SSB). These splicing events potentially affect receptor structure and consequently can also modify its function. While splicing at SSA has been shown to modify Lphn1 ligand-binding functions, the role of SSB in receptor functions remained elusive. However, because of its intracellular location SSB is likely to affect receptor trafficking to and from the cell membrane and/or its coupling to the cell signaling machinery. Thus, we analyzed the effect of SSB splicing on Lphn1 expression/trafficking, binding and signaling properties in HEK293T cells. Immunodetection of Lphn1 receptor variants revealed that SSB splicing did not affect their expression, their processing or their trafficking to the cell membrane, as steady state levels were identical for both proteins. Binding assays conducted with soluble ligands nrx-1β and FLRT3 unveiled that both SSB receptor variants possessed similar binding properties. Signaling properties of Lphn1 SSB variants were evaluated by measuring ligand-independent and ligand-dependent modulation of intracellular Ca\(^{2+}\) concentrations, activation of MAP kinases and regulation of cAMP levels. No changes between receptor variants were detected in both Ca\(^{2+}\) mobilization and MAP kinase activation assays. On the other hand, cAMP accumulation assays revealed that Lphn1+SSB expression constitutively and ligand-dependently increased intracellular levels of this second messenger in HEK293T cells while expression of Lphn1-SSB did not. Moreover, ligand-dependent inhibition of forskolin-induced cAMP production was observed for Lphn1-SSB and surprisingly for Lphn1+SSB expression, an effect that was mediated by the activation of G\(^{α/β}\)i/o proteins, thus suggesting that SSB splicing modulates Lphn1 dual activation of G\(^{α}\)i/o proteins leading to opposite cAMP signaling profiles. These data bring a greater understanding of the role of Lphn1 in coupling adhesion to intracellular signaling which support synapse formation events.


Poster

284. Synaptic Transmission: Modulation and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 284.09/B81
Title: Rapid actions of anti-Müllerian hormone in regulating synaptic transmission and long-term synaptic plasticity in the hippocampus


Abstract: Anti-Müllerian hormone (AMH) is a glycoprotein that was originally recognized as a fetal hormone generated by the testicular Sertoli cells to regulate sexual differentiation in mammalian species. Recent studies have shown that AMH acts as a neuroactive peptide factor in regulating neuronal viability and AMH-specific receptor AMHR2 is expressed in various brain regions including the hippocampus. However, little is known about the AMH effects on neuronal activities in hippocampus. In this study, we aimed to investigate AMH actions in regulating CA3-CA1 excitatory synaptic transmission and long-term synaptic plasticity in the hippocampus. Brain tissue was obtained from wild-type male and female CD-1 mice (6-8 weeks old).

Following transcardial perfusion and post-fixation by 4% paraformaldehyde, mouse brains were frozen-sectioned for immunohistochemistry using anti-AMHR2 antibody. The direct actions of AMH on neuronal activities were examined by extracellular field recordings in hippocampal slices with and without 0.4 nM recombinant AMH protein addition. The efficiency of synaptic transmission is determined from plots of the slope of the extracellular field excitatory postsynaptic potentials (fEPSPs) against the amplitude of fiber volley (FV) evoked by synaptic stimuli of varying intensities. Whole-cell current-clamp recordings in hippocampal slices were used to determine AMH-mediated postsynaptic modulation of synaptic transmission. Data from female mice showed that AMHR2 was primarily localized in the hippocampal pyramidal neurons. Acute slices exposed to AMH displayed a robust increase in the efficiency of synaptic transmission relative to controls (0.61 ± 0.06 ms^{-1} versus 0.29 ± 0.03 ms^{-1}; p < 0.001). AMH addition also significantly boosted long-term synaptic potentiation, measured by the ratios of fEPSP slopes 5 minutes before and 15 minutes after delivery of high frequency train, compared to controls (168.9 ± 7.0 % versus 140.8 ± 6.6 %; p < 0.01). These results suggested enhanced information coding following AMH exposure. In addition, AMH treatment acutely increased excitatory postsynaptic potentials in CA1 pyramidal neurons (163.1 ± 16.3 %; p < 0.01). Male mice followed the same trend as females in AMHR2 expression and electrophysiological properties upon AMH exposure. Our findings provide functional evidence that AMH directly regulates synaptic transmission and long-term synaptic plasticity in the hippocampus. These data suggest a possible role of AMH in learning and memory, and the potential of AMH as a therapeutic target in preventing memory lost or treating cognitive diseases in the general population.

Poster

284. Synaptic Transmission: Modulation and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 284.10/B82

Topic: B.06. Synaptic Transmission

Title: Signaling by microglia-enriched cytokine tweak through the neuronal receptor Fn14 modulates synaptic neurotransmission and plasticity

1Biomarkers, 2Acute Neurol., 3Biogen, Cambridge, MA

Abstract: Synaptic plasticity plays a critical role in the development of the CNS through synaptic strengthening and pruning, however these processes may become dysregulated or aberrant in the context of disease. While the molecular mediators of plasticity are not fully understood, emerging evidence may suggest bidirectional crosstalk between neurons and surrounding glia influence this neuroplasticity. We postulated that signaling by the microglia-enriched cytokine, TWEAK, through its neuronally-expressed receptor, Fn14, regulates synaptic physiology and pathophysiology based on the recent discovery of Fn14 as a molecular mediator of synaptic refinement in experience-dependent visual system development. Through the use of acute hippocampal (HC) brain slices and slice cultures, we demonstrate that recombinant TWEAK dose dependently dampens both basal synaptic neurotransmission (BSN) and long-term potentiation (LTP) while inhibition of endogenous TWEAK by an anti-TWEAK antibody augments these synaptic functions. We determined that the effects of TWEAK were Fn14-dependent by testing genetic Fn14 knockout (Fn14KO) mice as a tool to globally inhibit Fn14 signaling. We established that naïve adult Fn14KO mice do not exhibit any functional difference in normal synaptic physiology compared to wildtype control (WT) animals. In addition, we dissected the cell type specificity of the observed effects with the use of a hairpin targeted to knockdown Fn14. Hairpins were packaged into neuronal specific adeno-associated viruses and injected into WT animals, delineating that TWEAK modulates BSN and synaptic plasticity through neuronal Fn14. TWEAK treatment increased the paired-pulse ratio of excitatory postsynaptic potentials and decreased the frequency of spontaneous miniature excitatory postsynaptic currents while it had no effect on its amplitudes, suggesting that it modulates BSN through presynaptic mechanisms. To further understand the molecular mechanisms involved in the synaptic modulatory effect of the TWEAK/Fn14 pathway we ran phosphoproteomic analysis on TWEAK-treated acute HC slices from both WT and Fn14KO animals. We identified 243 Fn14-dependent TWEAK-induced phosphoproteomic hits, of which 68 (~27%) are synapse annotated. The broad impact of TWEAK/Fn14 signaling on the phosphorylation state of critical synaptic proteins supports a general role in synapse modulation. Activation of the pathway
dampens BSN and plasticity through presynaptic mechanisms while inhibition of the pathway improves synaptic physiology. We posit that inhibition of TWEAK/Fn14 signaling may be beneficial in diseases featuring synapse loss or reduced function.

**Disclosures:**  
**D. Nagy:** A. Employment/Salary (full or part-time); Biogen.  
**K. Ennis:** A. Employment/Salary (full or part-time); Biogen.  
**S. Su:** A. Employment/Salary (full or part-time); Biogen.  
**R. Gu:** A. Employment/Salary (full or part-time); Biogen.  
**R. Wei:** A. Employment/Salary (full or part-time); Biogen.  
**C. Hinckley:** A. Employment/Salary (full or part-time); Biogen.  
**K. Li:** A. Employment/Salary (full or part-time); Biogen.  
**B. Gao:** A. Employment/Salary (full or part-time); Biogen.  
**M. Hajos:** A. Employment/Salary (full or part-time); Biogen.  
**L.C. Burkly:** A. Employment/Salary (full or part-time); Biogen.

**Poster**

**284. Synaptic Transmission: Modulation and Mechanisms I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 284.11/B83

**Topic:** B.06. Synaptic Transmission

**Support:**  
KAKENHI JP26290006  
KAKENHI JP16K11482  
MSIP 2008-0062282  
NRF-2017M3C7A1025602

**Title:** Inhibition of GluR current in microvilli of sensory neurons via Na⁺-microdomain coupling among GluR, HCN channel and Na⁺/K⁺ pump

**Authors:**  
*Y. KANG*¹, M. SAITO², Y. BAE³, S. OH⁴;  

**Abstract:** Glutamatergic dendritic EPSPs evoked in cortical pyramidal neurons are depressed by activation of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels expressed in dendritic spines. This depression has been attributed to shunting effects of HCN current (Iₜ) on input resistance or Iₜ deactivation. Primary sensory neurons in the rat mesencephalic trigeminal nucleus (MTN) have the somata covered by spine-like microvilli that express HCN channels. In rat MTN neurons, we demonstrated that Iₜ enhancement apparently diminished the glutamate receptor (GluR) current (IₜGluR) evoked by puff application of glutamate/AMPA and enhanced a transient outward current following IₜGluR (OT-IₜGluR). This suggests that some outward current opposes inward IₜGluR. The IₜGluR inhibition displayed a U-shaped voltage-dependence with a
minimal inhibition around the resting membrane potential, suggesting that simple shunting effects or deactivation of \( I_h \) cannot explain the U-shaped voltage-dependence. Confocal imaging of Na\(^+\) revealed that GluR activation caused an accumulation of Na\(^+\) in the microvilli, which can cause a negative shift of the reversal potential for \( I_h \) (\( E_h \)). Taken together, it was suggested that \( I_{GluR} \) evoked in MTN neurons is opposed by a transient decrease or increase in standing inward or outward \( I_h \), respectively, both of which can be caused by negative shifts of \( E_h \), as consistent with the U-shaped voltage-dependence of the \( I_{GluR} \) inhibition and the OT-\( I_{GluR} \) generation. An electron-microscopic immunohistochemical study revealed the colocalization of HCN channels and glutamatergic synapses in microvilli of MTN neurons, which would provide a morphological basis for the functional interaction between HCN and GluR channels. Mathematical modeling eliminated the possibilities of the involvements of \( I_h \) deactivation and/or shunting effect and supported the negative shift of \( E_h \) which causes the U-shaped voltage-dependent inhibition of \( I_{GluR} \).

Disclosures: Y. Kang: None. M. Saito: None. Y. Bae: None. S. Oh: None.

Poster

284. Synaptic Transmission: Modulation and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 284.12/B84

Topic: B.06. Synaptic Transmission

Title: Cell type specific zinc modulation of excitatory synaptic transmission in mouse hippocampus

Authors: *J. N. TANG, G. SOLER-LLAVINA;

Abstract: Zinc is implicated in playing a vital role in neurophysiological homeostasis, with zinc dysregulation being associated with neurological and psychiatric disorders. Zinc in the brain is bound within proteins, where it plays catalytic, regulatory, and structural roles. In certain brain regions, 10-15% of zinc localizes to synaptic vesicles at excitatory terminals, where it is coreleased with glutamate in an activity-dependent manner. Accumulating evidence establishes zinc as a modulator of synaptic transmission, plasticity, and information processing. However, how zinc modulates synaptic properties in specific cell types remains poorly understood. In this study, we use transgenic mouse lines to assess the role of synaptic zinc in modulating excitatory synaptic transmission at defined interneuron populations relative to pyramidal neurons in mouse hippocampal brain slices. We find that zinc differentially modulates synaptic transmission at specific synapses, suggesting that zinc plays an important role in controlling excitatory-inhibitory balance and ultimately network function.
Disclosures:  J.N. Tang: None. G. Soler-Llavina: None.

Poster

284. Synaptic Transmission: Modulation and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 284.13/B85

Topic: B.06. Synaptic Transmission

Support: NIH Grant 5R01DA040630-02

Title: Heterosynaptic GABA<sub>B</sub> receptor function gates glutamatergic transmission in the nucleus accumbens

Authors: *K. M. MANZ;
Anesthesiol., Vanderbilt Univ. Sch. of Med., Nashville, TN

Abstract: The nucleus accumbens (NAc) integrates distinct synaptic inputs to facilitate goal-directed motivational output. Medium spiny neurons (MSNs), segregated based on the expression of D1 or D2 dopamine receptors, mediate NAc output by projecting to functionally divergent brain regions. Glutamatergic afferents to the NAc drive feedforward inhibition mediated by parvalbumin (PV)-expressing interneurons (INs) and are critically involved in reward-related behavioral states, including those elicited by drugs of abuse. The GABA<sub>B</sub> heteroreceptor (GABA<sub>B</sub>R), a G<sub>i/o</sub>-coupled G protein-coupled receptor (GPCR), is highly expressed at glutamatergic synapses throughout the mesolimbic reward network, yet its physiological role and molecular mechanism at these synapses remains unknown. Here, we explored GABA<sub>B</sub>R function at glutamatergic synapses within PV-IN-embedded microcircuits in the NAc core. Using transgenic reporter mice, optogenetics, and whole-cell patch-clamp electrophysiology, we found that presynaptically-expressed GABA<sub>B</sub>R recruits a non-canonical SNAP-25-dependent signaling mechanism to reduce glutamatergic synaptic efficacy at D1(+) and D1(-) [putative D2] MSN subtypes. Furthermore, PV-INs heterosynaptically regulate glutamatergic transmission onto D1(+) MSNs by targeting presynaptic GABA<sub>B</sub>R. Cocaine withdrawal abolished this plasticity and unmasked cell type-specific GABA<sub>B</sub>R-induced long-term depression (LTD). These findings extend the current model of how PV-INs regulate NAc circuit function and refine mechanisms by which GABA<sub>B</sub> heteroreceptors modulate glutamatergic transmission in the NAc core.

Disclosures:  K.M. Manz: None.
Title: Alterations of CB1-mediated excitatory neurotransmission following chronic mild stress during adolescence at the CA1 dendritic layer


Abstract: Work in our lab demonstrated that hippocampal CB1 receptor (CB1R) levels are lower in female adolescent animals compared to males (Reich et al., 2009). Following 21-day exposure to chronic mild stress, CB1R density increased in females while decreasing in males; thus suggesting that hippocampal CB1 responds differentially to stress in a sex-dependent manner. Several other lines of converging evidence clearly indicate a functional sex difference in the endocannabinoid system and behavioral reactions to exogenous cannabinoids in both humans and animals (Rubino and Paralaro, 2011). However, there remains a paucity of data exploring how these sex differences are manifested physiologically following chronic mild stress in either sex. We, therefore, investigated adolescent sex and stress-dependent variance in rat endocannabinoid function throughout the CA1 dendrosomatic layer. Field excitatory postsynaptic potentials (fEPSPs) were recorded from CA1 in male and female adolescent Sprague-Dawley rats (40-60 d.o.) 400 μm hippocampal slices. All drugs were bath applied after a 10-min baseline with appropriate positive and negative drug controls as necessary. All sample sizes were greater than n=5, the size needed for 80% statistical power. The present data demonstrates that exogenous activation of CB1 (CB1 agonist, WIN 55-212-2) enhances excitatory neurotransmission (fEPSPs) in the CA1 area of female hippocampal slices, while it classically decreases excitatory transmission in males. The latter is due to a CB1-mediated suppression of glutamate release. In females, we provide evidence that CB1-modulation of excitatory neurotransmission results from enhanced CB1-mediated suppression of GABAergic neurotransmission (inhibitory) that is attenuated following CMS introduction during adolescence. Further observations suggest that the following contribute to the mechanism for augmented suppression of inhibition in females: 1) an attenuation of constitutive CB1 activity at GABAergic synapses, 2) the persistent presence of tonic 2-AG release irrespective of CMS induction and 3) the cessation of CB1-mediated estrogen receptor α-driven eCB production.
These results clearly demonstrate sex and stress-dependent differences in the dendritic layer of CA1 and further elaborate upon a multitude of sex differences found throughout the entirety of the CA1 dendrosomatic axis.

**Disclosures:** A.R. Ferraro: None. P.A. Wig: None. S.M. O'Sullivan: None. N.M. Amada: None. J.R. Lopez: None. B. Haner: None. Z. Mall: None. A. Kemp: None. C.G. Reich: None.

**Poster**

284. Synaptic Transmission: Modulation and Mechanisms I

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program#/Poster #:** 284.15/B87

**Topic:** B.06. Synaptic Transmission

**Title:** Cholinergic gain control of the claustrum

**Authors:** *A. NAIR*¹,², M. GRAF¹, G. J. AUGUSTINE¹;

**Abstract:** The claustrum is the most interconnected region of the brain and therefore requires mechanisms to distinguish and weight its myriad of inputs and outputs. Cholinergic signaling from the basal forebrain is hypothesized to discern and selectively enhance neuronal circuit output through the control of gain, i.e. the sensitivity of the output of individual neurons to their input. We therefore investigated the ability of cholinergic drive to modulate the gain of claustrum neurons. Whole-cell patch clamp recordings were used to measure claustrum neuronal responses to photostimulation of cholinergic inputs in brain slices prepared from a ChAT-ChR2 transgenic mouse line. Input-output (I-O) relationships were measured in individual neurons as the frequency of action potentials evoked by depolarizing currents of different intensities. Fluorescent retrogradely transported beads were injected into cortical and subcortical structures to identify claustrum neurons that project to these targets. We observed target-specific regulation of claustrum neurons by cholinergic input: this input evoked significantly larger polysynaptic excitation and significantly smaller polysynaptic inhibition in claustrum subcortical (CS) projection neurons in comparison to claustrum-cortical (CC) projection neurons. Using synaptic blockers to isolate direct synaptic responses, we found that a sparse subset of CS neurons and VIP interneurons in the claustrum received excitation mediated by nicotinic receptors. This cholinergic excitation caused an increase in the slope of I-O curves, indicating an increase in the gain of these neurons. Remarkably, we also discovered a direct inhibition from cholinergic input that was mediated by GABA_A receptors and preferentially targeted a sparse subset of CC projection neurons. This inhibitory input elicited a decrease in the slope of I-O curves, representing a reduction in gain of these neurons. In summary, cholinergic neuromodulatory input is capable of eliciting opposing, cell-type specific effects on neuronal gain in the claustrum.
We hypothesize that such gain control may provide a general mechanism for the claustrum to selectively process incoming signals and maintain discernibility of its outputs under behavioral states of high cholinergic drive such as directed attention.

**Disclosures:** A. Nair: None. M. Graf: None. G.J. Augustine: None.

**Poster**

284. Synaptic Transmission: Modulation and Mechanisms I

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 284.16/B88

**Topic:** B.06. Synaptic Transmission

**Support:** NIH grant NS065957
NIH grant NS066932
JSPS KAKENHI grant 25860193
NSF grant IOS-0843585

**Title:** Adenosine protects neurosynaptic transmission against experimental ischemia, hypoxia, or hypoglycemia in the mouse hippocampus

**Authors:** M. KAWAMURA, Jr1, *D. N. RUSKIN2, S. A. MASINO2;
1Pharmacol., Jikei Univ. Sch. Med., Tokyo, Japan; 2Dept. of Psychology, Trinity Col., Hartford, CT

**Abstract:** Adenosine receptors are widely expressed in the brain and adenosine is a key bioactive substance for neuroprotection. Here, we clarify systematically the role of adenosine A1 receptors during a range of timescales and conditions where large amounts of adenosine are released. Using acute hippocampal slices obtained from mice that were wild type or null mutant for the adenosine A1 receptor we quantified and characterized the impact of varying durations of experimental ischemia, hypoxia and hypoglycemia on synaptic transmission in the CA1 subregion. In normal tissue, these three stressors markedly reduced synaptic transmission, and treatment of sufficient duration led to incomplete recovery. Whereas inactivation of adenosine A1 receptors delayed and/or lessened the reduction in synaptic transmission during all three stressors, the lack of functional adenosine A1 receptors resulted in incomplete recovery. We reproduced the differing responses to hypoxia and hypoglycemia by applying an adenosine A1 receptor antagonist, validating the clear effects of genetic receptor inactivation on synaptic transmission. These studies clarify the neuroprotective role of the adenosine A1 receptor during a variety of metabolic stresses, and reveal the consequences of adenosine A1 receptor activation on synaptic transmission during the acute phase of ischemia, hypoxia or hypoglycemia, and during recovery from these events.
Disclosures: M. Kawamura: None. D.N. Ruskin: None. S.A. Masino: None.

Poster

284. Synaptic Transmission: Modulation and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 284.17/B89

Topic: B.06. Synaptic Transmission

Support: Colorado State University

Title: Reactive oxygen species signaling modulates glutamate receptor transport

Authors: *R. L. Dosering1, G. AMBERG1, F. J. HOERNDL1;
1Biomed. Sci., 2CVMBS, Colorado State Univ., Fort Collins, CO

Abstract: In neurons, changes in the subcellular localization and expression of the AMPAR subtype of ionotropic glutamate receptors (AMPARs) is necessary for learning and memory. These receptors are primarily translated in the cell body and transported through neuronal processes to their destinations by molecular motors. This means that their localization, and therefore synaptic plasticity, requires proper intracellular transport to the synapse. Recent studies have determined that synaptic plasticity is abnormal in the presence of non-physiological levels of reactive oxygen species (ROS), byproducts of normal energy production. In some cell types, ROS are known to modulate voltage-gated calcium channels (VGCC). Our lab and others have shown that calcium signaling and CaMKII play an essential role in activity-dependent regulation of AMPAR transport. Together, these findings led us to ask: does physiological ROS-signaling modulate AMPAR transport and synaptic function in vivo? To visualize fluorescently tagged AMPARs in vivo in real-time we utilize the transparent genetic model C. elegans. This approach revealed that in the presence of increased ROS, AMPAR transport is decreased resulting in hindered AMPAR delivery and exocytosis at synapses. Using a genetic epistasis strategy, we determined that ROS regulate AMPAR transport by acting on or directly downstream of VGCC, but upstream of CaMKII. In addition, using the genetically encoded ROS sensor roGFP and calcium sensor GCaMP, we verified that pharmacological and genetic elevation of ROS leads to decreased intracellular calcium signaling in vivo. Using a combination of genetically-encoded tools and electrophysiology techniques, future experiments will determine if ROS regulate AMPAR localization via direct modulation of VGCC. Our in vivo studies will provide insight as to why elevation of ROS levels is correlated with abnormal synaptic plasticity and cognitive function in the aged and diseased brain.

Disclosures: R.L. Doser: None. F.J. Hoerndli: None.
Title: Cerebellar modulation of mesolimbic dopamine transmission is functionally asymmetrical

Psychology, Univ. of Memphis, Memphis, TN

Abstract: Cerebral and cerebellar hemispheres are known to be asymmetrical in structure and function, and previous literature supports that asymmetry extends to the neural dopamine systems. Using in vivo fixed-potential amperometry with carbon-fiber microelectrodes in anesthetized mice, the current study assessed hemispheric lateralization of stimulation-evoked dopamine in the nucleus accumbens (NAc) and the influence of the cerebellum in regulating this reward-associated pathway. Our results suggest that cerebellar output can modulate mesolimbic dopamine transmission, and this modulation contributes to asymmetrically lateralized dopamine release. Dopamine release did not differ between hemispheres when evoked by medial forebrain bundle (MFB) stimulation; however, dopamine release was significantly greater in the right NAc relative to the left when evoked by electrical stimulation of the cerebellar dentate nucleus (DN). Furthermore, cross-hemispheric talk between the left and right cerebellar DN does not seem to influence mesolimbic release given that lidocaine infused into the DN opposite to the stimulated DN did not alter release. These studies may provide a neurochemical mechanism for studies identifying the cerebellum as a relevant node for reward, motivational behavior, saliency, and inhibitory control. An increased understanding of the lateralization of dopaminergic systems may reveal novel targets for pharmacological interventions in neuropathology of the cerebellum and extending projections.

Title: The phosphorylation status of the Kv3.4 channel N-terminal inactivation domain regulates nociceptive signaling in the DRG and superficial dorsal horn

Authors: *M. COVARRUBIAS, L. ZHI, T. MUQEEM, T. ALEXANDER; Neurosci., Sidney Kimmel Med. Col. of Thomas Jefferson Univ., Philadelphia, PA

Abstract: The Kv3.4 potassium channel shapes the repolarization of the action potential (AP) in nociceptors of the dorsal root ganglion (Ritter et al., J. Physiol., 2012), and thereby determines the strength of synaptic transmission in the superficial dorsal horn (Muqeem et al., J. Neurosci., 2018). This regulation depends on inactivation of Kv3.4, which is modulated by phosphorylation of four serines within the Kv3.4 N-terminal inactivation domain (NTID) (Covarrubias et al., Neuron, 1994). If the NTID is phosphorylated, Kv3.4 inactivation is slowed/eliminated, the AP is shortened and nociceptive signaling is dampened. However, whether or not this modulation impacts nociception in vivo remains unknown, and the translational potential of manipulating the Kv3.4 phosphorylation status and activity has not been explored. We are making Kv3.4 phosphorylation mutants and implementing an AAV-based approach to test the effects of manipulating inactivation on nociceptive signaling in vitro, using electrophysiological and optogenetic approaches, and in vivo, using behavioral protocols. Initial results have shown that the expression of the phosphomimetic Kv3.4 in embryonic DRG neurons increases the total high voltage-activating outward current, which exhibits an increased sustained level. Furthermore, expression of the phosphomimetic Kv3.4 induces shortening of the AP and accelerated maximum rate of AP repolarization in these neurons. Ongoing work is characterizing a Cre-dependent mouse line and the Kv3.4-null mouse to investigate the in vivo roles of Kv3.4 in nociception.

Disclosures: M. Covarrubias: None. L. Zhi: None. T. Muqeem: None. T. Alexander: None.

Poster

284. Synaptic Transmission: Modulation and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 284.20/B92

Topic: B.06. Synaptic Transmission
Title: Patterning interfaces at the nanoscale promotes axonal regeneration in lesion hippocampal organ cultures

Authors: *I. CALARESU*¹, I. RAGO², L. BALLERINI³, D. SCAINI⁴; ¹Intl. Sch. For Advanced Studies (SISSA), Trieste, Italy; ²Elettra, Trieste, Italy; ³Neurosci., SISSA-ISAS, Trieste, Italy; ⁴Neurobio. Sector, Intl. Sch. For Advanced Studies, Trieste, Italy

Abstract: The ability of neurons to sense and adapt to environmental features at the nanoscale offers the opportunity to manufacture ad hoc substrate mechanical and topographical cues to investigate their role in promoting axonal regeneration and synapse formation. Among the variety of nanomaterials developed in these efforts, carbon nanotubes (CNTs) represent one of the more promising. In previous works we demonstrated that CNTs are excellent growth surfaces for neurons, boosting neuronal activity, synaptogenesis and enhancing neural signal transmission. We recently manufactured a novel CNT-based substrate, by growing CNTs as an ultrathin forest directly onto a fused silica substrate, resulting in a novel transparent interface enriched with CNTs (tCNTs); we further investigate tCNT ability in affecting hippocampal circuit formation in vitro. By using whole cell patch-clamp recording, we extensively explored neurons-material relationship with particular attention to the synaptic adaptation of dissociate hippocampal cells coupled to nanostructured substrates. Neuroglial and neuronal cell density and morphology have been investigated through immunofluorescence and confocal microscopy. We further exploited this interdisciplinary approach to test the ability of tCNT in interfacing more complex tissues, such as brain organ cultures. We used organotypic entorhino-hippocampal slice cultures, interfaced to tCNT intact (control) and upon a severe transection of the perforant path segregating the enthorinal cortex and hippocampal formation, which were co-cultured physically separated by a fixed distance (lesion). Simultaneous extracellular recordings were obtained by positioning two electrodes in the granule cells layer of the dentate gyrus and in the deep layers of the entorhinal cortex. Local field potential (LFP) were used to evaluate the degree of functional reconnection 10 days in vitro post lesion. We stimulated the superficial layers of the entorhinal cortex to evoke responses in the dentate gyrus and in the deep entorhinal cortex. Surprisingly, spontaneous LFPs from control and lesion cultures were always more synchronized when slices were interfaced to tCNTs, in respect to flat substrates. Evoked LFP were detected in 100% of lesion cultures on tCNT, indicating perforant path partial regeneration, through axons-nanomaterial interactions. This result is supported by a greater axonal regrowth when slices were interfaced to tCNT, measured by confocal microscopy. Such a multidisciplinary approach, combining surface nano-topography and neural cells assembly (dissociated and explant) may highlight new concepts for tissue engineering.

Poster

284. Synaptic Transmission: Modulation and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 284.21/B93

Topic: B.06. Synaptic Transmission

Title: Functional modulation of PV interneuron by sleep/wake cycle

Authors: F.-J. ZONG¹, Z. CHEN², X.-T. ZHANG¹, X. MIN¹, H. YAO², *K.-W. HE¹;
¹IRCBC, Chinese Acad. of Sci., Shanghai, China; ²ION, Chinese Acad. of Sci., Shanghai, China

Abstract: Parvalbumin positive (PV+) interneurons are the most abundant inhibitory neurons in cortices. They play pivotal roles in maintaining the stability of the neural network and increasing the computational power of the circuit. Dysfunction in PV interneuron is commonly associated with varied brain disorders, further underscoring its importance. However, how PV interneuron is regulated is far from clear. Here we found that both synaptic and neuronal function of PV interneurons are tightly modulated during the light/dark cycle, during which sleep and experience are critically involved. We further showed that such modulation have significant impact on the microcircuit properties and brain function, suggesting that it is a physiologically important regulatory mechanism.

Disclosures: F. Zong: None. Z. Chen: None. X. Zhang: None. X. Min: None. H. Yao: None. K. He: None.

Poster

285. Long-Term Depression and Spike Timing-Dependent Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 285.01/B94

Topic: B.07. Synaptic Plasticity

Support: Government “Ministerio de Economía y Competitividad” (SAF2013-47989-R)
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CIBERNED CB06/05/1104
PIE13/00027
Generalitat de Catalunya (2014SGR1609)
Fundació La Marató de TV3 (201414-30)
Title: SIVA 1 modulates XIAP interaction with the death receptor antagonist FAIM-L to regulate apoptosis and synaptic function

Authors: E. COCCIA¹, L. PLANELLS-FERRER¹, R. BADILLOS-RODRIGUEZ², M. PASCUAL³, M. F. SEGURA¹, R. FERNANDEZ-HERNANDEZ⁴, J. LOPEZ-SORIANO², E. GARI⁴, E. SORIANO³, B. BARNEDA-ZAHONERO², R. S. MOUBARAK², M. J. PEREZ-GARCIA², *J. X. COMELLA²;
¹VHIR- Vall D'Hebron Inst. of Res., Barcelona, Spain; ²VHIR - Vall D'Hebron Inst. of Res., Barcelona, Spain; ³Cell Biology, Physiol. and Immunol., Univ. de Barcelona, Barcelona, Spain; ⁴IRBlleida, Lleida, Spain

Abstract: Apoptosis is the main type of programmed cell death, essential for the correct nervous system development. Even if in adult neurons apoptosis has been related to the pathology of neurodegenerative diseases, apoptotic machinery activation has been also described as necessary in non-lethal regulatory events, such as synaptic plasticity. Apoptosis activation, which culminates with activation of effector caspases, is modulated by hundreds of proteins. One of the regulators of apoptosis is the long isoform of Fas Apoptosis Inhibitory Molecule (FAIM-L), a neuronal specific death receptor antagonist shown capable to modulate neuronal caspase activation. FAIM-L carries out its anti-apoptotic activity by binding to X-linked Inhibitor of Apoptosis Protein (XIAP). XIAP is an inhibitor of caspases, and its levels are modulated by the ubiquitin-proteasome pathway. FAIM-L interaction with XIAP prevents its ubiquitination and degradation, allowing therefore its anti-apoptotic activity. This interaction also modulates non-apoptotic functions of caspases, such as the endocytosis of AMPA receptor (AMPAR), the main mechanism in long-term depression (LTD). To date consensus binding motifs of FAIM-L, which could shed light on its molecular mechanism of action, are unknown. Here, we performed a two-hybrid screening to discover novel FAIM-L-interacting proteins. We found a functional interaction of FAIM-L with the pro-apoptotic protein SIVA-1. SIVA-1 is a protein described as pro-apoptotic and able to interact with XIAP. In this work we show that SIVA-1 modulates FAIM-L function by disrupting the interaction of FAIM-L with XIAP, thereby promoting XIAP ubiquitination. Modulating the inhibitors of apoptosis XIAP and FAIM-L, SIVA-1 induces caspase-dependent neuronal cell death. Furthermore, we show SIVA-1 to be a novel modulator of synaptic plasticity. After induction of LTD in an in vitro neuronal model, SIVA-1 protein levels are rapidly increased, and SIVA-1 overexpression is sufficient to induce caspase-dependent internalization of AMPAR. In summary, our studies uncover a new functional partner of FAIM-L, SIVA-1. We propose SIVA-1 as a caspase modulator in neurons, and therefore to be a crucial regulator in neuronal cell death and synaptic plasticity.

Poster

285. Long-Term Depression and Spike Timing-Dependent Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 285.02/B95

Topic: B.07. Synaptic Plasticity

Support: KAKENHI 17J07402
KAKENHI 17H05566

Title: Effects of β-adrenergic receptor on long-term depression at parallel fiber to purkinje neuron synapses in the cerebellar flocculus

Authors: *T. INOSHITA, T. HIRANO;
Kyoto University, Grad. Sch. of Sci., Kyoto, Japan

Abstract: The cerebellum is involved in motor learning, and long-term depression (LTD) at parallel fiber to Purkinje neuron (PF-PN) synapses in the cerebellum has been regarded as a main mechanism for motor learning. On the other hand, the involvement of noradrenaline (NA) in motor learning has also been reported in several cerebellum-dependent learning paradigms. However, the relationship between NA and LTD at PF-PN synapses has not been clarified. Here, we examined effects of β-adrenergic receptor (β-AR) on the synaptic transmission and LTD at PF-PN synapses. Applying patch-clamp recording to slice preparations prepared from the cerebellar flocculus, we recorded excitatory postsynaptic currents (EPSCs) at PF-PN synapses. Cerebellar flocculus is known to regulate adaptation of oculomotor reflexes including optokinetic response (OKR) which works to stabilize an image on the retina during movement of visual field. We previously reported that the activity of β-AR in the flocculus is involved in OKR adaptation (Wakita et al., 2017). In addition, we also showed that LTD occurs at PF-PN synapses in the flocculus during OKR adaptation (Inoshita & Hirano, 2018). Thus, we focused on effects of β-AR activity on PF-PN synapses in the flocculus. In order to clarify whether activation of β-AR changes the transmission at PF-PN synapses, a selective agonist for β-AR isoproterenol (ISO) was applied to the slice preparation of the flocculus, which did not change EPSCs at PF-PN synapses. Next, we examined whether β-AR activity changes the threshold of LTD induction or not. In the ISO containing solution, LTD was induced by a relatively weak conditioning stimulation which did not induce LTD in the control solution. Effects of endogenous agonist of β-AR NA were also examined. NA did not change EPSCs at PF-PN synapse, and facilitated the LTD induction as ISO did. These results suggest that activation of β-AR does not affect the synaptic transmission but facilitates LTD induction at PF-PN synapses in the flocculus. Effects of ISO or NA on EPSCs and LTD at PF-PC synapse in the vermis will also be presented.

Disclosures: T. Inoshita: None. T. Hirano: None.
**Poster**

**285. Long-Term Depression and Spike Timing-Dependent Plasticity**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 285.03/B96

**Topic:** B.07. Synaptic Plasticity

**Support:** IBRO Grant / Fellowship

**Title:** Age dependent metabotropic glutamate receptor mediated long term depression

**Authors:** *A. J. IDOWU*, M. SEGAL, A. KIRKWOOD;

**Abstract:** Preserved cognitive function during normal aging in rats has been shown to be associated with metabotropic glutamate receptor (mGluR) mediated plasticity. Recently, neurobehavioral characterization of C57BL/6 mice showed significant age-dependent variations in cognitive functions between juvenile and adult mice. However, the unique functional properties of mGluR plasticity in the adult compared to juvenile C57BL/6 mice are yet to be described. We have now compared mGluR mediated long term depression in young (1 to 3 months; n=12 slices) and adult (6 to 8 months; n=12 slices) in the hippocampus of the C57BL/6 mice. We show an increase in the magnitude of mGluR long term depression between young and adult C57BL/6 mice (p<0.05). This finding suggests an age-dependent increase in mGluR plasticity in hippocampal slices between juvenile and adult C57BL/6 mice.

**Disclosures:** A.J. Idowu: None. M. Segal: None. A. Kirkwood: None.

**Poster**

**285. Long-Term Depression and Spike Timing-Dependent Plasticity**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 285.04/B97

**Topic:** B.07. Synaptic Plasticity

**Support:** NMRC/CBRG/0099/2015
NMRC-OF-IRG-2016

**Title:** Serotonin facilitates plasticity and associativity in hippocampal area CA2
Abstract: Hippocampal area CA2 is a neuromodulatory hub of the hippocampal circuit, receiving inputs from several extra-hippocampal neuromodulatory centres including the serotonergic median raphe nucleus. CA2 also possesses a high density of inhibitory interneurons. This study investigates the modulation of synaptic depression, and synaptic tagging and cross-tagging mediated associative plasticity in hippocampal area CA2 under the influence of serotonin (5-HT). Using extracellular field potential recording in male Wistar rat (5-7 weeks old) hippocampal slices, we show that bath application of serotonin (10 µM) is able to transform weak low frequency stimulation (WLFS - 900 pulses (1 Hz, impulse duration 0.2 ms per half-wave, total number of stimuli 900)) induced transient early long-term depression (E-LTD) to protein synthesis dependent long-lasting late long-term depression (L-LTD) at the Schaffer collateral (SC) synapses onto CA2. Further, through synaptic tagging and capture of plasticity-related proteins (PRPs), the 5-HT facilitated L-LTD at SC synapses onto CA2 is able to transform WLFS induced E-LTD to L-LTD at the entorhinal cortical (EC) synapses onto CA2. In the absence of serotonin, WLFS only enables a short-lasting E-LTD at both the SC and EC synapses onto CA2. It was also shown that the 5-HT facilitated L-LTD at SC synapses onto CA2 enables weak tetanization (one 100 Hz train (21 biphasic constant-current pulses; pulse duration per half-wave, 0.2 ms)) induced early long-term potentiation (E-LTP) at EC synapses onto CA2 transform into long-lasting late LTP (L-LTP), through engaging in synaptic cross tagging and subsequent capture of PRPs. Furthermore, our results also suggest a role for BDNF in the 5-HT facilitated LTD at SC synapses onto CA2 as co-application of the BDNF scavenger, TrkB-Fc fusion protein with 5-HT inhibits LTD maintenance at SC synapses onto CA2. Blocking of 5-HT4 receptors abolishes the 5-HT facilitated LTD maintenance and agonists to 5-HT4 receptors alone facilitate E-LTD to L-LTD transformation at SC synapses onto CA2, pointing to a role of 5-HT4 receptors in the maintenance of LTD observed at SC synapses. Together, these findings reveal that serotonin facilitates long-term depression and associative plasticity in hippocampal area CA2.

Disclosures: A. Benoy: None. N. Ather: None. S. Sajikumar: None.
Authors: *A. KAMALI TAFRESHI*, S. ABDELMAGED, K. PRASAD, A. POLAVARAPU, N. LOZANO, C. KOCH, P. ALGEDIK, O. BOZDAGI; ¹Psychiatry, Rutgers Univ., Newark, NJ; ²Mol. Biol. and Biochem., Rutgers Univ., Piscataway, NJ

Abstract: Neurofibromatosis type 1 (NF1) is an autosomal dominant tumorigenic neurodevelopmental disorder associated with autism spectrum disorder (ASD) and intellectual disability (ID). Abnormalities in perineuronal nets (PNNs), a specialized extracellular matrix, have been linked to several neuropsychiatric conditions, but have not been investigated in mouse models of neurodevelopmental disorders associated with ASD and ID. In this study, we explored PNNs in the hippocampus in NF1 heterozygous mouse model (Nf1+/−) compared to wild type controls. Because hippocampus is associated with synaptic plasticity, cognition and social behavior, we focused our analyses on CA1 and CA2 subregions of the hippocampus. Using lectin, Wisteria floribunda agglutinin (WFA) to label PNNs, we found increased WFA labeled PNNs in the CA2 of Nf1+/− mice compared to controls. Hippocampal gelatinase enzymatic activity, which remodels extracellular matrix proteins, is also reduced in Nf1+/− mice compared to wild type animals, associated with increased PNN formation. Additionally, we showed that Schaffer collateral-CA1 LTP is impaired in Nf1+/− mice, whereas long-term depression (LTD) is enhanced compared to wild type mice. Behavioral characterization suggests impaired long-term social learning and inhibitory avoidance learning in Nf1+/− mice compared to wild type controls. Our data show that PNN abnormalities might contribute to synaptic plasticity and behavioral deficits associated with ASD and ID.


Poster

285. Long-Term Depression and Spike Timing-Dependent Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 285.06/B99

Topic: B.07. Synaptic Plasticity

Support: NIMH IRP MH002881

Title: Characterizing long-term synaptic depression at the entorhinal to hippocampal CA1 synapse

Authors: *K. M. KEARY, III*, Z. Li; ¹Brown Univ. NIH GPP, Bethesda, MD; ²NIMH, Bethesda, MD
Abstract: Long term synaptic plasticity is the cellular mechanism through which learning and memory are able to take place. Long term potentiation is responsible for strengthening active synapses, while long term depression is key for weakening or eliminating underused synapses. Both are necessary processes for maintaining overall circuit homeostasis and allowing for continual cellular changes. The hippocampal formation is a widely studied structure critical for learning and memory. CA1 is the primary output of the hippocampus, and while Schaffer collateral LTD at proximal dendrites has been extensively studied, the direct cortical input from entorhinal cortex to distal dendrites is far less represented in the literature. To fully investigate the plasticity at this synapse, we recorded field potentials from the Stratum Lacunosum-Moleculare of CA1 neurons in young (<P19) and adult (>P56) mice. Here we establish that LTD appears to take place at both the young and old entorhinal synapse, while Schaffer collateral LTD is abolished in adulthood. Entorhinal LTD appears to be blocked by administration of NMDA antagonist APV, implicating an NMDA dependent induction mechanism at the distal dendrites. Further pharmacological manipulations, as well as transgenic lines, will be utilized to fully explore the mechanism of Entorhinal to CA1 long term depression in the future.

Disclosures: K.M. Keary: None. Z. Li: None.

Poster

285. Long-Term Depression and Spike Timing-Dependent Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 285.07/B100

Topic: B.07. Synaptic Plasticity

Support: NIH R01-NS-1074210100

Title: Targeted disruption of NMDAr-dependent synaptic plasticity by intermittent hypoxia

Authors: *A. ARIAS-CAVIERES¹, C. NWAKUDU¹, A. J. GARCIA, III²;
¹Univ. of Chicago, Chicago, IL; ²Emergency Med., The Univ. of Chicago, Chicago, IL

Abstract: Long term potentiation (LTP) and long term depression (LTP) are two forms of synaptic plasticity whose underlying mechanisms regulate the efficacy of synaptic communication and serve as substrates central to learning and memory. Both LTP and LTD can be evoked through NMDAr dependent and NMDAr independent mechanisms. Intermittent hypoxia (IH), a hallmark of sleep apnea, is known to impair spatial learning and memory. Although IH has been shown to impair LTP evoked by high frequency stimulation (LTPHFS), it is unclear whether IH targets specific forms of synaptic plasticity or alternatively, acts broadly to impair synaptic function. Here, we test the hypothesis that IH reduces the contribution of NMDAr causing a targeted disruption in NMDAr-dependent plasticity. Electrophysiological recordings were made in
hippocampal slices prepared from control mice (control) and mice exposed to ten days of IH (IH10). While LTPHFS and LTP evoked by theta burst stimulation (LTPθ) was reliability produced in control slice, LTPHFS was attenuated and LTPθ could not be evoked in IH10 slices. Similarly, both LTD evoked by low frequency stimulation (LTDLFS) and metabotropic glutamate receptor activation (LTDDHPG) was reliably elicited in the control; whereas, only LTDDHPG could be produced in IH10 slices. Our findings indicate that IH, associated with sleep apnea, causes targeted disruption to forms of NMDAr-dependent synaptic plasticity. Thus, the loss of NMDAr mechanisms supporting learning and memory is a principal consequence of IH which reduces the threshold for neurocognitive decline is sleep apnea.

Disclosures: A. Arias-Cavieres: None. C. Nwakudu: None. A.J. Garcia: None.

Poster

285. Long-Term Depression and Spike Timing-Dependent Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 285.08/B101

Topic: B.07. Synaptic Plasticity

Support: DFG SFB/Transregio 58

Title: Impact on brain-derived neurotrophic factor on synaptic transmission and plasticity in the oval nucleus of bed nucleus of the stria terminalis

Authors: *D. FIEDLER*1, M. SASI2, R. BLUM2, C. KLINKE3, M. ANDREATTAT3, H.-C. PAPE1, M. D. LANGE1;

1Inst. of Physiol. I, Westfälische Wilhelms-University, Muenster, Germany; 2Inst. of Clin. Neurobio., Univ. Hosp. Wuerzburg, Wuerzburg, Germany; 3Inst. of Psychology I, Julius-Maximilians-University, Wuerzburg, Germany

Abstract: The neurotrophin brain-derived neurotrophic factor (BDNF) is well known to regulate the differentiation of neuronal precursor cells and synaptogenesis and to contribute to neuronal growth and survival. In addition, BDNF modulates synaptic transmission and synaptic plasticity of neuronal networks, including the so-called fear circuits mediating fear learning and extinction. Specifically, BDNF is highly present in the bed nucleus of the stria terminalis (BNST), a key region contributing to stress-modulated fear. Here, we combined immunohistochemical and electrophysiological in vitro approaches to identify the role of BDNF in BNST circuits. The focus has been on intrinsic properties of BNST neurons, as well as synaptic transmission and plasticity in local BNST networks. Immunohistochemically stainings revealed the existence and regional distribution of both BDNF and its main receptor Tropomyosin receptor kinase B (TrkB) within the BNST, with high levels in the oval nucleus (ovBNST). The influence of BDNF on ovBNST neurons was investigated by
performing electrophysiological whole-cell patch-clamp recordings. BDNF-TrkB interaction caused a significant hyperpolarizing shift of the membrane potential from resting values, associated with reduced excitability of ovBNST neurons. While no effect of BDNF on frequency or amplitude of spontaneous evoked postsynaptic currents (sEPSC) was detected, synaptic plasticity was found to be modulated by BDNF. Specifically, long-term depression (LTD, initiated by low-frequency stimulation of 10 Hz for 10 min) was abolished by the TrkB antagonist ANA-12 or by the kinase inhibitor K252a. Furthermore, LTD was absent when BDNF was captured by the BDNF scavenger TrkB-Fc chimera. In summary, BDNF is functional in ovBNST synaptic networks. BDNF reduces LTD via TrkB receptors, with no effect on basal synaptic activity in ovBNST neurons. These findings suggest a synaptic entry point of the BDNF system to ovBNST-mediated regulation of stress and fear responsiveness.


Poster

285. Long-Term Depression and Spike Timing-Dependent Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 285.09/B102

Topic: B.07. Synaptic Plasticity

Title: Inositol triphosphate receptors are required for DHPG induced LTD, but not low frequency stimulation induced LTD, in visual cortex


Abstract: We evaluated the role of inositol triphosphate receptors (IP3Rs) and somatic calcium in the expression of long-term depression (LTD) induced by electrical verses chemical stimulation. We made whole-cell current-clamp recordings from layer 2/3 pyramidal neurons in acute slices (300 µm) of primary visual cortex from 6-12 day old tri-color guinea pigs. Preconditioning (baseline) and post-conditioning synaptic responses (postsynaptic potentials, PSPs) were evoked at 0.1 Hz using an extracellular stimulating electrode placed in L4. For conditioning, LTD was induced by either: 1) low frequency stimulation (LFS, 1 Hz, 15 min) of L4, or 2) chemical stimulation with (S)-3,5-dihydroxyphenylglycine (DHPG, 100 µM, 10 min bath application). Plasticity was calculated using a post/pre-conditioning ratio (mean evoked PSP peak amplitude between 20-30 min post-conditioning / mean evoked PSP peak amplitude during the 10 min pre-conditioning). We defined long-term depression (LTD) as a significant (P<0.01, t-test) decrease (≥15%) in post/pre ratios, long-term potentiation (LTP) as a significant increase in post/pre ratios, or no change as post/pre ratios that changed <15% and/or were not
significantly different (P>0.01, t-test). In some cases, the IP3R blocker Xestospongin C (XeC, 1µM) and/or the ratiometric calcium indicator Fura-4F was added to the recording pipette. LFS resulted in a mean 16% reduction in PSP amplitude (post/pre=0.84, N=17 slices). Moreover, LFS-LTD was unaffected by the addition of XeC to the recording pipette (post/pre = 0.83, N=11 slices; p = 0.99, KS-test). In contrast, DHPG-LTD resulted in a mean 41% reduction in PSP amplitude (post/pre = 0.59), which was significantly attenuated (to just 21%) by XeC in the recording pipette (post/pre=0.79, N=10 slices; P<0.05, KS-test). DHPG-LTD induction, but not LFS-LTD induction, was accompanied by a large calcium transient (resting baseline concentration=79±13 nM, N=12 slices; DHPG-LTD=600±95 nM, N=5) which was significantly attenuated by XeC (190±78 nM, N=5; P<0.01, t-test). Finally, the magnitude of DHPG-LTD, but not LFS-LTD was positively correlated with somatic calcium concentration. Together these data indicate a mechanistic difference in the induction/expression of LTD induced by LFS and DHPG.


Poster

285. Long-Term Depression and Spike Timing-Dependent Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 285.10/C1

Topic: B.07. Synaptic Plasticity

Support: NSF IOS-1755071 to B.A.C

Title: Spike-timing-dependent plasticity alters sensory network connectivity in vivo

Authors: *A. J. LUBE, X. MA, B. A. CARLSON;
Washington Univ. In St. Louis, St. Louis, MO

Abstract: How do sensory systems optimize detection of behaviorally relevant stimuli when the sensory environment is constantly changing? The adjustment of synaptic connectivity via spike-timing-dependent plasticity (STDP) has been found in circuits across diverse invertebrate and vertebrate organisms. Further, neuronal connectivity changes consistent with STDP have been observed during development, for example in the establishment of receptive fields and direction selectivity within the visual system. Although it is possible that STDP underlies rapid changes in neural connectivity that alter stimulus tuning, STDP has not been directly implicated in vivo under behaviorally relevant conditions. We hypothesize that STDP drives changes in sensory tuning in real-time that enable sensory systems to remain maximally responsive to a changing environment. Our study organism, mormyrid weakly electric fish, produce and receive electric organ discharges (EODs) used to electrolocate and communicate. Thus, spiking patterns themselves are the behaviorally relevant stimulus and the electroreceptive system of these fish
allows us to precisely manipulate presynaptic spike timing using both sensory stimulation *in vivo* and afferent stimulation *in vitro*. Using whole-cell intracellular recordings *in vitro* and *in vivo* to repetitively pair afferent or sensory stimulation with intracellular current injection, we manipulated the relative timing of pre- and post-synaptic spiking in central sensory neurons. Using afferent stimulation *in vitro*, we found significant synaptic potentiation at pre-leads post-synaptic delays and significant depression at reversed delays. *In vivo*, we paired sensory stimulation with intracellular spiking at delays that resulted in potentiation and depression *in vitro*. We found that pairing with a sensory stimulus leading intracellular spikes significantly potentiated synaptic responses compared to the reverse delay condition and controls. Thus, our results suggest that STDP alters neural connectivity *in vivo*. Future studies will address how naturalistic patterns of activity in this network may drive changes in sensory tuning through STDP.

Disclosures:  A.J. Lube: None. X. Ma: None. B.A. Carlson: None.

Poster

285. Long-Term Depression and Spike Timing-Dependent Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 285.11/C2

Topic: B.07. Synaptic Plasticity

Title: The influence of LTP induction on back-propagated action potentials on dendrites: Analysis using optical imaging with voltage sensitive dye

Authors: *S. SUGIMOTO*¹, M. KAWAI¹, M. KONDO², Y. R. TANAKA¹, T. AIHARA¹; ¹Tamagawa Univ., Tokyo, Japan; ²Grad. Sch. of Med., The Univ. of Tokyo, Tokyo, Japan

Abstract: The hippocampus has several functions that is related to the integration and storage of sensory information. Previous studies reveal that the dentate gyrus has the projections of spatial information (e.g. place) and non-spatial information (e.g. odor) at medial dendrite (MD) and distal dendrite (DD) in molecular layer, respectively. On the other hand, many researchers investigate that spike-timing dependent plasticity (STDP), the order and temporal interval between an excitatory post synaptic potential (EPSP) and a back-propagated action potential(bAP) decide the sign and magnitude of the plasticity, long-term potentiation (LTP) or depression (LTD). Our previous study showed that the amplitude and transmission distance of bAP, one of factors for STDP induction, were modulated by induction of LTP along dendrites in hippocampal CA1 pyramidal neurons. The result suggested that the association between the context information from CA3 and information through a direct pass from EC was facilitated by the modulated bAP. On the other hand, the influence of LTP induction on bAP modulation in hippocampal dentate granule cells was not investigated so that the integration of spatial and non-spatial information from EC projection to MD and DD on dentate gyrus granule cells was not
clear. In this study, to investigate the bAP modulation related to information integration of two inputs of dentate gyrus, we analyzed the amplitude of bAPs before and after LTP induction along dendrites in the granule cells, using optical imaging method with voltage sensitive dye. As the result, the bAP transmission distances were spread from MD to DD and the magnitude of bAP was increased at DD. The result suggests that the cooperative integration of place information and the other sensory information was influenced by the bAP modulation based on the induction of synaptic plasticity on dendrites in dentate granule cell.


Poster

285. Long-Term Depression and Spike Timing-Dependent Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 285.12/C3

Topic: B.07. Synaptic Plasticity

Support: MINECO Grant BFU2015-68655P
           JUNTA DE ANDALUCÍA Grant CVI7290

Title: Adenosine receptor mediated developmental loss of spike timing dependent depression in the hippocampus

Authors: *A. RODRIGUEZ-MORENO*¹, M. PÉREZ-RODRÍGUEZ¹, L. E. ARROYO-GARCÍA¹, J. PRIUS-MENGUAL¹, Y. ANDRADE-TALAVERA¹, J. A. ARMENGOL¹, E. PÉREZ-VILLEGAS¹, G. FLORES²;
          ¹Univ. Pablo de Olavide, Sevilla, Spain; ²Univ. Autonoma de Puebla / Inst. de Fisiologia, Puebla, Mexico

Abstract: Critical periods of synaptic plasticity facilitate the reordering and refining of neural connections during development, allowing the definitive synaptic circuits responsible for correct adult physiology to be established. Presynaptic spike timing-dependent long-term depression (t-LTD) exists in the hippocampus, which depends on the activation of NMDARs and that probably fulfills a role in synaptic refinement. This t-LTD is present until the 3rd postnatal week in mice, disappearing in the 4th week of postnatal development. We were interested in the mechanisms underlying this maturation related loss of t-LTD and we found that at CA3-CA1 synapses, presynaptic NMDA receptors (preNMDARs) are tonically active between P13 and P21, mediating an increase in glutamate release during this critical period of plasticity. Conversely, at the end of this critical period (P22-P30) and coinciding with the loss of t-LTD, these preNMDARs are no longer tonically active. Using immunogold electron microscopy, we demonstrated the existence of preNMDARs at Schaffer collateral synaptic boutons, where a
decrease in the number of preNMDARs during development coincides with the loss of both tonic preNMDAR activation and t-LTD. Interestingly, this t-LTD can be completely recovered by antagonizing adenosine type 1 receptors (A₁R), which also recovers the tonic activation of preNMDARs at P22-P30. By contrast, the induction of t-LTD was prevented at P13-P21 by an agonist of A₁R, as was tonic preNMDAR activation. Furthermore, we found that the adenosine that mediated the loss of t-LTD during the fourth week of development is supplied by astrocytes. These results provide direct evidence for the mechanism that closes the window of plasticity associated with t-LTD, revealing novel events probably involved in synaptic remodeling during development.


Poster

285. Long-Term Depression and Spike Timing-Dependent Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 285.13/C4

Topic: B.07. Synaptic Plasticity

Support: CIHR grant

Title: A key role for metabotropic presynaptic NMDA receptor signaling in neocortical STDP

Authors: *A. THOMAZEAU¹, J. BROCK², P. SJÖSTRÖM¹;

Abstract: Postsynaptic NMDA receptors (NMDARs) are ideally suited for Hebbian coincidence detection, because they require both presynaptically released glutamate and postsynaptic depolarization to open, flux Ca²⁺, and trigger plasticity. Recent evidence, however, questions this conventional view: NMDARs can signal metabotropically during Mg²⁺ block and without the need for ion flux, and there are also presynaptically located NMDARs (preNMDARs), neither of which suits Hebbian coincidence detection. We recently unveiled a double dissociation of preNMDAR signalling at visual cortex layer-5 (L5) pyramidal cell (PC) connections. Here, Mg²⁺-sensitive preNMDARs rely on RIM1αβ to regulate release probability during high- but not low-frequency evoked release, while preNMDARs signal metabotropically via JNK2 to regulate spontaneous release independent of frequency. Timing-dependent long-term depression (tLTD) — which requires preNMDAR activation — also does not depend on frequency. We therefore
hypothesized that preNMDARs signal unconventionally in tLTD at L5 PC-PC connections. Using quadruple patch, we elicited tLTD at L5 PC-PC synapses in acute visual cortex slices using a spike-timing-dependent plasticity (STDP) protocol at 20 Hz with Δt = ±25 ms. RIM1αβ was genetically ablated by crossing flox-RIM1αβ and Emx1-Cre mouse lines, and compared to wildtype (WT) C57Bl/6 mice. We found that tLTD persisted after homozygous RIM1αβ ablation (65% ± 7%, n = 4 vs. tLTD in WT mice 65% ± 5%, n = 9, p = 0.95). The tLTD evoked in RIM1αβ ablation cells was expressed presynaptically, as determined by analysis of the coefficient of variation and paired-pulse ratio, just as in control tLTD experiments (RIM1αβ KO and WT tLTD pooled: 1/CV² normalized = 42% ± 5%, n = 13, p < 0.001; ΔPPR = 0.12 ± 0.02, n = 13, p < 0.001 vs. zero). In contrast, the JNK2-selective inhibitor SP600125 prevented the induction of tLTD in WT mice (96% ± 2%, n = 8 vs. tLTD control 65% ± 5%, n = 9, p = 0.34). Here, paired-pulse ratio remained unaffected (ΔPPR = -0.07 ± 0.05, n = 8, p = 0.25 vs zero, but p < 0.01 vs WT tLTD ΔPPR = 0.13 ± 0.03, n = 9). In conclusion, our findings suggest a critical requirement for JNK2-mediated metabotropic preNMDARs signaling in neocortical tLTD. Our results contribute to the emerging view that NMDARs may signal metabotropically in pre- as well as postsynaptic compartments. The textbook view of NMDARs as ionotropic coincidence detectors may thus need reassessment.

Disclosures: A. Thomazeau: None. J. Brock: None. P. Sjöström: None.

Poster

285. Long-Term Depression and Spike Timing-Dependent Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 285.14/C5

Topic: B.07. Synaptic Plasticity

Support: NIDCD DC012557

Title: Input-specific excitatory and inhibitory stdp of layer 2/3 pyramidal neurons of mouse auditory cortex

Authors: *S. C. SONG, R. C. FROEMKE;

Abstract: There are multiple forms of long-term synaptic plasticity in the cortex, and distinct inputs onto the same type of postsynaptic neuron can have qualitatively different learning rules (Maheux et al. 2016). There is growing evidence that cortical inhibitory synapses are also highly plastic, and a recent study in prefrontal cortex identified differences between cell-type-specific GABAergic inputs onto layer 2/3 pyramidal neurons (Chiu et al. 2018). Here we examined spike-timing-dependent plasticity (STDP) of different excitatory and inhibitory projections onto layer 2/3 pyramidal neurons of adult mouse auditory cortex in brain slices. STDP is a method for
inducing input-specific and long-term modifications of synaptic strength, and previously we showed that layer 5 pyramidal neurons of mouse auditory cortex can express STDP for both glutamatergic and GABAergic inputs (D’amour and Froemke, 2015). This mechanism might underlie the synchronization of stimulus-evoked excitatory and inhibitory input during development (Dorrn et al., 2010) and after conditioning or maternal experience (Marlin et al., 2015; Martins and Froemke, 2015). We utilized isolated expression of channelrhodopsin in various glutamatergic afferents and GABAergic interneurons in order to selectively activate these inputs. Whereas intracortical inputs seem to express STDP after a few minutes of pairing, glutamatergic thalamic inputs from the MGB were relatively aplastic. Similar to the results of Chiu et al. (2018) in prefrontal cortex, our data also indicate that individual inhibitory cell types might differentially express STDP after pre- and postsynaptic spike pairing, although the specific learning rules might vary across brain areas or cortical layers.

Disclosures: S.C. Song: None. R.C. Froemke: None.

Poster

285. Long-Term Depression and Spike Timing-Dependent Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 285.15/C6

Topic: B.07. Synaptic Plasticity

Support: the Chinese Academy of Sciences QYZDY-SSW-SMC001

Title: In vivo verification of Hebb cell assembly hypothesis for perceptual memory

Authors: *X. YAN¹, D. ZHANG², M.-M. POO³;
¹Inst. of Neurosci., Shanghai, China; ²Inst. of Neuroscience, CAS, Shanghai City, China; ³Inst. of Neuroscience, SIBS, CAS, Shanghai City, China

Abstract: The nervous system can store and retrieve temporal sequence information, but the neural circuit mechanism for this ability remains largely unknown. Hebb has proposed that perceptual experience, including temporal sequence information, can be stored in selective cell assemblies, which are established by selective strengthening of synaptic connections among the neurons within the assembly. His postulate was supported by the discoveries of long-term potentiation (LTP) and spike timing-dependent plasticity (STDP), as well as findings of sequence learning in hippocampal place cells and the primary visual cortical neurons (V1), but direct evidence at the level of cell assemblies is still elusive. In this study, we used V1 of anaesthetized mice as a model system to study sequence learning and memory, and designed an experimental system for sequence stimulation, using optogenetic approaches and in vivo multi-electrodes recording. To directly test the role of sequential spiking of V1 neurons in storing sequence information, we expressed light activatable ion channel ChR2 in V1 neurons through viral
injection, built a customized scanning mirror to control the laser light for sequential light stimulation, and recorded in vivo neuronal activities from a linear array of neuronal groups in V1 to assay the firing sequence of the assembly of neuronal groups. Our results showed that ChR2-expressing neurons in V1 could be activated to fire in a sequential manner during sequential light stimulation. After 100 repeats of uni-direction moving-spot laser stimulation, spot stimulation only at the starting point of the sequence induced more sequential firing as compared to that found prior to the conditioning, whereas spot stimulation at the end point stimulation had no such effect. This effect can last for about 2.5 minutes, representing a form of short-term memory. We also investigated whether neuromodulator acetylcholine could affect the sequence learning and recall in V1 by optogenetic activation of diagonal band of Broca (HDB) during visual sequence stimulation and found that sequential learning was reduced by HDB stimulation, indicating neuromodulator acetylcholine can modulate sequence learning. Taken together, these results support Hebb’s cell assembly hypothesis for perceptual memory formation and retrieval, and demonstrate that sequential firing of cortical neurons could imprint the sequence information into the cortical circuits.

Disclosures:  X. Yan: None. D. Zhang: None. M. Poo: None.

Poster

285. Long-Term Depression and Spike Timing-Dependent Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 285.16/C7

Topic: B.07. Synaptic Plasticity

Support: DFG FOR2419

Title: Optogenetic spike-timing-dependent plasticity over behaviorally relevant time scales

Authors: *M. ANISIMOVA, B. VAN BOMMEL, T. G. OERTNER, C. E. GEE; Inst. for Synaptic Physiol., Univ. Med. Ctr. Hamburg-Eppendorf (UKE), Hamburg, Germany

Abstract: Spatial memory in rodents depends on hippocampal function and can last for many days. Lasting changes in synaptic strength after coincident activity in the pre- and postsynaptic neurons is a candidate mechanism for information storage; a process is commonly referred to as a spike-timing dependent plasticity (STDP). With electrophysiology alone it is not possible to follow the long-term consequences of STDP, since intracellular recordings can only be maintained for about one hour. Here we present a new all-optical method to induce STDP, thus separating the induction of plasticity from the electrophysiological read-out by a user-defined time period (e.g. 3 days). We induced STDP at Schaffer collateral synapses in rat organotypic hippocampal slices transfected with red-light-sensitive ChrimsonR (local virus injection in CA3) and blue-light-sensitive CheRiff (single cell electroporation in CA1). First, we verified that light-
induced spiking produces STDP while patched on the postsynaptic cell. We found that red light flashes (300@ 5 Hz) paired with bursts of 3 violet light flashes (50 Hz) induced LTP after causal pairing (pre before post) and LTD after anti-causal pairing (post before pre). We constructed illumination towers, each containing collimated red (630 nm) and violet (405 nm) high-power LEDs to induce controlled spike patterns in the incubator. At the read-out point (3 hours to 3 days), we sequentially recorded EPSCs from CA1, including at least 2 non-transfected neurons, while re-activating ChrimsonR-expressing CA3 neurons with a laser. To determine relative input strengths, the initial EPSC slope from each CheRiff neuron was divided by the average EPSC slope from the non-transfected neurons in that slice. At 3 hours after pairing, input strength was not significantly changed by either causal or anti-causal pairing. Surprisingly, 3 days after pairing, input strength to the CheRiff neurons was significantly larger in slice cultures that underwent causal or anti-causal pairing. Plasticity was abolished when NMDA receptors were blocked during pairing. Reducing the pairing frequency from 5 Hz to 0.1 Hz also prevented plasticity induction. When spontaneous activity was blocked with tetrodotoxin in the period from 3 hours to two days after causal pairing, input strength no longer increased. These experiments suggest that the synaptic memory of a short episode of coincident activity may be actively maintained and amplified in the cultures, perhaps by spontaneous reactivation of the networks once driven to synchronous activity.

Disclosures: M. Anisimova: None. B. van Bommel: None. T.G. Oertner: None. C.E. Gee: None.

Poster

285. Long-Term Depression and Spike Timing-Dependent Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 285.17/C8

Topic: B.07. Synaptic Plasticity

Support: Italian Ministry of Education University and Research: 2017K2NEF4_002

Title: Chronic unpredictable mild stress mediates neuroplasticity of medium spiny neurons through the GSK3β pathway

Authors: G. ACETO¹, C. COLUSSI², L. LEONE¹, S. FUSCO¹, M. RINAUDO¹, F. SCALA⁴, F. LAEZZA⁵, C. GRASSI¹, *M. D'ASCENZO¹, M. D'ASCENZO¹, ¹Univ. Cattolica S. Cuore, Sch. of Med., Rome, Italy; ²Cell Biol. and Neurobio., Natl. Res. Council, Rome, Italy; ³Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy; ⁴Neurosci., Baylor Col. of Med., Houston, TX; ⁵Dept. of Pharmacol. & Toxicology, Univ. of Texas Med. Br. at Galveston, Galveston, TX
Abstract: Chronic unpredictable mild stress (CUMS) is a clinically relevant model of major depressive disorder (MDD). Yet, the mechanisms of neuroplasticity underlying depression-like behaviors in the CUMS model are still poorly understood. Here, we identified glycogen-synthase kinase 3β (GSK3β) and voltage-gated K+ channel Kv4.2 as key regulators of maladaptive plasticity in CUMS mice. We performed whole-cell patch-clamp recordings of medium spiny neurons (MSNs) in the nucleus accumbens (NAc), a brain region primary involved in the neurobiology of depression, and studied spike timing-dependent long-term potentiation (tLTP) in control and CUMS mice. We found that tLTP was increased in CUMS mice compared to control and that selective GSK3β knockdown in the NAc prevented these changes in tLTP. In addition, we found that A-type K+ current density was decreased in MSNs from CUMS mice, an effect that was also prevented by GSK3β knockdown. Immunohistochemical, biochemical and pharmacological experiments revealed that the observed GSK3β-dependent changes in tLTP in CUMS mice were mediated by direct phosphorylation at Ser-616 of the Kv4.2 subunit, a molecular determinant of A-type K+ currents. Furthermore, knockdown of GSK3β in the NAc ameliorated depressive-like behavior in CUMS mice measured as responses to forced swim test, elevated plus maze and sucrose preference. Collectively, our results identify GSK3β regulation of Kv4.2 as a molecular mechanism of MSN maladaptive plasticity underlying depression-like behavior and suggest that GSK3β/Kv4.2 axis may be an attractive therapeutic target for MDD.


Poster

285. Long-Term Depression and Spike Timing-Dependent Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 285.18/C9

Topic: B.07. Synaptic Plasticity

Support: ARO W911NF-17-1-0300

Title: A modeling study of the cellular and circuit-level mechanisms of emotional memory consolidation in the prefrontal-amygdala network during REM sleep

Authors: *Y.-A. RHO, S. VIJAYAN;
Sch. of Neurosci., Virginia Tech., Blacksburg, VA

Abstract: Rapid eye movement (REM) sleep, the stage of sleep in which vivid dreams occur, has been implicated in the consolidation of emotional memories. Our recent work has found a candidate system for REM-related memory consolidation. During REM sleep, the frontal cortices, in particular the anterior cingulate cortex (ACC) and the dorsolateral prefrontal cortex (DLPFC), are dominated by theta (4-8 Hz) oscillations and by bursts of beta (15-35 Hz) activity,
and both beta and theta oscillations are coherent between the two regions. Experimental work has shown that theta power in the frontal cortex during REM sleep is positively correlated with the strengthening of emotional memories. Furthermore, studies suggest that rhythmic interactions between the frontal cortices and limbic structures, in particular the amygdala, play a critical role in the consolidation of emotional memories. However, the underlying mechanisms responsible for the consolidation of emotional memories during these rhythmic interactions during REM sleep remain unknown. We used biophysically realistic models of the prefrontal cortex (PFC) and amygdala (developed previously) to build a large-scale network and incorporated synaptic plasticity mechanisms into the connections between these two regions. We were able to reproduce the oscillatory dynamics observed in experimental studies and identify cell type specific synaptic changes caused by spike-timing dependent plasticity (STDP). The rhythmic activities observed in the PFC and the amygdala during REM sleep are generated under different physiological conditions than during the awake state since, for example, norepinephrine (NE) and serotonin (SE) are much lower during REM sleep. This has major implications for plasticity; indeed, experimental studies have revealed that neuromodulators modulate the expression of synaptic plasticity. Using our large-scale network model, we show how the levels of neurotransmitters such as NE and SE during REM sleep affect oscillatory dynamics and in turn influence the strengthening of connections related to emotional memories. To summarize, our network modeling provides us with a system level and cellular level mechanistic understanding of how emotional memories are consolidated during REM sleep.

Disclosures: Y. Rho: None. S. Vijayan: None.

Poster

285. Long-Term Depression and Spike Timing-Dependent Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 285.19/C10

Topic: B.07. Synaptic Plasticity

Support: DARPA HR0011-17-2-0025
NIH R01 NS12542
NIH RR00166

Title: Vagus nerve stimulation and induced cortical plasticity in non-human primates

Authors: *I. R. REMBADO¹, D. SU², L. SHUPE¹, H. BOYD¹, B. AMOENI¹, I. KEMP¹, A. MORSE¹, C. BIRCH¹, S. I. PERLMUTTER¹, E. E. FETZ¹, S. ZANOS³;
Abstract: Vagus nerve stimulation (VNS) paired with behavioral experience promotes neuroplasticity which results in behaviorally relevant changes [1, 2]. VNS engages neuromodulatory systems in a temporally precise manner, which makes it a candidate for targeted plasticity therapy [5]. However, the mechanisms through which VNS promotes plasticity and enhances behavioral performance are not fully understood. We characterized how cortical stimulation-induced cortical plasticity, a form of activity-dependent synaptic plasticity [6], is affected by auricular (noninvasive) and cervical (invasive) VNS, in behaving monkeys. In three male macaque monkeys epidural and intracortical electrodes were implanted in prefrontal, pericentral (sensorimotor) and parietal cortical areas. The auricular branch of the vagus nerve (abVN) was stimulated with an earclip placed on either right or left cymba concha. One animal was also implanted with a bipolar cuff electrode on both left and right cervical VN (cVN). In order to induce cortical plasticity between 2 cortical sites, cortical stimulation (CS) in closed-loop mode was delivered to one site, triggered from the trough of beta oscillatory cycles (15-25 Hz) detected at the other site. Each experiment comprised 2 “conditioning” sessions, in random order: (a) only CS, (b) CS paired with VNS. Different VNS protocols (abVNS/cVNS, left/right, high/low frequency), at different time intervals relative to CS (before or paired with CS) were tested. In each experiment, the size of cortically evoked potentials (CEPs), a measure of cortical connectivity and cortical excitability, was measured before and after conditioning. We found that CEPs after 30-40 minutes of CS increased or decreased in size compared to pre-conditioning CEPs. When CS was paired with VNS, the difference in CEP size was reduced to zero. Even though additional controls are needed to established whether CS induced a change in cortical connectivity or it altered cortical excitability, the delivery of VNS seemed to suppress such an effect. This study adds new insights on the neurophysiology of VNS with regard to VNS timing, modality and dose. [1] Engineer et al., Nature 470: 101-104, 2011; [2] Clark et al. Neurobiol. Learn. Mem. 63: 213-216, 1995; [3] Henry, Neurology 59: S3-14, 2002; [4] Dawson et al. Stroke 47: 143-50, 2016; [5] Hays et al., Progress in Brain Research 207: 275-299, 2013; [6] Zanos et al., Current Biology 28: 2515-2526, 2018.


Poster

286. Synaptic Plasticity: Kinases and Intracellular Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 286.01/C11

Topic: B.07. Synaptic Plasticity

Support: NIMH RO1 MH 041083 grant to TJC
**Title:** ERK phosphorylation is uncoupled from ERK activation in a cellular model of long-term plasticity

**Authors:** *N. KUKUSHKIN, T. J. CAREW; 
Ctr. for Neural Sci., New York Univ., New York, NY

**Abstract:** Extracelluar signal-regulated kinase (ERK) is a central hub of cellular signaling whose activity underlies many forms of long-term memory. As a member of the mitogen-activated protein kinase (MAPK) family, ERK is activated in a switch-like manner by double phosphorylation of a remarkably conserved, yet distinctly unusual TxY motif. Typically, phosphorylation of this motif is viewed as a reporter of ERK activity. However, ERK phosphorylation is distributed, meaning that the cellular population of pERK consists not only of active, dually phosphorylated enzyme (dualP-ERK), but also of singly phosphorylated and therefore inactive ERK (pT-ERK and pY-ERK). We show that stimulating Aplysia sensory neurons with 5HT, which mimics sensitization training in the intact animal, produces temporally and spatially distinct perturbations in these forms of pERK. In particular, an early surge of dually phosphorylated ERK in stimulated cells is followed by a later wave of pY-ERK, but not pT-ERK, at the time when dualP-ERK levels returns to baseline. The increase in pY-ERK is due to ongoing phosphorylation rather than dephosphorylation of dualP-ERK, since the MEK inhibitor U0126 prevents the later wave of pERK even when administered after the earlier one. Given the importance of ERK in repeated-trial learning, we hypothesize that the accumulation of inactive but biochemically primed pY-ERK represents a potential mechanism for intertrial interactions.

**Disclosures:** N. Kukushkin: None. T.J. Carew: None.

**Poster**

**286. Synaptic Plasticity: Kinases and Intracellular Signaling**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 286.02/C12

**Topic:** B.07. Synaptic Plasticity

**Support:** NIMH RO1 MH 094792

**Title:** Proteolytic processing of TGFβ provides a mechanism for precise temporal orchestration in long term memory formation

**Authors:** *P. MIRANDA¹, N. KUKUSHKIN¹, A. A. MIRISIS², M. SCHREIBMAN¹, T. J. CAREW²; 
¹Ctr. for Neural Sci., ²New York Univ., New York, NY

**Abstract:** While canonically viewed as hormone-like molecules operating with little temporal resolution, growth factors in fact play key roles in orchestrating the timing of molecular events
required to form a long-term memory (LTM). Specifically, in a two-trial training paradigm that induces LTM for sensitization in the marine mollusk *Aplysia californica*, the growth factor TGFβ is required during Trial 2 but not Trial 1, suggesting temporally specific inter-trial regulation of TGFβ’s signaling cascade, either upstream or downstream of TGFβ release. Since TGFβ is necessary and sufficient to promote LTM, we hypothesized that it contributes to the inter-trial interactions that determine the temporally restricted outcome of repeated trial learning (Mirisis & Carew, 2019). However, the precise mechanisms that determine the differential requirement for TGFβ during two trial training remain unknown. To examine whether interactions between trials alter the magnitude of TGFβ signaling, we have developed a bioassay based on the nuclear translocation of Smad, a downstream target of the TGFβ cascade. Cultured *Aplysia* sensory neurons were treated with either one or two pulses of 5HT to mimic two trial sensitization training in the intact animal. We found that the rate of Smad nuclear translocation is indeed significantly increased after Trial 2 compared to Trail 1, suggesting differential activation of the TGFβ cascade during two trial training. To investigate the mechanisms underlying this effect, we monitored Smad translocation in the presence of BMP-1, a protease known to liberate secreted TGFβ from a latent state. While BMP-1 alone had no effect on Smad translocation, when it was combined with a single pulse of 5HT, it significantly enhanced Smad’s redistribution. These data suggest a model in which the interaction between trials involves both temporally segregated stages of TGFβ release and proteolytic activation, the combination of which is required for the precise temporal processing of repeated trials during LTM formation.

**Disclosures:** P. Miranda: None. N. Kukushkin: None. A.A. Mirisis: None. T.J. Carew: None. M. Schreibman: None.

**Poster**

**286. Synaptic Plasticity: Kinases and Intracellular Signaling**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 286.03/C13

**Topic:** B.07. Synaptic Plasticity

**Support:** NIMH RO1 MH 094792
Hellenic Medical Society of New York Research Grant

**Title:** Nuclear export of the RNA-binding protein ELAV provides a candidate mechanism for mRNA localization during long-term memory formation

**Authors:** *A. A. MIRISIS, T. J. CAREW;* Ctr. for Neural Sci., New York Univ., New York, NY

**Abstract:** In the marine mollusk *Aplysia*, signaling through distinct growth factor (GF) pathways is required for long-term memory (LTM) formation in unique temporal and spatial domains.
Specifically, in a two-trial sensitization training paradigm, TrkB signaling is required for gene expression of apc/ebp, an immediate-early gene, at 45 minutes following Trial 1, but prolonged apc/ebp gene expression necessary for LTM also requires TGFβ signaling during Trial 2 (Kopec et al., 2015). The apc/ebp transcript contains multiple AU-rich elements (AREs) in its 3’ UTR, conferring increased susceptibility to degradation and/or stabilization due to the AREs’ ability to bind various RNA-binding proteins (Yim et al., 2006). We have recently reported that the interaction between ELAV and apc/ebp is increased by Trial 2, and this interaction is required for LTM formation (Mirisis and Carew, 2018). Recent experiments have revealed that ELAV translocates from the sensory neuron nucleus into dendritic processes at 30 min following Trial 2. Moreover, this translocation is dependent on p38 MAPK activation downstream of TGFβ signaling. Taken together with previous findings of ARE-containing mRNAs in dendrites and at synapses (Alberini 2009; Meer et al., 2012; Arguello et al., 2013), this observation suggests a model in which ELAV-mediated transcript shuttling plays a critical role in LTM formation.

Disclosures: A.A. Mirisis: None. T.J. Carew: None.

Poster

286. Synaptic Plasticity: Kinases and Intracellular Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 286.04/C14

Topic: B.07. Synaptic Plasticity

Support: NIA AG051299-01

Title: NYX-458, a novel NMDA receptor modulator, improves age-related, hippocampal-dependent learning impairment and reverses changes in plasticity, spine morphology, and protein expression in rat hippocampus
Abstract: Age-related cognitive decline is a natural process that can particularly impact hippocampal function. This study was conducted to evaluate the effects of NYX-458, a novel N-Methyl D-Aspartate receptor (NMDAR) modulator with greatest affinity for NMDAR subtype NR2B, on hippocampal dependent cognitive function and associated cellular processes that contribute to cognitive decline with age. NMDARs have a critical role in hippocampal dependent learning and memory, and the depletion and dysfunction of these receptors are implicated in age-related cognitive decline. A single oral dose of NYX-458 (1 mg/kg) causes enhancement of hippocampal LTP that can be measured at both 24 hours and 72 hours post-dose in naïve young rats. In aged (26- to 27-month-old) rats, an oral dosing regimen of NYX-458 (1 mg/kg, daily) also improved performance in the hippocampal-dependent fixed-platform version of the Morris water maze. This dosing regimen also reversed the age-related deficits in hippocampal LTP and changes in spine morphology and enhanced the expression of proteins that regulate synaptic plasticity (specifically NMDAR2B, AMPAR1, and CAMKIIß). The long-term enhancement of hippocampal LTP and the reversal of age-related hippocampal impairments observed in the present study supports further investigation of NYX-458 in patients suffering from cognitive deficits associated with aging and for neurodegenerative diseases where synaptic plasticity and NMDAR function are impaired.
study, report that research relationship even if those funds come to an institution.; NIA AG051299-01. **P.K. Stanton:** 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.; NIA AG051299-01. F. Consulting Fees (e.g., advisory boards); Aptinyx. **J.R. Moskal:** A. Employment/Salary (full or part-time);; Aptinyx. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx. **C.N. Cearley:** A. Employment/Salary (full or part-time);; Aptinyx. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx.

**Poster**

**286. Synaptic Plasticity: Kinases and Intracellular Signaling**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 286.05/C15

**Topic:** B.07. Synaptic Plasticity

**Support:** NINDS/NIH Grant NS100047

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

**Authors:** *M. W. PORCH*¹, J.-Y. HWANG², A. E. CHAVEZ³, R. ZUKIN⁴;
¹Albert Einstein Col. of Med., Bronx, NY; ²Neurosci., Creighton, Omaha, NE; ³Neurosci., Univ. De Valparaíso, Valparaiso, Chile; ⁴Dept Neurosci, Albert Einstein Col. Med., Bronx, NY

**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 Ca²⁺. Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ permeability. Whereas the impact of Ser1166 on NMDAR Ca²⁺ 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. Zukin: None.

**Poster**

**286. Synaptic Plasticity: Kinases and Intracellular Signaling**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 286.06/DP03/C16

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**Topic:** B.07. Synaptic Plasticity

**Support:** NIH F32MH101954
NIH R01MH080047
NIH 1DP1NS096787
Max Planck Florida Institute

**Title:** Imaging compartmentalized and isozyme-specific PKC activity during plasticity

**Authors:** *L. A. COLGAN\(^1\), H. L. HOLMAN\(^1\), P. PARRA-BUENO\(^1\), X. TU\(^2\), J. MISLER\(^1\), R. YASUDA\(^1\);\n\(^1\)Max Planck Florida Inst., Jupiter, FL; \(^2\)Max Plank Florida Inst. For Neurosci., Jupiter, FL

**Abstract:** Synaptic plasticity is mediated by complex signaling cascades that transduce short-lived synaptic inputs into long-lasting changes of synaptic strength. The protein kinase C (PKC) family, consisting of more than 10 isozymes, has been shown to be involved in the induction, expression and maintenance of synaptic plasticity. However, poor isozyme discrimination, spatiotemporal resolution and sensitivity have limited our understanding of PKC function in
plasticity. Here, we have developed a suite of tools to discriminate the function of PKC isozymes through high resolution fluorescence resonance energy transfer (FRET) sensors optimized for 2-photon fluorescence lifetime imaging (2p-FLIM) and chemogenetic approaches. These tools reveal compartmentalized and isozyme-specific PKC activity and function during plasticity, clarifying the role of PKC in plasticity, learning and memory.


Poster

286. Synaptic Plasticity: Kinases and Intracellular Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 286.07/C17

Topic: B.07. Synaptic Plasticity

Support: NIH Grant ROINS103168
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Title: Dynamic analysis of CaMKII activity switch in living cells using FRET/FLIM sensors

Authors: C. ZHANG, *N. OTMAKHOV, R. KEPHARD, L. GRIFFITH; Brandeis Univ., Waltham, MA

Abstract: CaMKII plays a critical role in synaptic and behavioral memory. It has been suggested that its unique properties - operating as an activity switch and binding to multiple synaptic targets - are crucial for that role. In vitro studies demonstrated that CaMKII could undergo stable activation by means of its T286 autophosphorylation after a short pulse of Ca2+/CaM stimulation. It is also shown that binding to NR2B subunit of the NMDA receptor further supports this switch. The occurrence of a stable CaMKII switch, however, has not been demonstrated in living cells. Here we monitored CaMKII activity using the FRET/FLIM sensor Camui in Hela cells. Ca2+(i) transients, induced by Ionomycin application, resulted in a fast switch of Camui from a closed (‘inactive’) to an open (‘active’) state, which reversed back to its inactive state upon wash with EGTA/0 Ca2+/Ionomycin. Using different Camui mutants revealed that CaMKII T286 phosphorylation prolonged and T305/T306 phosphorylation shortened the reversal time, which was only 2 min longer than the reversal of Ca2+(i) transient. Adjusting the Ca2+(i) level at washout to match the basal level, prolonged the reversal time by 5-10 min. Inhibition of phosphatases with Calyculin A rendered a stable “active” Camui state for at least 15 min after Ca2+(i) reversal. The stable switch occurred in WT and 305A/306A but not in 286A or 286A/305A/306A Camui mutants indicating its dependence of T286 autophosphorylation. To test how binding to NR2B affects Camui activity switch, we
coexpressed Camui with the NR2B-C tail, which can bind CaMKII. We first coexpressed mls-NR2B-C, which binds the mitochondrial surface. Ca2+(i) transients produced translocation of Camui to mitochondria as observed by clustering. The Camui clustering, however, quickly disappeared upon wash with EGTA/Ionom. solution. Consistently, the Camui active state after Ca2+ stimulation lasted no longer than that without NR2B-C expression. For controls, we used GFPCaMKII, which also showed only transient clustering; the Camui (I205K) mutant, which does not bind to NR2B, did not cluster upon Ca2+(i) stimulation and its recovery from active state was not different from that of Camui WT. Since only a few subunits (of 12) in the CaMKII holoenzyme can bind to mitochondria-bound NR2B-C, our method might not resolve the effect of this binding. Therefore, we tested soluble GST-NR2B-C peptide, which should bind all subunits. Despite this, the Camui active state was not maintained beyond ~8 minutes after Ca2+ stimuli. Currently we are exploring different experimental conditions including more physiological Ca2+(i) stimulation to test the regulation of the CaMKII switch in living cells.

**Disclosures:** C. Zhang: None. N. Otmakhov: None. R. Kephard: None. L. Griffith: None.

**Poster**

286. Synaptic Plasticity: Kinases and Intracellular Signaling

**Location:** Hall A

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**Topic:** B.07. Synaptic Plasticity

**Support:**
- R01NS065856
- R21NS102661
- R01GM117034

**Title:** Endogenous role for Rem2 inhibition of CaMKII in neuronal synaptic development and plasticity

**Authors:** *R. ANJUM1, L. ROYER1, J. J. HERZOG1, K. M. KENNY1, B. TZVETKOVA1, J. C. COCHRANE2, M. T. MARR, II1, S. PARADIS1;


**Abstract:** In order to understand learning and memory at the molecular level, we must uncover the molecular mechanisms underlying synaptic development and plasticity. Ca2+/calmodulin-dependent protein kinase II (CaMKII) is a well-studied serine-threonine kinase that is highly expressed at the post-synaptic density, and is required for multiple types of synaptic plasticity, including long-term potentiation (LTP). Our lab recently determined that Rem2, a Ras-like GTPase from the RGK (Rad/Rem/Rem2/Gem/Kir) protein family, is a potent endogenous inhibitor of CaMKII. Previously, we have characterized several neuronal functions of Rem2,
including promoting excitatory synapse formation, inhibiting dendritic branching, and regulating homeostatic plasticity in the visual cortex. Moreover, like other members of the RGK protein family, Rem2 overexpression inhibits voltage-gated calcium currents in heterologous cells and cultured neurons. Interestingly, CaMKII function has also been implicated in these biological processes including facilitation of calcium flux through voltage-gated calcium channels. In addition, our work shows that Rem2 inhibition of CaMKII regulates dendritic branching in cultured hippocampal neurons. Thus, we seek to determine if Rem2 inhibition of CaMKII plays a role in other neuronal processes in addition to dendritic branching. We have also developed a N-terminus mutant of Rem2, which cannot inhibit CaMKII.


**Poster**

**286. Synaptic Plasticity: Kinases and Intracellular Signaling**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 286.09/C19

**Topic:** B.07. Synaptic Plasticity

**Support:** AFOSR FA9550-18-1-00

**Title:** Interactions between CaM and Ng govern the dynamics of CaMKII as a leaky integrator

**Authors:** *M. ORDYAN*¹, ³, T. M. BARTOL, JR², M. B. KENNEDY⁵, P. RANGAMANI⁴, T. J. SEJNOWSKI¹;
²CNL-S, ¹Salk Inst., La Jolla, CA; ³Inc, ⁴Mechanical and Aerospace Engin., UCSD, La Jolla, CA; ⁵Caltech, Pasadena, CA

**Abstract:** Calmodulin-dependent kinase II (CaMKII) has long been known to play an important role in learning and memory as well as long term potentiation (LTP). More recently it has been suggested that it might be involved in time averaging of synaptic signals, which can then lead to the high precision of the information stored at a single synapse. In this work, we adopt a rule-based modeling approach through Monte Carlo method to study the effect of Ca\(^{2+}\) signals on CaMKII phosphorylation in the postsynaptic densities (PSD). We study the differences between the CaMKII monomers and the dodecameric holoenzyme, as well as the effect of the scaffolding molecule neurogranin (Ng), which limits the availability of free Calmodulin (CaM), the protein which activates CaMKII in the presence of calcium. When Ng is present at significant concentration, we show that it plays an important modulatory role in CaMKII phosphorylation following a surge of high calcium concentration that is observed in the synaptic spines during an EPSP and back-propagating action potential due to the opening of NMDA receptors and voltage dependent calcium channels. We find a non-intuitive dependence of this effect on CaM
concentration that results from the different affinities of CaM for CaMKII depending on the number of calcium ions bound to the former. It has been shown previously that in the absence of phosphatase, CaMKII monomers integrate over Ca^{2+} signals of certain frequencies through autophosphorylation (Pepke et al, Plos Comp. Bio., 2010). We also study the effect of multiple calcium spikes on CaMKII holoenzyme autophosphorylation, and show that in the presence of phosphatase the CaMKII behaves as a leaky integrator of calcium signals, a result that has been recently observed in vivo. Our results also indicate the modulatory role the scaffolding protein Ng plays during signal integration: the area under the curve is significantly lower in the presence of Ng. Thus CaM scaffolds can make CaMKII phosphorylation a more robust process, resistant to noise in calcium signals.


Poster

286. Synaptic Plasticity: Kinases and Intracellular Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 286.10/C20

Topic: B.07. Synaptic Plasticity

Title: Deciphering IGF1 and insulin signaling within a single dendritic spine in long term potential

Authors: *X. TU, R. YASUDA;
Max Plank Florida Inst. For Neurosci., Jupiter, FL

Abstract: The insulin superfamily of peptides are well-known to regulate metabolism by regulating blood sugar levels, but only recently insulin and insulin-like peptides were shown to be crucial nervous system function as well. Decreased brain insulin levels or dysfunctional insulin and insulin-like growth factor 1 signaling are related to impaired learning, memory, as well as age-related neurodegenerative diseases. However, it remains unknown whether insulin receptor (IR) and insulin-like growth factor 1 receptor (IGF1R) are essential for synaptic plasticity and if so, when and how are they activated in dendritic spines during plasticity. To determine whether IR and IGF1R are required for plasticity, we assessed IR and IGF1R CRE-dependent knockout animals. We found that disruption of IGF1R or IR signaling in CA1 pyramidal neurons resulted in structural plasticity defects in those neurons. To further study the role of insulin and IGF1 signaling in plasticity, we developed fluorescence resonance energy transfer-based sensors for IGF1R and IR to measure the activity of the receptors during structural long-term potentiation (sLTP) in single spines. In response to sLTP induction, we discovered fast and sustained activation of IGF1R and IR in the stimulated spine. Elucidating insulin and IGF1 signaling mechanisms using two-photon fluorescence lifetime imaging microscopy in
combination with new biosensors will potentially allow to develop novel drug approaches to treat Alzheimer’s disease or enhance learning and memory.

Disclosures: X. Tu: None. R. Yasuda: None.

Poster

286. Synaptic Plasticity: Kinases and Intracellular Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 286.11/C21

Topic: B.07. Synaptic Plasticity

Support: Research Council of Norway, grant 248828

Title: Biochemically detailed single compartment model for neocortical postsynaptic long term plasticity

Authors: *T. MÄKI-MARTTUNEN¹, A. G. EDWARDS¹, G. T. EINEVOLL², K. L. BLACKWELL³;
¹Simula Res. Lab., Oslo, Norway; ²Norwegian Univ. Life Sci., Aas, Norway; ³Mol. Neurosci., George Mason Univ., Fairfax, VA

Abstract: Phenomenological models of plasticity have been widely applied in computational studies of cortical information processing. However, these models typically fall short of descriptions of molecular mechanisms behind the plasticity and are thus not useful for cellular-level or pharmacological studies of mental diseases that involve disruptions of plasticity. By contrast, biochemically detailed models for postsynaptic long-term plasticity exist for glutamatergic synapses in basal ganglia (Blackwell et al. 2019, Eur J Neurosci 49(6):768-783), cerebellum (Gallimore et al. 2018, Cell Rep 22(3): 722-733) and hippocampus (Jedrzejewska-SzMK, et al. 2017, PLoS Comput Biol 13(7): e1005657). In this work, we combine previously described molecular pathways that are required for cortical plasticity into a biochemically detailed single-compartment model of a postsynaptic spine in the neocortex. Our model describes the dynamics of the signaling networks mediating β-adrenergic receptor-dependent potentiation as well as cholinergic receptor-dependent depression, and includes descriptions for the dynamics of the key signaling molecules PKA, PKC, and CaMKII. To determine Ca^{2+} inputs to the model, we employed a multi-compartmental model of layer II/III pyramidal cells from the database of Markram et al. (2015, Cell, 163(2):456-492) to predict the interaction between the time of a spike in the post-synaptic neuron and the magnitude of the Ca^{2+} inputs at the synaptic site. We show that our single-compartment model reproduces the induction of plasticity as observed in experimental studies of cortical pyramidal cells. In particular, we reproduced central features of the spike-timing dependent LTP and LTD of glutamatergic synapses in visual cortex (Seol et al. 2007, Neuron 55(6):919-929). Our model offers a unique method for examining
contributions of mental disorder-associated genes to the induction of synaptic plasticity. It also allows modeling the effects of candidate pharmacological treatments that could be used to maintain a baseline level of cortical LTP/LTD induction under altered disease conditions.


**Poster**

**286. Synaptic Plasticity: Kinases and Intracellular Signaling**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 286.12/C22

**Topic:** B.07. Synaptic Plasticity

**Support:** NIH Grant 31771109
NIH Grant 31722023

**Title:** Camkiig drives long term potentiation of parvalbumin positive interneurons, boosting network oscillations and memory

**Authors:** *X. HE, G. ZHOU, J. LI, J. YANG, H. MA; Zhejiang Univ. Sch. of Medicine, Hangzhou, Hangzhou, China

**Abstract:** Long-term potentiation (LTP) is fundamental for information encoding, processing and storage in the brain. Most studies have focused on LTPE-E, the potentiation of excitatory synapses onto excitatory neurons, in which the “memory molecule” αCaMKII serves the central regulator. Inhibitory interneurons also receive excitatory synaptic input, but because these cells lack αCaMKII, it remains unclear whether, why and how LTPE-I occurs. Here, we report that γCaMKII is the long-sought “αCaMKII-like” molecule that is enriched in inhibitory interneurons. Deleting γCaMKII in parvalbumin* interneurons spares LTPE-E, but eliminates LTPE-I, disrupts experience-dependent oscillation strengthening at gamma and theta bands, and impairs memory consolidation. Re-expression of γCaMKII in hippocampal parvalbumin* interneurons repairs memory defects, whereas optogenetically driving gamma and theta oscillations following learning also exerts similar rescue effects. Thus, γCaMKII is the critical mediator of LTPE-I, and represents a novel synapse-specific target with unique roles in memory by linking synaptic activity to long-term network plasticity.

**Disclosures:** X. He: None. G. Zhou: None. J. Li: None. J. Yang: None. H. Ma: None.
Cannabinoid regulation of excitatory synaptic transmission at hippocampal TA-CA1 synapses

Authors: *A. FARAH¹, A. J. IRVING², J. HARVEY¹; ¹Univ. of Dundee, Dundee, United Kingdom; ²Univ. Col. Dublin, Dublin, Ireland

Abstract: It is known that cannabinoids produce their biological effects via activation of CB1 and CB2 receptor subtypes (Battista et al., 2012), however in the CNS, the predominant receptor is CB1. Numerous studies have examined the modulatory effects of cannabinoids on excitatory synaptic transmission at hippocampal Schaffer collateral (SC)-CA1 synapses. Moreover, increasing evidence suggests that hippocampal CB1 receptors play a role in learning and memory, and are also linked to neurodegeneration in Alzheimer’s disease (AD; Hájos & Freund, 2002). However the effects of cannabinoids on excitatory synaptic function at the anatomically-distinct temporoammonic (TA) input to hippocampal CA1 neurons is not clear. Standard extracellular recordings were used to examine the effects of different selective agonists for CB1 receptors on excitatory synaptic transmission at juvenile TA-CA1 synapses. Recordings were made from transverse hippocampal slices (350µM) prepared from 12-18 day old rats, perfused with oxygenated aCSF. Statistical analyses were performed using paired t test (two-tailed; 95% confidence interval) or repeated-measures ANOVA for comparison between multiple groups. P < 0.05 was considered significant with n representing the number of slices used from different animals. Application of methanandamide (100nM; 15min) induced a long term increase (LTP; to 148±5% of baseline; n=4; p<0.001) in excitatory synaptic transmission via activation of CB1 receptors, as application of a CB1R- antagonist blocked this effect (AM251; 102 ± 1.1% of baseline; n=4; p>0.05). CB1R-induced LTP has a postsynaptic locus of expression, and was NMDA receptor-dependent as 50 µM D-AP5 inhibited this effect (n = 4; p>0.05). These findings may be important as the TA pathway plays a role in episodic memory (Remondes & Schuman, 2004) and impairments in episodic memory is an early event in AD (Perry et al., 2000).

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Poster

286. Synaptic Plasticity: Kinases and Intracellular Signaling

Location: Hall A

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Program #/Poster #: 286.14/C24

Topic: B.07. Synaptic Plasticity

Support: American Heart Association (15SDG25700054)
National Institute of Mental Health (1R01MH113986-01A1)

Title: AMPA receptor endocytosis enhanced by transient acidification in retrieval-induced lability of an aversive memory

Authors: *J. DU, B. LIN, F. S. NAGHAVI, E. E. KOFFMAN, C. M. KRUSE, K. SINGH; The Univ. of Toledo, Toledo, OH

Abstract: Being able to predict threat by recollecting fear stimuli is crucial for most animals such that one can behave adaptively to the dangerous environmental situation. However, since fear memories developed due to traumatic events may trigger mental health conditions, attenuating such memories could be a solution to its resulting psychiatric disorders. Pavlovian conditioning is a common method to develop a reflex response by training with repetitive actions. It was used to assess the ability of lab mice to learn and remember an association between a conditioned stimulus (CS, i.e. an auditory cue) and an aversive unconditioned stimulus (US, i.e. an electric foot shock), and how that memory can be modified. A retrieval cue (CS alone) can recall and destabilize the previously coupled US-induced aversive memory, and at this labile state, the memory is subjected to diminish by the following extinction process, in which the animal receives consecutive auditory cues without coupling with foot shocks. Our earlier studies demonstrated that transient acidification by exposing animals to CO2 in conjunction with the retrieval cue significantly enhanced labilization of the target memory and more effectively weakened the aversive memory after memory extinction. By electrophysiological techniques, we found that during retrieval, the rectification on AMPA receptors (AMPARs) was enhanced, suggesting a structural change of these neuroreceptors. CO2 inhalation induced acid-sensing ion channels (ASICs) to further energize this process. Switching Ca2+-impermeable to more excitatory Ca2+-permeable membrane-embedded AMPARs via ubiquitination, endocytosis and proteasomal degradation of AMPAR subunit GluR2 at glutamatergic synapses is essential for neuroplasticity. To dissect this mechanism, amygdalae from mice receiving fear conditioning/retrieval/CO2 or sham controls were harvested, processed and assessed for relevant molecular events. Western blot analysis showed an increased CaMKII phosphorylation, the central of signaling cascades mediating learning and memory, when retrieval was coupled with CO2 inhalation. Results of 20S proteasome activity assay and Western blot analysis of phosphorylation of the 19S regulatory particle Rpt6 suggested an increased proteasome activity...
in mice exposed to CO2. Polyubiquitinated GluR2 was found more robust in mice exposed to CO2 compared to controls when a proteasome inhibitor was used to block the protein degradation. Supportively, knocking out predominant proton sensor ASIC1a to block CO2-induced signaling confirmed its regulatory effects on CaMKII phosphorylation, proteasome activity, and GluR2 ubiquitination.


Poster

286. Synaptic Plasticity: Kinases and Intracellular Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 286.15/C25

Topic: B.07. Synaptic Plasticity

Support: NIH Grant MH081935
      NIH Grant DA017392

Title: Assessing molecular mechanisms underlying NMDAR plasticity at hippocampal synapses

Authors: *S. LUTZU1, K. ALVINA2, P. E. CASTILLO3;

Abstract: NMDA receptors (NMDARs) are crucial for synaptic transmission, plasticity, and cognitive processes such as learning and memory. Like AMPA receptors, NMDARs can also undergo bidirectional long-term plasticity (NMDAR-LTP/LTD) at several key brain areas, but the molecular basis of NMDAR-plasticity remains poorly understood. In the hippocampus, the mossy fiber-to-CA3 pyramidal cell (MF-CA3) synapse expresses bidirectional NMDAR plasticity which can be elicited by physiologically-relevant coincident pre-postsynaptic burst activity. Both NMDAR-LTP and LTD induction require postsynaptic Ca2+ rise, but the Ca2+ requirements differ - e.g. NMDAR-LTP, but not NMDAR-LTD, requires Ca2+ release from internal stores. Different Ca2+ dynamics could determine the bidirectionality of NMDAR plasticity. To test this hypothesis, we combined electrophysiology with 2 photon microscopy in acute rat hippocampal slices and measured postsynaptic Ca2+ transients (CaTs) in CA3 pyramidal cells upon coincident pre-postsynaptic burst-activity used for the induction of NMDAR-LTP and LTD. At thorny excrescences (TEs), the postsynaptic target of MFs, CaTs were significantly larger during the induction of NMDAR-LTP than NMDAR-LTD. This difference was abolished by depleting Ca2+ release from internal stores, or blocking type 5 metabotropic glutamate receptors (mGluR5), two manipulations known to abolish NMDAR-LTP. Conversely, mGluR1 blockade, a manipulation that impairs NMDAR-LTD, did not affect CaTs associated to
NMDAR-LTP and LTD, suggesting distinct functions for mGluR1 and mGluR5 at the MF-CA3 synapse. Moreover, mGluR5 blockade strongly reduced synaptically-induced CaTs, which are largely mediated by NMDARs. In contrast, mGluR1 blockade only elicited a slight reduction of synaptic CaTs, consistent with a different intracellular coupling of mGluR1 and mGluR5 at MF-CA3 synapse. MFs also contact hilar mossy cells (MCs) which play key role in hippocampal function and behavior. While MF-MC and MF-CA3 synapses share structural and functional similarities, it is unknown whether MF-MC synapses express NMDAR plasticity. We found that the MF-MC synapse expresses NMDAR-LTP but not NMDAR-LTD. Intriguingly, CaTs associated with NMDAR LTP and LTD induction protocols resembled those observed at MF-CA3, suggesting that some molecular difference likely accounts for the unique learning rules at MF-CA3 and MF-MC synapses. Our findings indicate that similar patterns of activity can have a significantly different impact on NMDAR function in a target-specific manner.

Disclosures:  S. Lutzu: None. K. Alvina: None. P.E. Castillo: None.

Poster

286. Synaptic Plasticity: Kinases and Intracellular Signaling

Location: Hall A

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Program #: Poster #: 286.16/C26

Topic: B.07. Synaptic Plasticity

Support: NIH Grant 5R01NS037112-19
         NIH Grant 5R21NS102652-02

Title: The impact of rare human genetic variants of RGS14 on its binding to active Gαi1 and Rap2A

Authors: *G. TERZIOGLU1, C. MONTANEZ-MIRANDA2, K. L. FOWLER3, S. RAMINENI4, J. R. HEPLER1,2,3,4;
1Neurosci. and Behavioral Biol., Emory Univ., Atlanta, GA; 2Mol. and Systems Pharmacol.,
3Biochemistry, Cell and Developmental Biol., Emory Univ. Laney Grad. Sch., Atlanta, GA;

Abstract: The Regulator of G Protein Signaling14 (RGS14) is a multifunctional signaling protein that integrates G protein, MAPKinase, and Ca++/CaM signaling pathways in host cells. Expressed primarily in hippocampal neurons, RGS14 is a natural suppressor of synaptic plasticity and long-term potentiation (LTP) in area CA2 neurons, and linked hippocampal-based learning and memory. The RGS14 domain structure consists of an RGS domain that binds active Gαi/o-GTP, an H-Ras and Rap1/2 binding domain (R1), and a GoLoco/GPR motif that binds inactive Gαi1/3-GDP. Genetic variations account for diverse traits and individual predispositions to disease. We have identified genetic variants of RGS14 that confer changes within functional
binding domains. Here we investigate the functional consequences of two human variants of RGS14, Q116P and K334N, on their interactions with related binding partners. Variant Q116P encodes a missense point mutation located in the RGS domain that may impact RGS14 interactions with active Gαi/o-GTP. Variant K334N encodes a missense point mutation in the R1 domain and that may alter RGS14 interactions with active Rap2A. To test this, wild type RGS14 or variant Q116P were co-expressed with Gai1 in HEK cells in the presence of aluminum fluoride (AlF₄⁻) and co-immunoprecipitated, followed by SDS-PAGE and immunoblotting. In parallel, we tested RGS14-Luciferase interactions with Gai-YFP in live cells by bioluminescence resonance energy transfer (BRET). We found that wild type RGS14 and Q116P both interact with Gai1 similarly, indicating the variant did not alter RGS14 function. By contrast, similar studies performed comparing RGS14 or variant K334N interactions with a constitutive active mutation (G12V) of Rap2(G/V) found that the K334N variant blocked RGS14’s capacity to bind active Rap2A(G/V) as measured by both co-immunoprecipitation and BRET studies. Ongoing and future experiments will test the impact of K334N on RGS14 interactions with active H-Ras, hippocampal neuron physiology, and on synaptic plasticity, dendritic development and maintenance.


Poster

286. Synaptic Plasticity: Kinases and Intracellular Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program#/Poster #: 286.17/C27

Topic: B.07. Synaptic Plasticity

Support: Health Research Council of New Zealand
German Academic Exchange Service scholarship

Title: Arc protein synthesis is promoted by secreted amyloid precursor protein-alpha in primary hippocampal neurons

Authors: *R. LIVINGSTONE¹, M. K. ELDER⁴, M. BARRETT¹, C. WESTLATE¹, K. PEPPERCORN², W. TATE², W. C. ABRAHAM³, J. M. WILLIAMS¹;
¹Anat., ²Biochem., ³Psychology, Univ. of Otago, Dunedin, New Zealand; ⁴Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Secreted amyloid precursor protein-α (sAPPα) is a neuroprotective and memory-enhancing molecule, however the mechanisms through which sAPPα generates these effects are not well understood. Recently, we have shown that sAPPα enhances cell-surface expression of glutamate receptors in a protein synthesis-dependent fashion. Activity-related cytoskeletal-
associated protein Arc (Arg3.1) is an immediate early gene (IEG) capable of modulating synaptic plasticity, through internalization of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors. Accordingly, we hypothesized that sAPPα may enhance synaptic plasticity, in part, by stimulating de novo synthesis of Arc mRNA and protein. Using FUNCAT-PLA and immunohistochemistry to quantify newly made and overall levels of Arc protein, we found that sAPPα (1 nM, 2 h) enhanced levels of Arc in primary hippocampal neuron cultures. Arc protein levels were increased in both the neuronal somata and dendrites in a Ca\textsuperscript{2+}/calmodulin-dependent protein kinase II-dependent manner. Additionally, dendritic Arc expression was dependent on activation of mitogen-activated protein kinase and protein kinase G. The enhancement of dendritic Arc protein was significantly reduced by antagonism of N-methyl-D-aspartate (NMDA) and nicotinic acetylcholine (α7nACh) receptors, and fully eliminated by dual application of these antagonists. These data suggest sAPPα-regulated plasticity within hippocampal neurons is mediated by cooperation of NMDA and α7nACh receptors to engage a cascade of signal transduction molecules to enhance the transcription and translation of Arc.


Poster

286. Synaptic Plasticity: Kinases and Intracellular Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 286.18/C28

Topic: B.07. Synaptic Plasticity

Support: NIMH Grant R37 MH057068
NIMH R01MH115304

Title: Weak high-frequency synaptic stimulation induces persistent long-term potentiation in rat hippocampal slices if preceded or followed by a brief application of 4% sevoflurane

Authors: *P. TSOKAS\textsuperscript{1}, L. M. RODRIGUEZ VALENCIA\textsuperscript{2}, C. HSIEH\textsuperscript{3}, I. S. KASS\textsuperscript{1}, J. E. COTTRELL\textsuperscript{2}, T. C. SACKTOR\textsuperscript{4};
\textsuperscript{1}Physiol. and Pharmacology; Anesthesiology; The RF Furchgott Ctr. for Neural & Behavioral Sci., \textsuperscript{2}Anesthesiol., \textsuperscript{3}Physiol. and Pharmacology; The RF Furchgott Ctr. for Neural & Behavioral Sci., SUNY Downstate Med. Ctr., Brooklyn, NY; \textsuperscript{4}Physiol. and Pharmacology; Neurology; Anesthesiology; Furchgott Ctr. for Neural & Behavioral Sci., SUNY Downstate, Brooklyn, NY

Abstract: In addition its role as a molecule maintaining memory, studies have shown that PKM\textsubscript{ζ}--a brain-specific, autonomously active member of the atypical protein kinase C family--also serves a neuroprotective role. Specifically, sevoflurane preconditioning is associated with a \textit{de novo} increase in PKM\textsubscript{ζ} protein in hippocampal slices that confers neuroprotection by
mitigating the aversive effects of hypoxia on neuronal health (Wang et al., J Physiol. 590:4093, 2012). However, postsynaptic whole-cell infusion of PKMζ in CA1 neurons has been shown to cause potentiation of postsynaptic responses throughout the neuron. Such wide-spread increases in synaptic strength throughout the neuron would be expected to introduce noise that could corrupt memory traces, but interestingly no LTP is observed with sevoflurane. This suggests that the sevoflurane-induced newly synthesized PKMζ exerts its neuroprotective effects without affecting pre-existing synaptic pools of PKMζ, and that the integrity of pre-existing engrams in the hippocampus may be achieved via compartmentalization of PKMζ. To study the mechanisms that control compartmentalization of PKMζ we examined whether there is communication between different pools of neuronal PKMζ. We hypothesized that PKMζ synthesized by a brief sevoflurane application can be driven into the synaptic compartment by a subsequent weak, sub-threshold high-frequency synaptic stimulation (weak HFS), through a process similar to “synaptic tagging and capture”. Results: We find that a 20 min application of 4% sevoflurane transforms a transient LTP induced by weak HFS into persistent LTP that lasts several hours, if the weak HFS is delivered 15, 45, or 75 min after sevoflurane washout, but not at 180 min post-sevoflurane. Remarkably, 4% sevoflurane can also transform transient into persistent LTP if the weak HFS precedes sevoflurane application by 15 or 45 min (but not 75 min). Conclusion: The underlying mechanism of this phenomenon could involve “synaptic capture” by the weakly tetanized synapses of new PKMζ synthesized in the dendrite in response to sevoflurane. Under normal physiological conditions PKMζ synthesized in response to sevoflurane is excluded from synapses and plays a role in neuroprotection without directly affecting synaptic output. This compartmentalization could break down due to pathological conditions, spontaneous ripple activity (resembling HFS) in the anesthetized brain, or during emergence from anesthesia. Breakdown of compartmentalization would corrupt pre-existing engrams by introducing noise, and may therefore contribute to post-operative cognitive dysfunction.


Poster

286. Synaptic Plasticity: Kinases and Intracellular Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 286.19/C29

Topic: B.07. Synaptic Plasticity

Support: NIH Grant NS102490

Title: Simulations suggest combined-drug treatments that enhance synaptic plasticity and potentially improve learning and memory
Abstract: Genetic disorders such as Rubinstein-Taybi syndrome (RTS) and Coffin-Lowry syndrome (CLS) cause lifelong intellectual disability, including deficits in learning and memory. Can pharmacological therapies be suggested that improve learning and memory in these disorders? To address this question, we simulated drug effects within a computational model describing induction of late long-term potentiation (L-LTP). Biochemical signaling pathways impaired in these and other disorders converge on a common target, histone modification by acetyltransferases such as CREB binding protein (CBP), which facilitates induction of genes necessary for L-LTP. We focused on four classes of drugs: TrkB agonists, cAMP phosphodiesterase inhibitors, histone deacetylase inhibitors, and ampakines. Results suggested that each of these drugs alone may rescue, in part, deficits in L-LTP and learning. A potential disadvantage, however, was the necessity of simulating strong drug effects (i.e., high dosages), which could, in practice, produce adverse side effects. Thus, we also investigated the effects of the six drug pairs that use these four classes. These combination treatments normalized impaired L-LTP with substantially smaller individual drug ‘dosages’. In addition, two of these combinations, using a cAMP phosphodiesterase inhibitor paired with a TrkB agonist or an ampakine, exhibited strong synergism in simulated L-LTP rescue. Drugs simulated in these combinations correspond to specific small molecules that are either clinically approved for other disorders, or have demonstrated effects in animal models. Therefore, we suggest these drug combinations are promising candidates for further empirical studies in animal models of genetic disorders that impair histone acetylation, L-LTP, and learning.

Disclosures: P.D. Smolen: None. D.A. Baxter: None. J.H. Byrne: None.

Poster

286. Synaptic Plasticity: Kinases and Intracellular Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 286.20/C30

Topic: B.07. Synaptic Plasticity

Title: Calcium dependent remodeling of PSD-95 by competitive binding for PDZ domains

Authors: *P. GIOLANDO, B. DAVIS;
Biomed. Engin., Purdue Univ., West Lafayette, IN

Abstract: Introduction: About 1 in 60 children in the US have been diagnosed with Autism Spectrum Disorder (ASD). Of the genes associated with ASD, those most correlated with the disorder produce proteins involved in synaptic function. Among these SYNGAP1 is a major
constituent and a downstream target of glutamate. Also highly expressed is the scaffolding protein PSD-95, which binds synGAP at all three of its PDZ domain. SynGAP has been found to occupy up to 15% of these domains at one time (1), potentially restricting the binding of other proteins to these sites including TARP and LRRTM proteins involved in regulating synaptic functions. To gain a full understanding of how synGAP competes for binding to PDZ domains, we present a computational model of the dynamic binding of synGAP and other key synaptic proteins (TARP, and LRRTM) with PDZ domains. Our model focuses on two events influencing the binding of synGAP to PDZ domains: binding of Ca\(^{2+}/\text{Calmodulin (Ca}^{2+}/\text{CaM)}\) to synGAP, and phosphorylation of synGAP by CAMKII. We hypothesize these events provide a mechanism for regulating the availability of PDZ domains at the synapse.

**Materials and Methods:** The dynamic interactions among synGAP, CaM, CaMKII, TARP, and LRRTM are modeled according to mass action kinetics. We define the time dependent binding for each protein by numerically solving a system of ordinary differential equations. Model parameters are derived from previous literature. To quantify model robustness, we use Latin hypercube sampling to perform a global sensitivity analysis.

**Results and Discussion:** To validate our model, we compared the concentration of synGAP bound to PSD-95 at basal conditions with known values and found synGAP bound up to 15% of the PSD-95 domains, in agreement with previous studies (Figure 1). The model accurately predicted the reduction in synGAP bound to PSD-95 by the activation of CaM and CaMKII, which previous *in vitro* studies determined to be 20% and 54% respectively.

To test whether phosphorylation of synGAP by CaMKII causes significant rearrangement of proteins bound to PSD-95, we stimulated activation of CaMKII by Ca\(^{2+}/\text{CaM}\) at 100 Hz Ca\(^{2+}\) spike frequency for 10 s, to mimic LTP and at 10 Hz for LTD. We find significant rearrangement in the binding pattern of synGAP, TARP and LRRTM occurs on the order of seconds and is thus on a timescale relevant for AMPAR receptor trafficking.

**Figure 1.** Rearrangement of proteins bound to PSD-95 during LTP stimulation (100 Hz), at 20 s for a duration of 10 s. Each protein is normalized to its steady state bound concentration.

**Disclosures:** P. Giolando: None. B. Davis: None.

**Poster**

286. Synaptic Plasticity: Kinases and Intracellular Signaling

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 286.21/C31

**Topic:** B.07. Synaptic Plasticity

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generous donations from Cure Medical, Research for Cure,
Conditional genetic deletion or knockdown of PTEN in neurons leads to persistent activation of mTOR, which enables regeneration of CNS axons and sprouting of intact axons after injury and induces de novo growth of cell bodies and dendrites of adult neurons in the absence of any injury. It is unknown how PTEN deletion, persistent activation of mTOR, and de novo growth affect the way neurons are activated in functional circuits in the brain. As a first step to address this question, we assessed consequences of PTEN knockdown in cortical neurons and granule cells of the dentate gyrus, focusing on activity-induced kinase signaling and immediate early gene (IEG) induction. Adult rats received unilateral injections of AAV expressing shRNA against PTEN and a reporter (either zsGreen or GFP) into the motor cortex or dorsal dentate gyrus. To assess IEG induction in response to intense neuronal activity, rats were anesthetized with urethane and prepared for acute neurophysiology. Rats with intra-cortical injections of AAVshPTEN received high frequency stimulation (HFS) to the side of the cortex that received AAVshPTEN. Stimulation was delivered via surface electrodes, and consisted of trains of 8 pulses at 400hz delivered 1/10sec for 10 minutes at an intensity sufficient to induce muscle twitch. Rats were perfused 20-60 minutes post-stimulation. For rats with injections of AAVshPTEN into the dentate gyrus, HFS was delivered as above via a stimulating electrode positioned in the entorhinal cortex to activate the perforant path while monitoring performant path evoked potentials via a recording electrode positioned in the dentate gyrus. Rats were perfused 60 minutes after the initiation of HFS. Brains were prepared for immunocytochemistry using antibodies for PTEN to reveal the area of deletion, IEGs (Arc and c-fos), and phospho-specific antibodies for kinase signaling (p-ERK, p-S6). In rats with intra-cortical injections of AAVshPTEN, HFS of the cortex strongly induced IEG expression throughout the cortex on the side of the stimulation but IEG induction was attenuated in areas of PTEN deletion. In rats with injections of AAVshPTEN into the dentate gyrus, HFS of the perforant path massively induced Arc and c-fos expression throughout the dentate gyrus on the side of the stimulation, but this induction was almost completely abrogated in areas of PTEN deletion. Immunostaining for p-ERK and p-S6 revealed abrogation of both mTOR and ERK activation in PTEN-deleted granule cells, which may account for the abrogation of IEG induction. It remains to be determined whether how attenuation of activity-dependent kinase activation and IEG induction alter overall neuronal function.

Disclosures:  O. Steward: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Axonis Inc. K.M. Yee: None.
Poster

286. Synaptic Plasticity: Kinases and Intracellular Signaling

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 286.22/C32

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

Support: R01 grants DA017392
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Fondation pour la Recherche Médicale (Postdoctoral Fellowship for a research abroad)
Fondation Bettencourt Schueller (Prix pour les Jeunes Chercheurs 2016)

Title: LTP at mossy cell-granule cell synapse requires adenosine/A2a receptor retrograde signaling

Authors: *K. NASRALLAH1, Y. HASHIMOTODANI3, M. GULFO2, R. LUJAN4, P. E. CASTILLO5;
1Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., New York, NY; 2Albert Einstein Col. of Med., Bronx, NY; 3Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Medecine, New York, NY; 4Univ. of Castilla-La Mancha, Albacete, Spain; 5Albert Einstein Coll Med., Bronx, NY

Abstract: The dentate gyrus, a major input area of the hippocampus, contains two types of excitatory neurons: dentate granule cells (GCs) and hilar mossy cells (MCs). MCs receive inputs from GCs and project back to GCs locally (intralamellar), contralaterally, and along the dorsoventral axis of the hippocampus, thereby establishing an associative positive-feedback loop (GC-MC-GC). However, how MCs contribute to dentate gyrus function remains poorly understood. We recently reported that MC-GC synapses express a robust form of presynaptic long-term potentiation (MC-GC LTP) that can increase the dentate gyrus output. Mechanistically, MC-GC LTP is NMDA receptor-independent and requires postsynaptic BDNF/TrkB and presynaptic cAMP/PKA signaling. MC-GC LTP also requires retrograde signaling but the identity of the retrograde signal remains unknown. To address this issue, we first determined whether postsynaptic firing alone could induce LTP in rodent hippocampal slices. We found that theta-burst firing (TBF) of a single GC by direct depolarization is sufficient to induce plasticity in an input-selective manner, namely, MC inputs but not neighboring medial perforant path (MPP) inputs undergo LTP. Using this single cell manipulation and selective pharmacology, we discarded a role for conventional retrograde messengers, such as endocannabinoids and nitric oxide. Given the requirement for presynaptic cAMP/PKA activity, we explored whether a Gs-coupled receptor could be involved. We discovered that activation of A2a receptors is necessary and sufficient for MC-GC LTP, whereas activation of the Gi/o-coupled...
A1 receptor dampens LTP induction. In addition, using immunoelectron microscopy, we found that A2A receptors are highly expressed at MC axon terminals but not at MPP axon terminals. Remarkably, interfering with adenosine release from GCs abolished LTP. Altogether, these results strongly suggest that adenosine may act as a retrograde messenger to mediate MC-GC LTP through activation of presynaptic, Gs-coupled A2A receptors. By increasing the strength of MC inputs onto GCs, A2A receptor-mediated LTP may contribute to memory formation and epilepsy.


Poster

287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 287.01/C33

Topic: B.07. Synaptic Plasticity

Support: Uehara Memorial Foundation, Takeda Science Foundation Takeda Medical Research Foundation Novartis Stiftung für Medizinisch-Biologische Forschung (MEXT) of Japan (25293043 and 17H04014 to MS, 15k08151 and 23590232 to M-J X).

Title: Phosphoinositide responsive Phldb2 is crucial for LTP regulating synaptic NMDA and AMPA receptor density and PSD95 turnover

Authors: *M. XIE1,2,4, Y. ISHIKAWA5, H. YAGI6, Y. FUKAZAWA3, M. SATO7,4; 
1Res. Ctr. for Child Mental Develop., 2Life Sci. Innovation Ctr., Univ. of Fukui, Yoshida-gun, Japan; 3Dept. of Morphological and Physiological Sci., Univ. of Fukui, Fukui, Japan; 4United Grad. Sch. of Child Develop., Osaka University, Kanazawa University-Hamamatsu Univ. Sch. of Medicine, Chiba Univ. and Univ. of Fukui., Osaka, Japan; 5Maebashi Inst. of Technol., Gunma, Japan; 6Dept. of Anat. and Cell Biol., Hyogo Col. of Med., Nishinomiya, Japan; 7Dept. of Anat. and Neurosci., Osaka Univ. Grad. Sch. of Med., Suita, Japan

Abstract: The essential involvement of phosphoinositides in synaptic plasticity is well-established, but incomplete knowledge of the downstream molecular entities prevents us from understanding their signalling cascades completely. Here, we determined that Phldb2, of which pleckstrin-homology domain is highly sensitive to PIP3, functions as a phosphoinositide-signalling mediator for synaptic plasticity. BDNF application caused Phldb2 recruitment toward postsynaptic membrane in dendritic spines, whereas PI3K inhibition resulted in its reduced
accumulation. Phldb2 bound to postsynaptic scaffolding molecule PSD-95 and was crucial for localization and turnover of PSD-95 in the spine. Phldb2 also bound to GluA1 and GluA2. Phldb2 was indispensable for the interaction between NMDA receptors and CaMKII, and the synaptic density of AMPA receptors. Therefore, PIP3-responsive Phldb2 is pivotal for induction and maintenance of LTP. Memory formation was impaired in our Phldb2−/− mice.


Poster

287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 287.02/C34

Topic: B.07. Synaptic Plasticity

Support: Ministry of Science and Technology (MOST 106-2321-B-006-018)

Title: Transcranial direct current stimulation induces hippocampal metaplasticity mediated by BDNF-dependent TrkB activation

Authors: *T.-H. YU1, Y.-J. WU2,3, M.-E. CHIEN2, K.-S. HSU1,4;

Abstract: Transcranial direct current stimulation (tDCS) is a non-invasive stimulation which can modulate brain function via applying electric fields. It has been used on several neurological disorders in humans. However, the detail cellular and molecular mechanisms is unclear. We investigated mechanism of the aftereffects after tDCS which can enhance the long-term potentiation (LTP) at Schaffer collateral-CA1 synapses, also can alter the performance of learning and memory. Anodal tDCS was applied using a constant current stimulator connected with a head electrode of 0.25 cm² on the skull over the hippocampus for 30 min at 0.25 mA. There were no significant changes on hippocampal EEG and neuronal excitation in tDCS-treated group by using electroencephalogram (EEG) and immunohistochemistry. Extracellular field potential recordings were used to examine the induction of LTP at hippocampal CA1 region. We found that hippocampal CA1 LTP were enhanced in tDCS-treated group compared to sham-treated group. This enhancement could be lasting for 12 hours and inhibit by the TrkB inhibitor ANA-12. It also increased brain derived neurotrophic factor (BDNF) protein levels which were measured with ELISA kit. To test whether tDCS influenced learning and memory, we compared memory performance in one-trial passive avoidance learning task between sham- and tDCS-treated group. Anodal tDCS enhanced memory performance in the behavior test. This
enhancement also could be inhibited by the TrkB inhibitor. Altogether, our results suggest that the aftereffects of anodal tDCS could facilitate the induction of hippocampal CA1 LTP and enhance the memory performance mediated by BDNF/TrkB pathway. These findings may give a further understanding on the cellular and molecular mechanisms of the aftereffects induced by tDCS and provide the therapeutic potential of tDCS for neurological disorders.

Disclosures:  T. Yu: None. Y. Wu: None. M. Chien: None. K. Hsu: None.

Poster

287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 287.03/C35

Topic: B.07. Synaptic Plasticity

Support: JSPS Overseas Research Fellowships

Title: Synaptotagmin11 is essential for long term synaptic plasticity and spatial memory

Authors: *M. SHIMOJO1,2, J. C. MADARA4,2, S. PANKOW3, J. R. YATES, III3, M. HIGUCHI1, T. C. SUDHOFT5,6, A. MAXIMOVO2,6;

Abstract: Synaptotagmin-11 (Syt-11) is an uncharacterized member of synaptotagmin protein family that lacks apparent ability to bind calcium, phospholipids and SNARE proteins. Recent genetic studies suggest that mutations in the Syt-11 locus links to schizophrenia and Parkinson’s disease. However, the biological role of Syt-11 in the mammalian brain still remains uncertain. Here, we demonstrate a detailed analysis of expression and localization of Syt-11 in cultured neurons and central nervous system, and phenotype of genetic mutant mice that lack Syt-11 either constitutively or in specific neuronal population. We observed that Syt-11 is broadly expressed in the mouse brain and distributed as a membrane constituent of mobile secretory vesicles that recycle in an activity-dependent manner. Importantly, while constitutive deletion of Syt-11 in all tissues resulted in early postnatal lethality, mice lacking Syt-11 in forebrain neurons exhibited normal lifespan but an impaired ability of spatial learning and memory. Moreover, ablation of Syt-11 had no effect on basal neurotransmission but blocked the induction of hippocampal long-term synaptic potentiation. Our findings indicate that Syt-11 acts on a secretory pathways essential for synaptic plasticity and memory, and provide important insights into the molecular mechanisms underlying neurological disorders.
Poster

287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 287.04/C36

Topic: B.07. Synaptic Plasticity

Title: Cholinergic modulation of the corticostriatal synaptic plasticity in sub-group of stratal projection neurons

Authors: *A. TAMURA, J. A. CHOUINARD, K. KURIMA, Y. AKAMINE, J. R. WICKENS; Neurobio. Res. Unit, Okinawa Inst. of Sci. and Technol., Onna-son, Japan

Abstract: The cholinergic interneurons (CINs) of the striatum are crucial for behavioral flexibility. CINs of the dorsomedial striatum (DMS) play a role in strategy switching. However, how CINs modulate the neural circuitry underlying strategy switching is unclear. The glutamatergic afferents from the cerebral cortex to the striatum display activity-dependent plasticity in the corticostriatal synapses, and may be involved in certain types of learning. One hypothesis is that strategy switching may be realized by a modulatory effect of CINs on corticostriatal plasticity. Here, we investigated the effect of CINs on activity-dependent plasticity in the corticostriatal synapses. To control tonic firing of CINs, adeno-associated virus (AAV) encoding halorhodopsin (NpHR) was injected into DMS of ChAT-cre mice. AAV injected mice expressed NpHR in CINs and we can optogenetically inactivate CINs firing. We made whole-cell ex vivo slice recordings from spiny projection neurons (SPNs), which are the output neurons of the striatum, and recorded EPSPs induced by electrical stimulation of the corpus callosum. Activity dependent synaptic plasticity was induced by high-frequency stimulation under the Mg-free conditions. This conditioning stimulus combined with optogenetic inactivation of CINs during HFS induced long-term potentiation in some SPNs. However, other group of SPNs showed long-term depression in response to the same conditioning stimuli. These results might indicate that CIN activity modulates corticostriatal plasticity differentially in direct and indirect SPNs.

287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 287.05/C37

Topic: B.07. Synaptic Plasticity

Support: FAPESP 2016/01607-4

Title: A short episode of high intensity sound decreases BDNF levels in the hippocampus, which impairs LTP

Authors: J. L. DE DEUS¹, M. R. AMORIN², A. O. CUNHA³, L. G. S. BRANCO², *R. M. LEAO¹;
¹Physiol., ²Morphology, Physiol. and Basic Pathology, ³Univ. of Sao Paulo, Ribeirao Preto, Brazil

Abstract: Hippocampal neurons can respond to acoustic dimension clues, and that their function is affected by auditory stimulation and deprivation. High-intensity sound is a noxious stimulus, which can produce intense emotional reactions in animals and humans. We have previously found that a single minute exposure to a 110 dB sound inhibits Schaffer-CA1 LTP in hippocampal slices from rats. Here we investigate the possible mechanisms of this effect Whole-cell recordings were performed in hippocampal slices from rats sacrificed 2 hours after sound stimulation. We found that both GABAergic and glutamatergic (AMPA/kainate and NMDA receptor mediated) neurotransmission were unaffected by high intensity sound. Neurotrophins such as brain-derived neurotrophic factor (BDNF) are known to promote LTP in the hippocampus. We then measured the BDNF content in CA1 area using ELISA and found lower levels of BDNF in the CA1 from rats exposed to high intensity sound (2344 ± 89 pg/mg protein vs. 1732 ± 77 pg/mg protein, n= 8 and 7 respectively; p<0.001). We then studied whether BDNF can reverse LTP inhibition after a single episode of high-intensity sound. LTP was induced by 3 high-frequency stimulation trains (100 Hz; 1 second each) on the Schaffer-CA1 and then field postsynaptic excitatory potentials were recorded in the stratum radiatum of the CA1. BDNF (50 ng/ml) and the trk-B agonist, LM 22A4 (5 μM) were perfused for 5 minutes before and for 5 minutes after induction of LTP. LTP in animals exposed to a single sound stimulus is inhibited (1.04 ± 0.11, n = 5) in contrast to sham-stimulated rats (1.41 ± 0.11, n = 4; p <0.05). Perfusion with BDNF restored the LTP to its original levels (1.55 ± 0.12, n = 6). Application of BDNF in slices from sham-stimulated animals did not induced further potentiation of LTP (1.4 ± 0.07, n=7; p=0.9). The agonist of the BDNF target, the trk-b receptor, LM 22A4, also rescued LTP from sound-stimulated animals (1.62 ± 0.17, n = 5). Our results strongly suggest that the exposure to high intensity sound inhibits the BDNF production in the hippocampus, which could be the mechanism of the inhibition of LTP by high-intensity sound exposure.

Poster

287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 287.06/C38

Topic: B.07. Synaptic Plasticity

Support: NMRC-CBRG-0099-2015
NMRC-OFRG-0037-2017
MOE2017-T3-1-002

Title: Modulation of synaptic plasticity in hippocampal CA1 region by basolateral amygdala

Authors: *Y. CHONG, C. GOH, S. SREEDHARAN;
Natl. Univ. of Singapore, Singapore, Singapore

Abstract: Amygdala plays a vital role in emotional events which is known to affect memory consolidation. Hippocampus is a critical brain region involved in memory consolidation. Evidence has shown that a specific sub-region of amygdala, the basolateral amygdala (BLA), has reciprocal connections to ventral hippocampus proving that priming on the BLA alters the long-term potentiation (LTP) in hippocampus. To investigate time- and activity-dependent modulation of synaptic plasticity in the hippocampal CA1 region by the BLA and its underlying mechanisms, we used field electrophysiological recordings in acute horizontal brain slices from 5 to 7 weeks old C57BL/6J mice to study synaptic plasticity in the CA1 area under different BLA stimulation conditions. First, we studied the effect of timing dependence of the effect of BLA co-stimulation on synaptic plasticity in hippocampal area CA1. Co-induction of high frequency stimulation (100 Hz) in BLA and late-LTP in CA1 resulted in an elevated LTP while stimulation of BLA prior to late-LTP induction at a specific time scale (10 min and 30 min) suppressed late-LTP. No significant effect was observed when BLA stimulation was given 5 min prior to late-LTP induction in CA1. Next, we observed that the enhancement of late-LTP due to BLA co-stimulation (100 Hz) ameliorated LTP impairments in the synaptic competition phenomenon in CA1. However, stronger BLA co-stimulation (200 Hz) resulted in the impairment of late-LTP and associative plasticity in hippocampal area CA1. The modulation of BLA on synaptic plasticity in CA1 required protein synthesis and NMDAR as application of the inhibitors and antagonist, respectively, impaired late-LTP maintenance. The ongoing biochemistry study suggests that Arc protein level in CA1 was altered in the BLA co-stimulation conditions. Our finding confirms that amygdala has positive and negative influences on long-term synaptic plasticity in the hippocampus and it is time- and activity-dependent. Unravelling
its cellular mechanisms may provide insights into the mechanisms of emotional disorders such as post-traumatic stress disorder (PTSD).

Disclosures: Y. Chong: None. C. Goh: None. S. Sreedharan: None.

Poster

287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 287.07/C39

Topic: B.07. Synaptic Plasticity

Support: MINECO BFU2017-82375-R

Title: Long-term potentiation can be trans-synaptically evoked in the hippocampal formation

Authors: *M. T. Romero-Barragan, J. M. Delgado-Garcia, A. Gruart; Div. of Neurosciences, Pablo De Olavide Univ., Sevilla, Spain

Abstract: Long-term potentiation (LTP) evoked by high frequency stimulation (HFS) is a very well-known experimental procedure that shares certain mechanisms with learning and memory processes. LTP is a typical example of synaptic plasticity, which appears after applying an HFS train to the afferent pathway of a CNS synapse. Basically, the LTP consists in an increase of the synaptic response to a control stimulus following the presentation of the HFS trains. This technique was described for the first time in the hippocampus and it is still studied mostly there, since those synaptic connections are very susceptible to LTP induction. Besides, the highly laminar nature of the hippocampus provides a suitable model to study synapses with different ultrastructural dispositions. Although most of preceding studies have been performed in vitro, we have developed a new experimental approach to carry out these experiments in behaving animals. The main goal of this study was to ensure that there are synaptic changes in strength not only in the first synapse where LTP is induced, but also in those which are contiguous to it. Field excitatory post-synaptic potentials (fEPSP) evoked in five hippocampal synapses, located both ipsi- and contralateral hemispheres, were studied in alert behaving male mice. HFS was presented to the perforant pathway (PP) and recordings were carried out in the CA1 and CA3 areas of the ipsilateral hippocampus and in the CA1 area of the contralateral side. The five studied synapses were: PP-CA1i, PP-CA3i, PP-CA1c, CA3-CA1i, and CA3-CA1c. Animals were prepared for chronic recordings in the mentioned synapses following procedures described elsewhere (Gruart et al., J. Neurosci., 2006). We have characterized input/output curves, pair pulse facilitation (PPF) and LTP of these five synapses. Data from input/output curves and paired-pulse facilitation proved that the five studied synapses have similar basic properties, which makes their later comparison easier for subsequent histological, pharmacological, and genetic analysis. Importantly, regarding HFS of the PP, we observed the presence of significant
LTP both at the CA3-CA1c and PP-CA1c synapses. In conclusion, these results indicate that LTP can be evoked at synapses located far away from the stimulated afferent pathways.

**Disclosures:** M.T. Romero-Barragan: None. J.M. Delgado-Garcia: None. A. Gruart: None.

**Poster**

**287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 287.08/C40

**Topic:** B.07. Synaptic Plasticity

**Support:** 5R01MH099085-05

**MH115188**

**Title:** Effects of social isolation rearing and ketamine on hippocampal synaptic plasticity

**Authors:** *J. B. LOGUE*\(^1\), K. J. SCHOEPFER\(^1\), Y. ZHOU\(^2\), M. KABBAJ\(^1\);

\(^1\)Biomed. Sci., \(^2\)Florida State Univ., Tallahassee, FL

**Abstract:** Social isolation rearing is a long-term stressor that produces lasting effects including memory deficits, cognitive impairments, and hippocampal functional and structural alterations. Ketamine (KET) is a non-competitive N-methyl-d-aspartate receptor (NMDAR) antagonist currently of interest for its antidepressant effects at subanesthetic doses. Previous work from our lab has shown that rats’ sex and gonadal hormone status play critical roles in mediating sensitivity to the antidepressant-like effects of KET, as females behaviorally respond to a lower dose of KET (2.5 mg/kg, i.p.) than males (5 mg/kg, i.p.), an effect that requires both estradiol (E2) and progesterone (P4) on-board. However, electrophysiological correlates to this female-specific dose sensitivity remain unclear. This study aims to investigate the interaction between social isolation rearing stress and low-dose KET on hippocampal activity and synaptic plasticity in rats of both sexes. We hypothesize that: 1. The field excitatory post-synaptic potentials (fEPSPs) in dorsal hippocampus Schaffer collateral (CA3-CA1) synapses may be impaired by isolation stress in both sexes. 2. A single low dose of KET (2.5 mg/kg, i.p.) given \textit{in-vivo} 3hr prior to slice recording may rescue stress-induced fEPSP deficits in females, but not males. 3. Female-specific KET rescue effects may be modulated by estrous stage (Proestrus: high E2/P4, Diestrus: low E2/P4), and 4. Stress-induced fEPSP deficits in both sexes may be ameliorated with a single treatment of 5 mg/kg KET (i.p.). Postsynaptic plasticity was evaluated by measuring tetanic stimulation-induced long-term potentiation (LTP), the responsivity of the synapse to electrical stimulation was measured by fEPSP input-output curves, and presynaptic transmitter release probability was measured by paired-pulse facilitation experiments. Completion of this project will generate insights into the interactions between gonadal hormones and KET at this synapse and the potential restoration of neural plasticity.

Poster

287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 287.09/C41

Topic: B.07. Synaptic Plasticity

Title: Hippocampal long-term potentiation is modulated by exercise-induced alterations in dopaminergic neurotransmission in mice selectively bred for high voluntary wheel running

Authors: *K. D. PARFITT¹, J. M. PHAN², J. YI⁴, J. H. A. FOOTE¹, S. K. OLSON³, T. GARLAND, Jr⁵;
¹Dept of Neurosci., ²Mol. Biol., ³Biol., Pomona Col., Claremont, CA; ⁴Neurosci., Washington Univ., Saint Louis, MO; ⁵Biol., Univ. of California Riverside, Riverside, CA

Abstract: Mice from lines selected for high voluntary wheel-running activity (high-runner; HR) display behaviors that resemble features of human attention deficit hyperactivity disorder (ADHD). They exhibit increased motor activity and are highly motivated to run, running almost three times farther per day than non-selected control (C) counterparts. Our previous work revealed that HR mice with running-wheel access show significantly increased hippocampal long-term potentiation (LTP) compared to HR mice without wheel access, and to C mice with or without wheel access. Because hyperactivity and motivation are associated with alterations in dopaminergic neurotransmission, and because running is reduced in HR mice treated with methylphenidate, we were interested in possible changes in dopamine transporters and receptors in the brain of HR mice. Here, we examined the influence of this intense running on dopamine D1 receptors (D1R) and dopamine transporters (DAT) in hippocampus of HR vs C mice with wheel access. Pretreatment of hippocampal slices with the D1R agonist SKF-38393 prevented the elevated LTP seen in HR mice that had wheel access, such that it was similar to LTP seen in slices from C mice with or without wheel access; the magnitude of LTP in the absence vs presence of SKF was 83 ± 12% vs 41 ± 11% respectively (p<0.05), whereas the magnitude of LTP was not significantly different in SKF-treated or untreated C mice with wheels. SKF-38393 did not affect basal synaptic transmission. Using Western blot analyses, we also examined the expression of D1Rs and DAT in the hippocampus, prefrontal cortex, striatum, and cerebellum and found that hippocampal levels of D1Rs were significantly reduced in HR mice compared to C mice; levels of D1Rs were 30% lower in HR mice without wheels and 20% lower in HR mice with wheels, compared to C mice with or without wheels. Levels of DAT in hippocampus were elevated 21% in HR mice, with or without wheel access. D1R and DAT levels were not altered in other brain regions examined. Taken together, these results suggest that the increased hippocampal LTP seen in HR mice with wheel access may be related to alterations in
dopaminergic synaptic transmission that underlie the neurophysiological basis of hyperactivity and/or addictive behaviors.


**Poster**

**287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 287.10/C42

**Topic:** B.07. Synaptic Plasticity

**Title:** Computational analysis of cholinergic mechanisms for disinhibition of CA1 pyramidal cells and induction of hippocampal plasticity

**Authors:** *I. GUERREIRO*¹, Z. GU², J. L. YAKEL², B. GUTKIN¹;
¹Ecole Normale Superieure, Paris, France; ²Natl. Inst. of Envrn. Hlth. Sci., Research Triangle Park, NC

**Abstract:** Studies of induction of hippocampal plasticity have shown that blockade of GABA inhibition can greatly facilitate the induction of LTP in excitatory synapses (Wingstrom and Gustafsson, 1983). It was shown experimentally that repeated inhibition of hippocampal CA1 somatostatin-positive interneurons can induce lasting potentiation of Schaffer collateral (SC) to CA1 EPSCs, suggesting that repeated dendritic disinhibition of CA1 pyramidal cells plays a role in the induction of synaptic plasticity. It was also shown experimentally that repeated cholinergic activation enhanced the SC-evoked EPSCs through a7-containing nicotinic acetylcholine receptors (a7 nAChRs) expressed in oriens lacunosum-moleculare (OLMa2) interneurons. We used a biophysically-realistic computational model to examine mechanistically how inhibitory inputs to hippocampal pyramidal neurons can modulate the plasticity of the SC-CA1 excitatory synapses. We found that locally-reduced GABA release (disinhibition) paired with SC stimulation could lead to increased NMDAR activation and intracellular calcium concentration sufficient to upregulate AMPAR permeability and potentiate the excitatory synapse. Repeated disinhibition of the excitatory synapses could lead to a larger increase of the AMPAR permeability. This results in the potentiation of the SC-CA1 excitatory synapse, which can be maintained when disinhibition period is over through repeated stimulation of the SC that keeps a balance between the down and upregulation of the AMPARs. We then used our model to show how repeated cholinergic activation of a7 nAChR in stratum oriens OLMa2 interneurons paired with SC stimulation can induce synaptic plasticity at the SC-CA1 excitatory synapses. Activation of pre-synaptic a7 nAChRs in OLM cells activates these interneurons which, in turn, inhibit fast-spiking stratum radiatum interneurons that provide feed-
forward inhibition onto pyramidal neurons after SC excitation, and thus disinhibiting the CA1 pyramidal neurons and inducing synaptic plasticity.

**Disclosures:** I. Guerreiro: None. J.L. Yakel: None. B. Gutkin: None. Z. Gu: None.

**Poster**

**287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 287.11/C43

**Topic:** B.07. Synaptic Plasticity

**Support:** NIH Grant DA017392
 NIH Grant MH081935

**Title:** Presynaptic LTP at mossy cell-dentate granule cell synaptic transmission requires pre- and postsynaptic BDNF and it can be induced by experience

**Authors:** *C. BERTHOUX*¹, K. NASRALLAH², P. E. CASTILLO³;
¹Dominick P. Purpura Dept. of Neurosci., ²Rose F. Kennedy Ctr., ³Albert Einstein Col. of Med., Bronx, NY

**Abstract:** The dentate gyrus (DG), the main input region of the hippocampus, plays a critical role in memory formation by transforming patterns of cortical inputs into new patterns of output to the CA3 area. The DG includes two principal cells, hilar mossy cells (MCs) and dentate granule cells (GCs), which are reciprocally connected thereby establishing a GC-MC-GC associative circuit. Remarkably, MCs send extensive projections—intralamellar, contralateral and along the longitudinal axis of the hippocampus—that synapse onto GC proximal dendrites. Given this wide distribution, as well as the proximal localization of MC-GC synapses, activity-dependent plasticity of MC-GC transmission may contribute significantly to information processing and DG-dependent cognitive functions. Our lab recently reported that physiologically-relevant patterns of MC activity *in vitro* induce robust presynaptic LTP of MC-GC transmission. While MC-GC LTP requires postsynaptic BDNF/TrkB signaling, a pathway known to regulate protein synthesis, it is unknown whether BDNF is derived from the pre- and/or postsynaptic neuron, and the downstream signaling pathways remain poorly understood. Moreover, it is unclear whether this form of LTP can occur *in vivo*. Here, we combined electrophysiology and 2-photon live imaging in acute rodent hippocampal slices to investigate the molecular basis underlying BDNF/TrkB-dependent LTP at MC-GC synapses. Using the fluorescent BDNF sensor BDNF-pHluorin and cell-specific conditional knock-out for *Bdnf* and *TrkB*, we found that BDNF is released from both MC axonal boutons and GC proximal dendrites following repetitive activation of MCs, suggesting that both anterograde and retrograde BDNF/TrkB signaling are involved in MC-GC LTP. In addition, blocking protein synthesis (80
μM cycloheximide) during induction but not 20 min post induction abolished MC-GC LTP, indicating the involvement of fast protein synthesis. Lastly, we found that exposing mice to enriched environment for 2 weeks increased MC-GC synaptic efficacy and occluded MC-GC LTP, strongly suggesting that this form of plasticity can be induced in vivo. Altogether, our findings highlight a novel mechanism by which the DG processes information and may contribute to hippocampal-dependent learning and memory.

Disclosures:  C. Berthoux: None.  K. Nasrallah: None.  P.E. Castillo: None.

Poster

287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 287.12/C44

Topic: B.07. Synaptic Plasticity

Support: NIH Grant MH094896

Title: Regulation of synaptic plasticity by BDNF-endocannabinoid interactions

Authors: F. LEMTIRI-CHLIEH, T. M. ROBINSON, *E. S. LEVINE;
Neurosci., Univ. of Connecticut Sch. of Med., Farmington, CT

Abstract: The goal of these studies is to explore the functional interactions between brain-derived neurotrophic factor (BDNF) and endogenous cannabinoids (eCBs) in regulating activity-dependent synaptic plasticity in the neocortex. Both BDNF and eCBs have been implicated in a diverse range of physiological processes, including sensory perception, motor coordination, memory, and cognitive abilities. Disruption of BDNF and/or eCB signaling may play a role in several neurologic and psychiatric disorders, including anxiety, depression, schizophrenia, and seizure disorders, and these neuromodulatory systems are currently major targets for the development of novel therapeutics. Although there is evidence for crosstalk between BDNF and eCB signaling, little is known regarding potential synaptic interactions. We have recently shown that BDNF suppresses presynaptic GABA release at cortical inhibitory synapses and this effect is mediated by the BDNF-induced release of eCBs from postsynaptic pyramidal cells that act as retrograde signals. We have also found that BDNF induces eCB release at cortical excitatory synapses, and the suppressive effects of eCBs on glutamate release can mitigate the direct enhancing effects of BDNF at these synapses. We are now poised to explore the functional relevance of these synergistic as well as antagonistic interactions in regulating activity-dependent synaptic plasticity. In the present studies, we examined the interactions between BDNF and eCB signaling at cortical layer 5 excitatory synapses, using a pharmacologically-induced long-term potentiation (LTP) protocol that increases intracellular cAMP and enhances NMDA receptor activation. We found that this chemical-LTP requires endogenous BDNF and TrkB signaling.
because it was prevented by the TrkB antagonist ANA-12 or the Trk tyrosine kinase inhibitor K-252a. The response to this induction protocol was also impaired in mice engineered to express a common human variant in the BDNF gene (Val66Met) that reduces activity-dependent BDNF release. The role of eCB signaling will be explored through the use of CB1 receptor antagonists and inhibitors of eCB synthesis and metabolism. Release of eCBs at these excitatory synapses may diminish plasticity whereas eCB suppression at inhibitory synapses could enhance excitatory plasticity. Our overarching hypothesis is that the balance of BDNF and eCB signaling in specific circuits regulates the direction and magnitude of synaptic plasticity.

**Disclosures:** F. Lemtiri-Chlieh: None. T.M. Robinson: None. E.S. Levine: None.

**Poster**

287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 287.13/C45

**Topic:** B.07. Synaptic Plasticity

**Support:** DFG, CRC1080

**Title:** Retinoic acid modulates the ability of cultured dentate granule cells to express synaptic plasticity through intracellular calcium stores

**Authors:** *M. Lenz*¹, A. Strehl², A. Eichler¹, J. Müllerleile², P. Jedlicka², T. Deller², A. Vlachos¹,³;
¹Dept. of Neuroanatomy, Inst. of Anat. and Cell Biology, Fac. of Medicine, Univ. of Freiburg, Freiburg, Germany; ²Inst. of Clin. Neuroanatomy, Neurosci. Ctr., Goethe-University Frankfurt, Frankfurt, Germany; ³Ctr. for Basics in Neuromodulation, Fac. of Medicine, Univ. of Freiburg, Freiburg, Germany

**Abstract:** All-trans retinoic acid (atRA) has been recently linked to the ability of neurons to express synaptic plasticity. It is involved in mediating the accumulation of AMPA-receptors at excitatory postsynapses during the expression of Hebbian and homeostatic plasticity. The precise mechanisms through which atRA mediates its effects on plasticity remain not well understood. In this study, we sought to test for the role of intracellular calcium stores in atRA-mediated excitatory synaptic plasticity. In organotypic tissue cultures treated for 3 days with 1 µM atRA an accumulation of AMPA-receptors at excitatory postsynapses of dentate granule cell is observed. These changes depend on protein synthesis and are impaired if intracellular calcium stores are blocked. As a result of atRA-mediated changes in AMPA-receptor composition of excitatory synapses, the ability of neurons to express synaptic plasticity is improved. Thus, we conclude that atRA promotes metaplasticity, i.e., it improves the ability of neurons to express plasticity via a mechanism that requires functional intracellular calcium stores.
Poster

287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 287.14/C46

Topic: B.07. Synaptic Plasticity

Title: Lipopolysaccharide (LPS) induced neural inflammation is associated with alterations in synaptic plasticity probed by 10 Hz repetitive magnetic stimulation

Authors: *A. EICHLER*1,2, M. LENZ1, C. GALANIS1, D. KLEIDONAS1,3,4, N. MAGGIO5,6, A. VLACHOS1,7

1Dept. of Neuroanatomy, Inst. of Anat. and Cell Biology, Fac. of Medicine, Univ. of Freiburg, Freiburg im Breisgau, Germany; 2MOTI-VATE Grad. School, Fac. of Medicine, Univ. of Freiburg, Freiburg im Breisgau, Germany; 3Spemann Grad. Sch. of Biol. and Medicine, Univ. of Freiburg, Freiburg im Breisgau, Germany; 4Fac. of Biology, Univ. of Freiburg, Freiburg im Breisgau, Germany; 5Dept. of Neurol., Sagol Sch. of Neurosciences, Talpiot Med. Leadership Program, the Chaim Sheba Med. Ctr., Tel HaShomer, Israel; 6Sackler Fac. of Med. and Sagol Ctr. for Neurosciences, Tel Aviv Univ., Tel Aviv, Israel; 7Ctr. for Basics in Neuromodulation, Fac. of Med., Freiburg im Breisgau, Germany

Abstract: Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation technique used in clinical practice for diagnostic and therapeutic purposes. It is based on the physical principle of electromagnetic induction and allows for the activation of cortical neurons through the intact skin and skull. Despite its clinical use, the cellular and molecular mechanisms of TMS-based diagnosis and therapy remain not well-understood. Based on our previous work, which demonstrated that 10 Hz repetitive magnetic stimulation (rMS) induces structural and functional changes consistent with a long-term potentiation of excitatory neurotransmission, we here tested whether LPS-mediated neural inflammation affects the ability of neurons to express rMS-induced synaptic plasticity. Indeed, in three-week old entorhino-hippocampal tissue cultures treated with LPS for 3 days, 10 Hz-rMS-induced synaptic plasticity of CA1 pyramidal neurons is not observed. Mechanistically, these changes are accompanied by activation of microglia, changes in pro-inflammatory cytokines and neuronal plasticity markers. Hence, our results provide a biological basis for the diagnostic use of rTMS in the context of neural inflammation.

Title: Distinct role of mGluR1α and mGluR5 in synaptic plasticity of somatostatin positive interneurons in dentate gyrus

Authors: *G. GRIGORYAN, M. BARTOS;
Systemic and Cell. Neurophysiol., Inst. of Physiol. I, Univ. of Freiburg, Freiburg, Germany

Abstract: Experience-dependent modifications in excitation-inhibition balance enable a selected group of neurons to form a new cell association during learning, which represent the new memory trace. GABAergic inhibitory interneurons are proposed to play a key role in this process by influencing the activity of individual pyramidal cells and cell associations. However, how interneurons are recruited during learning is largely unknown. Here we addressed this open question in the dentate gyrus (DG), which is essential for the acquisition of new memories. The DG is characterized by a sparse activity, which is tightly controlled by synaptic inhibition. Somatostatin-expressing interneurons (SOMIs) are a major GABAergic cell type in the DG. DG-SOMIs receive synaptic inputs from granule cells (GCs), the so-called mossy fibers (MFs), and provide feedback inhibition onto GC dendrites. Here we studied the recruitment of DG-SOMIs by MF-inputs and the cellular and molecular mechanisms underlying long-term potentiation (LTP) at MF-SOMI synapses. We performed whole-cell patch-clamp recordings from morphologically and physiologically identified DG-SOMIs in acute hippocampal slice preparations. Synaptic plasticity was evoked by extracellular stimulation of pharmacologically identified MF synapses in association with action potential generation in SOMIs. An associative burst-frequency stimulation reliably induced post-tetanic potentiation followed by LTP in DG-SOMIs (151.88 ± 3.77 %, 7 SOMIs). Antibody labeling revealed that DG-SOMIs express group I mGluRs (mGluR1a/5). Incubating slices with LY367385 (10 µM), a selective mGluR1a antagonist, led to a complete abolishment of LTP (106.98% of baseline, 7 SOMIs) whereas MPEP (10 µM), a selective antagonist of mGluR5, increased LTP magnitude (p<0.001; 5 SOMIs). Examination of the paired-pulse ratio, CV analysis and failure rate revealed the presynaptic expression of LTP. Intracellular SOMI loading with the G protein inhibitor GDP-β-S (0.5 mM) or the protein kinase C (PKC) inhibitor PKC 19-36 (10 µM) resulted in a loss of LTP, indicating the importance of both downstream mediators in LTP induction at this synapse. Moreover, stereotaxic injection of rAAVs containing a shRNA expression cassette into the ventral DG of SOM-Cre-tdTomato mice confirmed the selective loss of mGluR1a-mediated LTP
at MF-SOMI synapses. Thus, we show that associative synaptic plasticity at MF-SOMIs synapses requires postsynaptic mGluR1a activation of G protein and PKC-mediated second messenger cascades, but the activation of mGluR5 is dispensable.

**Disclosures:**  
G. Grigoryan: None. M. Bartos: None.

**Poster**

287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 287.16/C48

**Topic:** B.07. Synaptic Plasticity

**Title:** Presynaptic increase in IP3 receptor type 1 concentration in the early phase of hippocampal synaptic plasticity

**Authors:**  
*H. RINGSEVJEN*¹, H. UMBACH HANSEN¹, S. HUSSAIN¹, V. JENSEN¹, S. WALAAS², S. DAVANGER³;
  ¹Dept. of Anat., ²Anat., ³Univ. of Oslo, Oslo, Norway

**Abstract:** The inositol 1,4,5-trisphosphate receptor (IP3R) subtype IP3R1 is highly enriched in the brain, including hippocampal neurons. It plays an important function in regulating intracellular calcium concentrations. Residing on the smooth endoplasmic reticulum (sER), the IP3R1 mobilizes calcium into the cytosol upon binding the intracellular signaling molecule IP3, whose concentration is increased by stimulating certain metabotropic glutamate receptors. Increased calcium may mediate synaptic changes occurring during long-term plasticity, which includes molecular mechanisms underlying memory encoding. The exact synaptic localization of IP3R1 in the central nervous system remains unclear. We hypothesized that IP3R1, in addition to its known expression in soma and dendritic shafts of hippocampal CA1 pyramidal neurons, also may be present in postsynaptic spines. Moreover, we hypothesized that IP3R1 may be present in presynaptic terminals as well, given the importance of calcium in regulating presynaptic neurotransmitter exocytosis. To test these two hypotheses, we used IP3R1 immunocytochemistry at the light and electron microscopical levels in the CA1 area of the hippocampus. Furthermore, we hypothesized that induction of long-term potentiation (LTP) would be accompanied by an increase in synaptic IP3R1 concentrations, thereby facilitating synaptic mechanisms of long-term plasticity. To investigate this, we used quantitative immunogold electron microscopy to determine possible changes in IP3R1 concentration in sub-synaptic compartments before and five minutes after high frequency tetanizations. Firstly, our data confirm localization of IP3R1 in both presynaptic terminals and postsynaptic spines. Secondly, the concentration of IP3R1 after tetanization was significantly increased in the presynaptic compartment, suggesting a presynaptic role of IP3R1 in early phases of synaptic plasticity. It is therefore possible that IP3R1 is involved in modulating neurotransmitter release by regulating calcium homeostasis presynaptically.
**Disclosures:** H. Ringsevjen: None. S. Davanger: None. S. Hussain: None. V. Jensen: None. S. Walaas: None. H. Umbach Hansen: None.

**Poster**

**287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 287.17/C49

**Topic:** B.07. Synaptic Plasticity

**Support:** Wellcome-DBT India alliance fellowship

IISER Pune

**Title:** Acetylcholine mediated modulation of long-term plasticity at Schaffer-collaterals

**Authors:** R. SHARMA, *S. NADKARNI;

Biol., Indian Inst. of Sci. Educ. and Res. Pune, Pune, India

**Abstract:** Acetylcholine is a crucial neuromodulator in the brain that activates multiple receptors; triggering biochemical changes that affect signalling within neurons across various time scales. Blocking cholinergic receptors in the hippocampal formation has been shown to impair the encoding of new memories. Hippocampal pyramidal neurons primarily express M1 and M4 receptors. The M4 receptors are localized presynaptically, where their activation is shown to cause suppression of neurotransmitter release. On the other hand, M1 receptors are found on the soma and dendrites of pyramidal neurons. Activation of M1 receptors has been shown to cause increased neuronal excitability via suppression of potassium channels like KCNQ2/3 and calcium-activated potassium channels (SK channel). Separately suppression of SK channels in the spine has been reported to enhance LTP induction for a theta-burst protocol. Additionally, M1 activation also leads to calcium release via IP3 receptors by the way of triggering IP3 production. We have developed a detailed biophysical model that incorporates multiple signalling pathways associated with M1 and M4 activity to investigate acetylcholine-mediated modulation of synaptic signaling at a CA1 dendritic spine. The predicted change in membrane potential and calcium signal in response to a single vesicle release in our model is in quantitative agreement with experimental data. Schaffer-Collaterals typically undergo both LTP and LTD, with LTD induction taking place at low frequency sustained stimuli and LTP at short bursts of high-frequency stimulus. Upon M1 activation we report a clear change in this response and show enhanced potentiation. Further, we show that synapses are more likely to undergo LTP instead of LTD in the presence of acetylcholine, with the threshold for LTP getting reduced. We also show that concurrent activation of presynaptic M4 receptors, that suppresses neuronal release, can further modify the long-term plasticity profile in a stimulus-dependent manner.

**Disclosures:** R. Sharma: None. S. Nadkarni: None.
**Poster**

**287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms**

**Location:** Hall A  
**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM  
**Program #/Poster #:** 287.18/C50  
**Topic:** B.07. Synaptic Plasticity  
**Support:** NSERC (RGPIN/436190-2013)  
NSERC (RGPIN/203175-2012)

**Title:** 17β-estradiol enhances long-term potentiation in the rat primary auditory cortex *in vivo*

**Authors:** *C. N. SOUTAR*¹, P. GRENIER², M. C. OLMSTEAD¹², C. D. BAILEY³, H. C. DRINGENBERG¹²;  
¹Ctr. for Neurosci. Studies, ²Psychology, Queen's Univ., Kingston, ON, Canada; ³Dept. of Biomed. Sci., Univ. of Guelph, Guelph, ON, Canada

**Abstract:** The neuroactive steroid hormone 17β-estradiol (E2) is synthesized and released in the mammalian forebrain where it rapidly alters structural and synaptic plasticity in both males and females. A growing body of evidence from songbirds suggests that E2 synthesis within the auditory forebrain is regulated by auditory experience and that locally-synthesized E2 contributes to auditory processing and memory consolidation. Despite the established role of E2 as a modulator of synaptic activity and plasticity in the forebrain, as well as its role in auditory learning, the modulatory actions of E2 on long-term plasticity mechanisms underlying learning and memory have not been directly investigated in the auditory system. Thus, the objective of the current study was to investigate the potential role of E2 in gating long-term synaptic plasticity in the mammalian primary auditory cortex (A1). Specifically, we tested the hypotheses that the adult rat A1 is a site of E2 synthesis and that acute alterations in local E2 levels modulate long-term synaptic plasticity (long-term potentiation; LTP) in this region. To investigate E2 synthesis within A1, we performed immunohistochemistry (3,3′-diaminobenzidine and immunofluorescence) to probe for the expression of the estrogen synthetic enzyme aromatase. IHC experiments revealed that aromatase is widely expressed by A1 neurons across layers II-VI, with over 56% of NeuN-expressing cells being immunoreactive for aromatase. To test the effects of acute alterations in local E2 levels on LTP in A1, LTP was induced by theta-burst stimulation (TBS) of the medial geniculate nucleus *in vivo* during application of E2 or the aromatase inhibitor letrozole by reverse-microdialysis in A1. E2 application enhanced the magnitude of TBS-induced LTP in the thalamocortical auditory pathway, particularly at layer IV thalamorecipient synapses. In contrast, local reductions in E2 synthesis by letrozole application suppressed LTP induction in A1, particularly at layer II/III intracortical synapses. Ongoing experiments utilize whole-cell recording to investigate the effects of acute E2 manipulations on the electrophysiological properties of A1 pyramidal neurons, such as glutamatergic and
GABAergic receptor currents and spontaneous postsynaptic currents. Preliminary findings suggest that synaptic currents in A1 are sensitive to changes in E2 levels, with E2 application facilitating AMPA responses in layer II/III pyramidal cells. Collectively, these results indicate that the adult rat A1 is a site of E2 synthesis and that E2 functions as a modulator of synaptic activity and long-term plasticity in this region.


Poster

287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 287.19/C51

Topic: B.07. Synaptic Plasticity

Title: The distribution of synaptic strengths and mechanism for synaptic plasticity in the model of inter-spine molecular transport of PSD-95 molecules

Authors: *D. TSIGANKOV*¹,²;
¹Massachusetts Eye and Ear, Harvard Med. Sch., Boston, MA; ²Max-Planck Inst. for Dynamics and Self-Organization, Goettingen, Germany

Abstract: The scaffolding protein PSD-95 is the most abundant molecule in the post-synaptic density (PSD) located in the spine, where it forms a cluster to which the membrane synaptic receptors are bound. The amount of PSD-95 molecules inside an individual spine determines the size of the PSD cluster and is strongly correlated with the synaptic strength. It is observed that these molecules have high turnover rates and that neighboring spines are constantly exchanging individual molecules. Here we present a model of non-equilibrium molecular transport between spines in neuronal dendrites describing the dynamics of PSD-95 molecules. When the molecules interact with each other inside the spines of a dendrite due to binding to PSD cluster, the corresponding trapping times inside the spines depend on the size of the PSD cluster and become much longer than the diffusion times in the dendritic shaft. This allows us to obtain the stationary distributions of PSD cluster sizes that emerge from such inter-spine molecular dynamics. Our results suggest that spines are competing for a shared pool of PSD-95 molecules in a weak “winner-take-all” regime that is restrained by the finite lifetimes of the PSD-95 molecules. Furthermore, we propose that in the model non-equilibrium inter-spine dynamics of PSD-95 molecules can provide the basis for locally controlled synaptic plasticity through activity-dependent ubiquitination of PSD-95. Thus local rapid destruction of a fraction of the PSD-95 cluster can lead to its growth due to self-organization phenomena, providing the molecular mechanism for maintenance of late long-term potentiation (LTP) required for synaptic plasticity.
In this scenario, the geometrical filling fraction of the PSD cluster is suggested to be an important characteristic of the synapse that carries the information of the previous LTP events.

Disclosures: D. Tsigankov: None.

Poster

287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 287.20/C52

Topic: B.07. Synaptic Plasticity

Support: NIH Grant GM118801

Title: Etomidate suppression of LTP does not depend on modulation of α5-GABA_A receptors on Sst or OLM interneurons

Authors: *A. R. FRELKA¹, A. G. FIGUEROA¹, G. SURGES¹, M. PERKINS¹, K. KULLANDER², R. A. PEARCE¹;¹Anesthesiol., Univ. of Wisconsin, Madison, WI;²Uppsala Univ., Uppsala, Sweden

Abstract: GABA_A receptors containing α5 subunits (α5-GABA_ARs) are instrumental in controlling learning and memory. We previously reported that etomidate (ETOM) targets α5-GABA_ARs of interneurons (INs) to suppress long-term potentiation (LTP) of the Schaffer collateral (SC) pathway in the CA1 region of the dorsal hippocampus (SFN abstracts 2018), but the specific interneuron types that contribute to this effect are unknown. Here, we tested whether α5-GABA_ARs on Sst-INs, and more specifically on one subset of these cells (OLM-INs), are involved in ETOM suppression of LTP by selectively eliminating α5-GABA_ARs and measuring effects on LTP in vitro.

We eliminated α5-GABA_ARs in specific classes of INs by crossing Sst-ires-Cre or Tg(Chrna2-cre)1Kldr mice with fl/fl-α5-GABA_AR mice. For LTP studies, coronal hippocampal slices were obtained from 60-100 day old Sst - and Chrna2-α5-KO and fl/fl-α5 (pseudo-wild type) mice. LTP of the SC pathway was induced using a theta-burst stimulus (TBS), and defined as the change in the mean field EPSP slope between 51-60 minutes post-TBS compared to baseline, in groups of 8 slices under each condition.

In Sst-α5-KO mice, the selective loss of α5-GABA_ARs did not alter LTP under drug-free conditions (CTRL), nor did it influence the ability of ETOM (1μM) to suppress LTP (fl/fl-α5 CTRL 131 ± 6% vs. ETOM 109 ± 5%, p= 0.01; Sst-α5-KO CTRL 133 ± 7% vs. ETOM 112 ± 1%, p = 0.02). In Chrna2- fl/fl-α5 mice, ETOM (1μM) failed to suppress LTP even in the pseudo-wild type mice (fl/fl-α5 CTRL 122 ± 4% vs. ETOM 117 ± 4%, p = 0.33) and the loss of α5-GABA_ARs did not influence LTP or its modulation by ETOM (Chrna2-α5-KO CTRL 115 ± 4% vs. ETOM 111 ± 2%, p = 0.66).
Our results indicate that α5-GABA\textsubscript{A}Rs on Sst-INS are not essential to the ability of ETOM to suppress LTP \textit{in vitro}. Therefore, ETOM either targets a different class of interneurons in the dorsal hippocampus to suppress LTP \textit{in vitro}, or its action on any one of the multiple classes, including Sst-INS, suffices. The lack of effect of ETOM on LTP in the Chrna2-fl/fl-α5 (Cre\textsuperscript{-} pseudo-wild type) mice was a surprise and remains unexplained but does not alter our conclusions.


**Poster**

287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 287.21/C53

**Topic:** B.07. Synaptic Plasticity

**Support:** NIH R01 NS042595 to RWG
McDonnell Center for Cellular and Molecular Biology to BC
APS Future Leaders to BC

**Title:** Alternative splicing of synaptic adhesion molecules in human somatosensory neurons

**Authors:** *J. J. YOO, R. W. GEREAU, B. A. COPITS; Anesthesiol., Washington Univ. Sch. of Med., St. Louis, MO

**Abstract:** Processing external information from distinct sensory modalities is vital for generating the diversity of somatic sensations that we perceive. However, altered communication within these pathways can result in chronic pain states. While the plasticity of nociceptive circuits is well established, we possess a limited understanding of the molecular mechanisms that assemble sensory circuits, and how this connectivity may be altered in chronic pain. Neurexins (\textit{Nrxns}) are presynaptic cell-adhesion molecules essential in coordinating synapse formation through trans-synaptic interactions with myriad post-synaptic ligands. Alternative splicing of \textit{Nrxns} generates thousands of unique isoforms, which has been proposed to impart a “splice-code” for connectivity. Alternative splicing of \textit{Nrxn} splice site 4 (SS4) is critical for regulating synaptic plasticity by altering binding affinity for different post-synaptic ligands, resulting in altered glutamate receptor content. In the central nervous system, \textit{Nrxn} splicing has been shown to regulate synaptic properties critical to coordinating neuronal connectivity, yet \textit{Nrxn} splicing in somatosensory circuits remains largely unexplored. Here, we found that \textit{NRXN} alternative splicing of SS4 in sensory neurons from human organ donors is altered after loss of spinal grey matter. Using a reverse translational approach, we used mouse models to understand the mechanisms underlying these splicing changes. We found that culturing sensory neurons
produced similar alterations in SS4 exclusion, suggesting that these splicing may be regulated by changes in activity, neuronal injury, or synaptic connectivity. While direct membrane depolarization enhanced SS4 exclusion, we found that persistent inflammation induced hypersensitivity rendered splicing unchanged. To model the loss of connectivity observed in human donors, we severed axons of dorsal root ganglia onto spinal neurons, and found that SS4 splicing in mice reflects the changes observed in human sensory neurons one week after loss of synaptic contacts. To test whether these changes in splicing resemble those found during initial development of somatosensory circuits, we examined splicing of embryonic sensory neurons which have not yet formed connections in the spinal cord. We found significant differences in splicing between embryonic and adult mice, suggesting changes in post-transcriptional regulation during development. We are now testing whether these changes in splicing resemble those found in neurons during initial circuit assembly or instead represent a maladaptive response that leads to circuit rewiring during the development of chronic pain.

**Disclosures:** J.J. Yoo: None. R.W. Gereau: None. B.A. Copits: None.

**Poster**

287. Synaptic Plasticity: Pre- and Postsynaptic Mechanisms

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 287.22/C54

**Topic:** B.07. Synaptic Plasticity

**Support:** NIH Grant 2R44TR001326

**Title:** Differentiation and characterization of hiPSC-cortical neurons and their application to drug evaluation in CNS disease models

**Authors:** *K. AUTAR*, X. GUO, N. AKANDA, A. GOSWAMI, M. JACKSON, J. RUMSEY, C. LONG, J. J. HICKMAN;

1Univ. of Central Florida, Orlando, FL; 2Hesperos, Inc., Orlando, FL; 3Nanosci Technol. Ctr., Univ. Central Florida, Orlando, FL

**Abstract:** The differentiation of functional cortical neurons from human induced pluripotent stem cells in vitro easily lends itself to a serum-free, drug delivery platform advantageous for testing novel chemicals for safety and efficacy in disease treatment. Initially, cortical neuron cultures were characterized morphologically by phase microscopy and immunocytochemistry and functionally by patch-clamp electrophysiology. Specifically, the expression of neuronal markers and neuronal activity increased throughout maturation. On day 0 of maturation, 50 percent of the culture expressed layer V cortical neuron marker ctip2 and neuronal marker beta-III tubulin and displayed spontaneous and repetitive firing through whole-cell patch clamp. By day 28 of maturation, 90 percent of the culture expressed the aforementioned markers and
displayed electrical activity. Subsequently, neurons were cultured on multi-electrode arrays (MEAs) to determine the effects of chemicals on neural circuit physiology for modeling brain disease phenotypes. In this system, we tested GABA\(_A\) receptor antagonists and agonists as chemical convulsants or anti-convulsants, respectively. GABA\(_A\) receptor antagonist administration enhanced spontaneous activity mimicking an epileptic phenotype, while GABA\(_A\) receptor agonist administration quieted spontaneous activity. The versatility of this model lies in its ability to present an array of brain diseases characterized by functional brain deficits. Chemicals affecting receptor binding can be added to either enhance or inhibit neuronal activity. This serum-free, hiPSC cortical neuron model establishes a platform for the evaluation of neuron activity as well as a platform for drug testing in vitro.

Disclosures: K. Autar: None. X. Guo: None. N. Akanda: None. A. Goswami: None. M. Jackson: A. Employment/Salary (full or part-time); Hesperos, Inc. J. Rumsey: A. Employment/Salary (full or part-time); Hesperos, Inc. C. Long: A. Employment/Salary (full or part-time); Hesperos, Inc. J.J. Hickman: A. Employment/Salary (full or part-time); Hesperos, Inc.

Poster

288. Human Functional Imaging

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 288.01/C55

Topic: B.09. Network interactions

Support: MEXT KAKENHI Grant Number 15H05877

Title: Changes in electroencephalogram phase dynamics induced by transcranial magnetic stimulation

Authors: Y. SASAOKA\(^1\), Y. IWAI\(^1\), D. FUTAGI\(^2\), K. KITAOJ\(^3\), *K. KITANO\(^1\);
\(^1\)Ritsumeikan Univ., Kusatsu, Japan; \(^2\)Grad Sch. Information Sci. and Engin., Ritsumeikan Univ., 1-1-1 Noji-higashi, Kusatsu, Japan; \(^3\)Dept. of Syst. Neurosci., Natl. Inst. for Physiological Sci., Okazaki, Japan

Abstract: Inter-regional interactions of the brain activity obtained by non-invasive recording such as fMRI or EEG are drawing more attention in the whole brain-level studies. In the inter-regional interactions, the phase information of rhythmic activity such as alpha-band oscillations is suggested to play an important role. However, it is generally difficult to see how different brain regions interact thorough the phases in "passively" recorded data because the direct cause to modulate phase relations is not clear. On the other hand, transcranial magnetic stimulation (TMS) enables us to evaluate the causal relation between the controllable direct perturbation and modulated neural activity. In the present study, we investigated how TMS for subjects in the
resting state influenced neural activities in each brain region and the phase interactions between
the regions. To this end, we analyzed TMS-EEG data by the model-based approach using
coupled phase oscillators as known as Kuramoto model. We assumed that a time-series signal
recorded by an EEG electrode was generated by a nonlinear oscillator (presumably a neural
population below the electrode) and derived a model to reproduce recorded EEG data by
exploring model parameters such as coupling strengths between phase oscillators. The EEG data
were obtained by delivering TMS at the visual cortex (Oz electrode) of subjects (n = 4) for real
TMS trials (about 50 trials) or sham TMS trials (about 50 trials). Trial variability of derived
model parameters were large due to noise inherent in EEG data as is the case for other EEG
analyses. Coupling strengths averaged over trials indicated that the averaged coupling strengths
in the alpha- and beta-band dynamics were more enhanced during real TMS trials than sham
TMS trials. Furthermore, as for changes in coupling strengths before and after TMS, connections
between adjacent regions were increased in alpha- and beta-band dynamics whereas those
between distant regions were increased in theta-band dynamics. Thus, TMS could affect the
phase dynamics between brain regions, which depended on the frequency band and distances
between brain regions.

Kitano: None.

Poster

288. Human Functional Imaging

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 288.02/C56

Topic: B.09. Network interactions

Title: The interbrain EEG synchronization during a joint action reflects the sense of joint agency

Authors: *M. SHIRAISHI, S. SHIMADA;
Meiji Univ., Kawasaki, Japan

Abstract: In a cooperative joint action, people feel the sense of joint agency (e.g., a sense that
“we did it”) rather than the sense of self-agency (e.g., a sense that “I did it”). However, the
neural correlates of the sense of joint agency have not been fully investigated. In this study, we
examined whether the inter-brain synchronization during a joint action reflects the sense of joint
agency. Eighteen pairs of healthy right-handed male subjects (aged 21.6 ± 1.3, mean ± SD)
participated in the experiment. As a joint action, pairs of participants produced sequence of eight
tones, four tones each, at a constant pace by the mouse clicks in the two task (alternating and
sequential) conditions. In the alternating task, participants produced tones in alternation with
each other. In the sequential task, one participant produced the first four tones and the other
produced the last four tones. We defined the role of participants who produced the first tone as
leader and the role of the other as follower. After the joint action, participants rated their feelings of agency from 1 (self-agency) to 9 (joint agency). The brain activities of participant pairs were simultaneously recorded by an EEG system (g.USBamp, g.tec, Austria) with 14 active electrodes (Fp1, Fp2, F5, Fz, F6, T7, C3, Cz, C4, T8, P5, Pz, P6, Oz) for each participant. The two-way ANOVA (task × role) revealed that participants felt significantly stronger joint agency in the alternating task than in the sequential task ($F(1, 25) = 44.2, p < 0.001$). For inter-brain synchronization analyses, we calculated the inter-brain phase synchronization index (PSI). The result showed that the PSI value in theta rhythm (3-8 Hz) between the leader’s frontal region (Fz) and the follower’s right temporal parietal junction (P6) in the alternating task were marginally greater than that in the sequential task ($t(12) = 1.72, p < 0.10$). Moreover, we found a significantly positive correlation between the joint agency score and the PSI value ($r = 0.57, p < 0.005$). These results suggest that the functional connectivity in theta rhythm between the leader’s frontal lobe and follower’s right temporal parietal junction reflects the sense of joint agency.

**Disclosures:** M. Shiraishi: None. S. Shimada: None.

**Poster**

**288. Human Functional Imaging**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 288.03/C57

**Topic:** B.09. Network interactions

**Support:**
- NIH/NIBIB (P41-EB018783)
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- NIH/NIMH (P50-MH109429)
- US Army Research Office (W911NF-14-1-0440)

**Title:** Low-frequency alpha/beta oscillations: The same side of the same coin?

**Authors:** *M. ADAMEK$^1$, P. BRUNNER$^{1,2}$, G. SCHALK$^{1,2,3}$*,


**Abstract:** Ever since Hans Berger’s seminal work on EEG in 1928, thousands of studies have characterized low-frequency brain oscillations by different frequency bands such as alpha and beta. This categorization assumes that the brain generates multiple functionally independent
sinusoidal oscillations. However, recent findings have provided evidence that cortical oscillations are indeed non-sinusoidal and that, consequently, different frequency bands may not be functionally independent. In view of this realization, we aim to replace the conceptual generalization of sinusoidal oscillations and their categorization into defined frequency bands with a physiologically-informed characterization of oscillatory activity. Working towards this goal, we recorded electrocorticographic (ECoG) signals from the surface of the brain while human subjects performed a hand motor and a passive auditory listening task. To characterize the effects of oscillatory activity in task-related locations, we: 1) established the relationship between low-frequency activity (assessed by power, phase or instantaneous voltage) and population-level activity; 2) distinguished between true oscillations and their harmonics by calculating the power-power and phase-phase correlations across the entire low-frequency spectrum; and 3) recomposed the physiologically-informed oscillatory activity from its frequency components to determine the fraction of the variance of cortical excitability that it explains. Our analysis shows that cortical excitability in auditory and motor cortex is modulated by an oscillation that spans the alpha and theta frequency range (6-10Hz, $r^2=0.4$, permutation test, Bonf. corrected $p<0.01$). The analysis of harmonic components revealed power-power coupling in 99%, and phase-phase coupling in 77% of all task-related locations (permutation test, Bonf. corrected $p<0.05$). Finally, our results show that the recomposed physiological informed oscillatory activity is a better predictor of cortical excitability than the traditionally-used oscillatory power (45% increase, $p<<0.01$; paired Wilcoxon signed rank test).

We conclude that oscillations in traditional frequency bands (such as alpha and beta) are not independent of each other, but are (at least partially) different measurements from the same non-sinusoidal source. These results encourage further work on replacing frequency-based definitions of oscillatory activity with a functional definition, and suggest careful reinterpretation of past studies on low-frequency oscillatory activity.

Disclosures:  M. Adamek: None. P. Brunner: None. G. Schalk: None.

Poster

288. Human Functional Imaging

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 288.04/C58

Topic: B.09. Network interactions

Support: WashU ICTS, grant UL1TR002345 from NCATS of the NIH

Title: Synchronization of millihertz electrophysiological modulation revealed from bilevel spectral analysis of the pediatric EEG

Authors: *M. E. LOE¹, S. KHANMOHAMMADI³, R. MATHER¹, S. TOMKO⁴, M. MORRISSEY⁴, R. M. GUERRIERO⁴, S. CHING²;
Abstract: EEG modulation occurs when oscillatory patterns of disparate frequencies interact multiplicatively, often resulting in fast electrophysiological activity being systematically nested in a slow modulatory oscillation. We recently created an analysis pipeline to automatically detect very slow (millihertz) EEG modulation in critically ill young children; we termed this pattern macroperiodic modulating oscillations (MOs). MOs were generally detected in patients with recurrent seizures and status epilepticus (SE), conditions associated with mortality and poor outcomes. We studied the temporal and spatial distribution of these patterns, including their spatial synchronization.

From October 2015 to September 2018, we identified n=36 subjects in pediatric, neonatal, or cardiac intensive care units whose EEGs exhibited slow cycling on density spectral array (DSA). Our pipeline used two levels of spectral analysis to detect millihertz slow EEG modulation: first, a time-series of 5-15Hz band-limited power was extracted for each channel using a sliding window, multi-taper spectral estimation (Fast Fourier Transform). A second time-frequency analysis was performed on these power envelope signals, revealing slow, harmonic processes modulating the 5-15Hz activity, i.e., MOs. Our detection criteria were applied to the second spectrogram: a high power, narrowband signal (occurring in a small, consistent range of very slow frequencies), persisting across multiple consecutive time windows. After assigning each time window a binary categorization (MO present vs. absent) for each channel, we analyzed synchronization at three temporal scales: synchronization of “MO present” epochs, of the modulatory process within “MO present” epochs, and of the fast EEG signal during each modulatory cycle.

MOs were consistently and reliably identified at a frequency between 0.005Hz and 0.009Hz in the second level spectral analysis. In EEGs with MOs present for a large proportion of the time, synchronization of epochs was high. Furthermore, when epochs were synchronized across channels, it was generally at all three levels of synchronization.

MOs are a two-time scale modulatory EEG pattern with a millihertz time-scale that is considerably slower than classical patterns observed in critically ill patients. Our analysis suggests that MOs have a slow, narrowband modulatory effect on faster activity and appear to manifest synchronously across the cortex. Since the MOs pattern was detected in patients with poorer outcomes, this may represent a novel EEG biomarker for impending SE and recalcitrant seizures and may provide insight into mechanisms underlying these events.


**Poster**

288. Human Functional Imaging

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 288.05/C59

**Topic:** B.09. Network interactions

**Title:** Spatio-temporal characterization of TMS-EEG responses to antiepileptic drugs

**Authors:** *G. DARMANI*¹, J. O. NIEMINEN²,³, U. ZIEMANN¹;

¹Neurol. and Stroke, Hertie Inst. for Clin. Brain Research, Univ. of Tuebingen, Tuebingen, Germany; ²Neurol. and Stroke, Hertie Inst. For Clin. Brain Research, Univ. of Tuebingen, Tuebingen, Germany; ³Neurosci. and Biomed. Engin., Aalto Univ. Sch. of Sci., Espoo, Finland

**Abstract:** Combination of transcranial magnetic stimulation (TMS) and electroencephalography (EEG) to assess direct effects of central nervous system active drugs on TMS-evoked potentials (TEP) has provided a characterization of several GABAergic and anti-epileptic drugs (AEDs) based on their ability to modulate specific TEP components. Here, we complement these findings by analyzing time–frequency representations (TFR) of neuronal activity and by evaluating the complexity of cortical responses to TMS before and after administration of an AED.

Fifteen healthy males volunteered to a placebo-controlled, double-blind crossover study. TMS, with concurrent high-density EEG, was applied to the left primary motor cortex at an intensity of 100% resting motor threshold of the relaxed abductor pollicis brevis muscle. Single oral doses of the following drugs were studied: carbamazepine (600 mg), a voltage-gated sodium channel (VGSC) blocker, brivaracetam (100 mg), a modulator of GABAergic neurotransmission through binding to the synaptic vesicle protein SV2A, and tiagabine (15 mg), a selective GABA reuptake inhibitor. TFR of TMS-induced oscillations was calculated to assess non-phase-locked oscillatory responses, and the complexity of TEPs was quantified with the perturbational complexity index (PCI).

All drugs reduced significantly early (20–200 ms) beta oscillations mostly over the stimulated sensory–motor cortex; furthermore, only tiagabine decreased the early alpha and theta oscillations as well as late (200–400 ms) theta oscillations. For placebo, no changes were observed. The widespread cortical desynchronization observed in the tiagabine condition was accompanied by significantly reduced PCI values. Overall, our results revealed that AEDs decrease early beta oscillations independent of their modes of action; given previous findings showing that modulators of GABA receptors (alprazolam and baclofen) also reduce early beta activity, we may conclude that both GABAergic inhibition and VGSCs contribute to these TMS-induced oscillations. In addition, the observed reduction of the complexity of the TMS-evoked responses, which so far has been associated to a reduced level of consciousness, with tiagabine seems to relate to wide-spread cortical low-frequency TMS-induced oscillations.
Disclosures: G. Darmani: None. J.O. Nieminen: None. U. Ziemann: None.

Poster

288. Human Functional Imaging

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:/Poster #: 288.06/C60

Topic: B.09. Network interactions

Support: ISF 51/11 (I-CORE cognitive sciences)
Adelis Foundation (YN)

Title: Transcutaneous vagus nerve stimulation in humans induces pupil dilation and modulates EEG brain rhythms

Authors: *O. SHARON¹, F. FAHOUM³, Y. NIR¹,²;
¹Physiol. and Pharmacol., ²Sagol Sch. of Neurosci., Tel-Aviv Univ., Tel-Aviv, Israel; ³EEG and Epilepsy Unit, Dept. of Neurol., Tel Aviv Sourasky Med. Ctr., Tel-Aviv, Israel

Abstract: Background: Vagus nerve stimulation (VNS) is widely used to treat intractable epilepsy and depression. While the long-term therapeutic mechanisms remain unclear, VNS effect may involve stimulation of Locus Coeruleus (LC) via the nucleus of the solitary tract (NTS) that receives afferent vagal inputs. In rats, VNS elevates LC firing and forebrain noradrenaline (NE) levels, and LC lesions suppress VNS therapeutic efficacy. In recent years, non-invasive transcutaneous VNS (tVNS) has been used: electrical stimulation targets the auricular branch of the vagus nerve located at the cymba conchae of the left ear. Here, we set out to evaluate tVNS effects in healthy volunteers. Given VNS effects on LC-NE in animals, we hypothesized that human tVNS will promote markers of NE including pupil dilation and activation of the EEG. To test tVNS effects beyond tactile stimulation, we compared tVNS to sham stimulation at the earlobe (far from the vagus nerve branch) while recording pupillometry and high-density EEG. Participants were blind to study objectives.

Results: When tVNS was applied in a clinical 30s-ON/30s-OFF protocol during visual fixation (n=23) we observed a trend (p=0.09) for higher pupil dilation upon tVNS (5%±1.8%) compared to sham stimulation. During a closed eyes condition (n=14), tVNS attenuated theta (4-8Hz) power (-13dB±6, p=0.04) and increased alpha (8-12Hz) power (24dB±9, p=0.02) in occipital electrodes compared to sham, likely representing a shift from sleep onset to idle wakefulness rhythms. Significant differences between ON and OFF intervals were lacking, possibly due to carryover effects of the long stimulation.

To better understand phasic effects of tVNS, a second experiment employed shorter 3s-ON/30s-OFF tVNS during visual fixation (n=24). Stimulation intensity was adjusted to a maximal comfortable level for each participant and location separately. Electrical current was slightly higher in sham condition (by 0.62 mA, p=0.03), whereas subjective ratings were not significantly
different (p>0.05). However, tVNS led to robust pupil dilation, significantly higher than in sham stimulation (peak = 267%±44 increase from baseline in tVNS vs. 116%±30 increase in sham, p<0.01). tVNS-evoked pupil dilation was evident in most individual subjects. We are now assessing the phasic effects of tVNS on EEG activity by quantifying the degree of occipital alpha. The alpha topography is identified in each participant, and the effects of stimulation are compared relative to pre-stimulation baseline. **Conclusion:** tVNS affects pupillary and EEG markers of arousal beyond sham stimulation, supporting the notion that it elevates noradrenaline signaling

**Disclosures:** O. Sharon: None. F. Fahoum: None. Y. Nir: None.

**Poster**

**288. Human Functional Imaging**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 288.07/C61

**Topic:** B.09. Network interactions

**Support:** NRF-2019R1A2C1009674

**Title:** Suppression of the hippocampal gamma activity in general anesthesia

**Authors:** *M. CHOE*¹, S.-H. JIN², S. JUN¹, J. KIM³, C. CHUNG¹,²,⁴;

**Abstract:** Memory and consciousness are the two manifestations of higher cognitive function that are most closely associated with our self-awareness as individuals. Amnesia (loss of memory) is a major component of general anesthesia (GA) along with loss of consciousness and analgesia. Some patients unexpectedly become aware during surgery and form a traumatic memory. Gamma rhythm of hippocampus plays a crucial role in selecting inputs during memory formation. However, the effect of anesthetics on gamma oscillation has been controversial. In some studies, hippocampal gamma oscillation is preserved or increased under GA, while in others, gamma oscillation attenuated. However, gamma oscillation in hippocampus has not been directly compared between during GA and memory encoding in human. In this study, we calculated power spectrum of hippocampal gamma frequency (32-100 Hz) from the intrahippocampal depth electrode in 9 epilepsy patients (age: 24-55 years) during propofol-induced GA (3~5 ug/ml concentration), resting state, and long-term memory task. Friedman test was performed for intergroup comparisons of gamma power in hippocampus. Gamma power in hippocampus was attenuated during propofol-induced GA compared to those in resting state and
long-term memory task state \((p < 0.01)\). Suppression of the hippocampal gamma activity may be the cause of amnesia incurred by general anesthesia.

**Disclosures:** M. Choe: None. S. Jin: None. S. Jun: None. J. Kim: None. C. Chung: None.

**Poster**

288. Human Functional Imaging

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 288.08/C62

**Topic:** B.09. Network interactions

**Support:** Department of Anesthesiology, University of Michigan

**Title:** Level of consciousness is dissociable from cortical complexity and slow oscillations

**Authors:** *J. DEAN*¹, D. PAL², D. LI², M. BRITO³, A. FRYZEL², A. G. HUDETZ², G. A. MASHOUR²; ¹Dept. of Mol. and Integrative Physiol., ²Dept. of Anesthesiol., ³Dept. of Neurosci., Univ. of Michigan, Ann Arbor, MI

**Abstract:** Measures of complexity, used as a surrogate of information processing in the brain, have reliably indexed level of consciousness, e.g., cortical complexity is high during wakefulness but constrained during anesthetic-induced unconsciousness. We recently demonstrated that reverse dialysis delivery of the cholinergic agonist carbachol into prefrontal cortex of sevoflurane-anesthetized (1.9-2.4%) male SD rats \((n=11)\) produced signs of wakefulness and electroencephalographic (EEG) activation despite continued anesthetic exposure. Delivery of carbachol into posterior parietal cortex \((n=11)\) or noradrenaline into either prefrontal \((n=11)\) or posterior parietal \((n=11)\) cortex in sevoflurane-anesthetized rats (1.9-2.4%) was sufficient to produce EEG activation but failed to produce signs of wakefulness. Thus, we generated a model in which the presence of anesthetic in the brain can be dissociated from level of consciousness. Here, we tested the hypotheses that (1) higher cortical complexity would correlate with wakefulness, and (2) lower cortical complexity and increased slow oscillations \((0.5-1.0 \text{ Hz})\) would correlate with general anesthesia. We analyzed temporal Lempel-Ziv Complexity (LZC), which computes the number of unique binary patterns within finite length sequences, within frontal and parietal EEG signals collected before, during, and after sevoflurane and carbachol/noradrenaline infusion into prefrontal and posterior parietal cortices. Similar to prior studies, LZC of the EEG signal in frontal and parietal cortices was reduced from waking during sevoflurane-induced unconsciousness in all cohorts \((p<0.005)\). Compared to sevoflurane-induced unconsciousness, both carbachol and noradrenaline-induced EEG activation in frontal and parietal cortices increased LZC to above anesthetized levels, which returned to (frontal EEG signal following carbachol infusion into prefrontal cortex; \(p=0.22\)) or approached (all other
conditions; p<0.05) waking values and continued to increase following cessation of anesthesia. As expected, sevoflurane increased the power of cortical slow oscillations in all cohorts (p<0.05). However, there was dissipation of power following administration of carbachol or noradrenaline into both prefrontal and posterior parietal cortices independent of behavioral change (p>0.05). Thus, cortical complexity and slow oscillations correlated with changes in EEG activation rather than level of consciousness (i.e., wakefulness). This dissociation between level of consciousness and cortical dynamics prompts a reevaluation of current candidates for the neural correlates of consciousness and anesthesia.


Poster

288. Human Functional Imaging

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 288.09/C63

Topic: B.09. Network interactions

Support: Newcastle University

Title: Oscillatory correlates of affect expectation using EEG

Authors: *E. BENZAQUEN, T. D. GRIFFITHS, S. KUMAR;
Inst. of Neurosci., Newcastle Univ., Newcastle upon Tyne, United Kingdom

Abstract: Predictive coding (PC) has been highly successful in explaining perceptual processes [1]. Here, we addressed whether a predictive coding account can explain measured neural correlates of predictions and expectation during affective sound processing. We used a classical trace conditioning paradigm with two categories of sounds; highly aversive scraping sounds, and neutral/pleasant water sounds. We manipulated the predictability of the stimuli such that two (certain) cues were always followed by aversive or neutral sounds while a third (uncertain) cue failed to predict the subsequent stimuli. Participants' expectations were recorded on a trial-by-trial basis after the presentation of the cue while 64-channel EEG data were acquired. Time-frequency responses (TFRs) were acquired from 22 normal subjects. Brain activity during the processing of the cue was characterized by a marked beta-band suppression, which was stronger for both 'certain' conditions. To further explore if the observed beta suppression corresponded to the degree of expectation for individual subjects, TFRs were averaged over all channels for the complete duration of the cue, and correlated with the reported expectancy scores. A significant correlation indicating greater beta suppression with greater certainty was observed. This relation was further corroborated by correlation between standard deviation of expectancy across trials and beta power; subjects who were more consistent (lower SD) showed
greater beta suppression. To reject the possibility that beta activity reflects a preparation for a motor response, we correlated it with individuals’ reaction times, which failed to reach significance. Thus, beta suppression appears to index the certainty of a future outcome or the strength of predictions.

Our data strongly suggest a role of beta band activity in backward predictions consistent with findings from previous predictive coding work.


Disclosures: E. Benzaquen: None. T.D. Griffiths: None. S. Kumar: None.

Poster

288. Human Functional Imaging

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 288.10/C64

Topic: B.09. Network interactions

Title: Differential effects of prestimulus alpha power on perceived stimulus intensity and initial cortex excitation in a somatosensory discrimination task

Authors: *T. STEPHANI1,2, A. HODAPP1, A. VILLRINGER1,3,4, V. V. NIKULIN1,5;
1Dept. of Neurol., Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany;

Abstract: Brain responses to identical sensory stimuli vary from moment to moment which has been attributed to instantaneous fluctuations of cortical excitability. Ongoing neuronal oscillations in the alpha band (8-13 Hz) of the human EEG have been suggested to indicate changes of cortical excitability, shaping behavioral performance in paradigms with near-threshold stimulus detection. It remains unclear, however, whether prestimulus alpha power also affects perceived stimulus intensity of supra-threshold stimuli.

To further investigate the relationship of prestimulus alpha power and cortical excitability, we propose to use short-latency somatosensory evoked potentials (SEP). The N20 component of the SEP reflects the first excitatory post-synaptic potentials arriving from the thalamus to the cortex, thus being a direct measurement of cortex excitation when stimulus information enters primary sensory areas.

We measured 64-channel EEG from 32 human participants while they performed a somatosensory discrimination task using electrical median nerve stimuli of two intensities.
Prestimulus alpha band activity was measured over the somatosensory cortex between 200 and 10 ms prior to stimulus onset. Behavioral performance was assessed by sensitivity $d'$ and criterion $c$ derived from Signal Detection Theory. Higher prestimulus alpha power was associated with lower perceived stimulus intensity (criterion $c$) but had no effect on the discriminability (sensitivity $d'$). In contrast, initial cortex excitation reflected in N20 amplitudes did not predict behavioral performance although it was related to prestimulus alpha power and absolute stimulus intensity.

The present results confirm the relationship between prestimulus alpha power and sensory detection criterion, and extend it to perceived stimulus intensity. We conclude, however, that the effect of prestimulus alpha power on behavioral performance is not mediated by changes of initial cortical excitability and may play a role only at later stages of stimulus processing.

**Disclosures:**  
T. Stephani: None. A. Hodapp: None. A. Villringer: None. V.V. Nikulin: None.

**Poster**

**288. Human Functional Imaging**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 288.11/C65

**Topic:** B.09. Network interactions

**Support:**  
NIH Grant K01-ES026839  
NIH Grant R01-NS094399  
Doris Duke Charitable Foundation Clinical Scientist Development Award #2015096

**Title:** Quantitative EEG features during interictal HFO events provide complementary information to HFO rates when identifying pathological tissue

**Authors:** *S. V. GLISKE, W. C. STACEY;  
Neurol. and Biomed. Engin., Univ. of Michigan, Ann Arbor, MI

**Abstract:** Objective. High frequency oscillations (HFOs) are a promising biomarker of tissue instigating seizures. One challenge to improving the specificity of HFOs is that high frequency oscillations are caused by both pathological and normal processes. Our computational models have suggested quantitative features of the EEG signal during HFO events than can help disambiguate which channels are associated with pathological tissue. The goal of this project is to assess one of these features in a large cohort of human subjects.

**Methods.** Data were acquired from 29 patients (13 ILAE Class I), each with multiple days of intracranial EEG recording, sampled at over 4 kHz. HFOs were detected using a previously published HFO detector. The intercranial, interictal EEG was band pass filtered within 80-500 Hz, and the skewness of the curvature of on all channels during each HFO event was computed.
Parameters were tuned using 23 subjects, with 6 class I subjects held out as validation data. Fuzzy clustering using non-negative matrix factorization on the distribution of the skewness of the curvature was used to provide a pathological-score for each channel.

Results. Among subjects with ILAE Class I outcomes, an increase of 0.1 in the pathological score increased the odds a channel was in the clinically-identified seizure onset zone by 15% (p=0.003, logistic regression, adjusted for HFO rate) and increased the odds a channel was resected by 29% (p=10^{-15}, logistic regression, adjusted for HFO rate).

Conclusion. The skew-curve index of intracranial EEG during HFO events provides independent information to HFO rates and can help identify pathological tissue. Further work is needed to optimize prospective, clinical use of this information to identify the epileptogenic zone.

Significance. Complementary information from additional biomarkers can help improve clinical confidence and improve localization of the epileptic focus, potentially improving surgery outcomes.

Disclosures: S.V. Gliske: None. W.C. Stacey: None.

Poster

288. Human Functional Imaging

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 288.12/C66

Topic: B.09. Network interactions

Support: CIHR

Title: Sex related differences in cortical EEG, hippocampal LTD and de-depression, following prolonged exposure to surgical levels of isoflurane anesthesia

Authors: *R. TADAVARTY^1, T. MARIAM^1, X. DONG^1, S. LIU^2, T. CHEUNG^2, *P. J. SOJA^1;
^1Fac. of Pharmaceut. Sci., The Univ. of British Columbia, Vancouver, BC, Canada; ^2Simon Fraser Univ., Burnaby, BC, Canada

Abstract: Exposure to general anesthesia (GA) can not only cause post-operative cognitive dysfunction but also increase the risk of dementia and Alzheimer’s disease. Ensuing behavioral cognitive deficits can last up to several months or indefinitely. Surprisingly, female brains resist the manifestation of these deficits; the reason(s) for this discrepancy are not known. Mechanistically, memories are thought to be stored in the brain as representations of synaptic weights with long-term potentiation and long-term depression (LTD) playing a key role in activity dependent up or downscaling synaptic strength, respectively. Reversibility of synaptic strength is necessary for reconsolidation of memories. The cortical EEG signature under surgical anesthesia is primarily comprised of continuous large-amplitude slow wave (0.5-8 Hz) activity
Moreover, < 1 Hz oscillations emerge in SWA presumably due to alternating highly active or near silent states of cortical neurons. GA can uncouple such oscillations, disrupt connectivity-patterns and hamper information flow between neural networks, a crucial aspect of mnemonic processing in the brain. We therefore explored whether sex-related differences occur in hippocampal LTD and de-depression, EEG SWA and EEG oscillation dynamics, following prolonged exposure to surgical levels of isoflurane (ISO) anesthesia. Adult male or female SD rats were initially anesthetized in an induction chamber, their trachea intubated and head mounted in a stereotaxic frame. Cortical EEG was recorded using stainless steel screw electrodes positioned in S1 bilaterally. Hippocampal slices were then prepared from either naïve or ISO-exposed (for 5 h) rats. Field excitatory postsynaptic potentials were evoked by stimulating CA3 afferents, and recorded from the apical dendritic layer of CA1 pyramidal neurons. A low-frequency stimulation (1 Hz, 1200 pulses) was used to induce LTD. After following the timecourse of LTD for 30 min, LTD was reversed using a high-frequency tetanic stimulation (4 X 100 Hz, 10 s inter-stimulus interval). Our results indicate that while there is no difference in the timecourse of LTD between male and female rats, LTD could only be reversed in female rats, suggesting a potential lack of cognitive flexibility in male rats following ISO exposure. FFT analyses of EEG SWA revealed sex-related differences in δ and β band relative power. Furthermore, there is a ~ 3 fold increase in the occurrence of Up/Down States in males vs females. Our results disclose for the first time that males and females differ in EEG SWA oscillation dynamics, spectral content and hippocampal synaptic plasticity following exposure to ISO anesthesia.


Poster

288. Human Functional Imaging

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 288.13/C67

Topic: B.09. Network interactions

Title: EEG microstates analysis associated with cognitive thinking

Authors: *R. MAHAJAN¹, T. AZMY¹, C. DEMAREE¹, D. GUPTA², F.-S. CHOA³; ²Computer Sci. and Electrical Engin., ¹Univ. of Maryland Baltimore County, Baltimore, MD; ³UMBC, Baltimore, MD

Abstract: Keywords: Electroencephalography, EEG microstate, Global Field Power (GFP), MicroGFPpeakData
Electroencephalography (EEG) microstates are short duration quasi-stable states of the dynamically changing electrical field topographies recorded via array of EEG electrodes from
the human scalp. EEG microstate analysis offers characterization of the spatio-temporal features of large-scale brain network activity. The aim of the research is to use EEG Microstate Analysis to study brain dynamics when subjects are engaged in performing mathematical calculations. We collected EEG data while three healthy subjects performed a cognitive task of a series of twenty mathematical calculations by each subject. The EEG data recording was performed in a pattern of 10 seconds resting state and 10 seconds mathematical calculations state. We recorded 20 sets of each state for each subject. So totally 400 seconds of EEG data was recorded per subject. In microstate analysis the goal is to segment the recorded EEG time samples into microstate classes. The microstate analysis was performed in Matlab using interactive plug-in of Microstate Analysis Toolbox in EEGLAB. The selected EEG data (MicroGFPpeakData) is segmented into predefined number of microstate prototype topographies by applying the method of K-means clustering to investigate the microstates conveyed by the event-related potential extracted from EEG data during mental cognitive calculations. We studied the clustering algorithm time samples that peaked in the Global Field Power (GFP) time curve, as they are considered to have the "cleanest" representations of their microstate. The number of microstates into which the EEG data to be clustered is set to 4 microstates prototypes. All the prototypes are sorted by Global Explained Variance (GEV). Based on the evaluation of prototype topographies and measures of fit, we selected GEV and CV criterion to identify active number of microstates. We analyzed different measures, including microstate sequences, topographical map, hemispheric lateralization, and duration of microstate, to characterize the dynamics of microstates during mental cognitive tasks. GFP of active microstates is obtained. From GFP active microstates plot it was observed that the activation of right frontal parietal region was stronger according to microstates mode 2. The obtained brain states from EEG microstates will be compared with EEG source information obtained from sLoreta 2D to 3D studies.


Poster

288. Human Functional Imaging

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 288.14/C68

Topic: B.09. Network interactions

Support:  NIMH R01MH111889
          NIMH R01MH101547

Title: Causal evidence for theta-gamma and delta-beta cross frequency coupling in cognitive control
Authors: *J. RIDDLE, A. MCFERREN, F. FROHLICH;
Psychiatry, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Cognitive control is modulated by the number of rules and the level of abstraction of a given task. We used a hierarchical cognitive control paradigm that manipulated taskset in a two by two design: the number of rules, or set-size, and the abstraction of the task (Badre & D’Esposito, 2007). We found that increased set-size increased theta frequency oscillations and theta-gamma phase amplitude coupling (PAC); whereas, an increase in abstraction increased delta and beta oscillations and delta-beta PAC. In order to causally test these correlational findings, we conducted a crossover double-blind placebo-controlled experiment (N=24) that delivered transcranial alternating current stimulation (tACS) to right prefrontal cortex (PFC) with an individualized custom waveform of gamma frequency at the peak of theta phase (Alekseichuk et al., 2016) or beta frequency at the peak of delta phase. TACS was delivered during performance of the cognitive control task and we recorded resting state EEG after each block to quantify the lasting impact of tACS on PAC in the targeted frequencies. The frequency of theta-gamma and delta-beta tACS was individualized for each participant based on their PAC calculated from an initial baseline session. We hypothesized to find a frequency specific modulation of tACS on behavior for the two dimensions of cognitive control. We found that theta-gamma tACS increased accuracy as a function of set-size (t=2.99; p=0.007) and delta-beta tACS increased reaction time as a function of abstraction (t=2.31; p=0.031). While we did not find an increase in PAC with tACS in the right PFC, we found that theta-gamma tACS increased theta-gamma PAC in the left PFC (t=1.72; p=0.049). To capture participant variance in their response to tACS, we ran a brain to behavior correlation analysis and found that the degree to which theta-gamma tACS altered reaction time and theta-gamma PAC as a function of set-size were positively correlated (r=0.50; p=0.014). In addition, the degree to which delta-beta tACS altered accuracy was predicted by the strength of delta-beta PAC as a function of abstraction during the baseline session (r=0.38; p=0.074). Altogether, cross frequency coupling is an essential mechanism by which the PFC orchestrates cognitive control and can be systematically targeted with tACS.

Disclosures: J. Riddle: None. A. McFerren: None. F. Frohlich: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pulvinar Neuro LLC.

Poster

288. Human Functional Imaging

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 288.15/C69

Topic: I.07. Data Analysis and Statistics
Title: Tuning of SOBI recovered ocular artifact components by the direction and distance of directed saccadic eye movement

Authors: *R. SUN¹, C. CHAN², J. HSIAO², A. C. TANG³;
²Psychology, ³Lab. of Neurosci. for Educ., ¹The Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Much of our understanding of brain functions are based on carefully controlled experimentation carried out in the laboratory context with bulky and expensive imaging modalities. Generalizability of findings made with such constraints cannot be assumed but requires field-friendly brain imaging tools, such as EEG, to enable observation of brain function in more natural context, such as during free eye movement. Yet, ocular artefact in human scalp EEG has been an enduring problem in cognitive neuroscience and biomedical research for nearly a century with a large literature mainly focused on how to remove the ocular artifact from the EEG signals associated with neural activities. Here building upon previous work, we used a hybrid method consists of Second Order Blind Identification (SOBI) for decomposing the EEG into components and a novel Discriminant-index ANd Similarity-index based (DANS) method for automatic identification of the horizontal and vertical ocular components. Going beyond artifact removal, for the first time we show for each individual participant that when correctly extracted and identified, ocular artifact components in the EEG are surprisingly finely tuned by the direction and distance of saccadic eye movement and saccade-related potential (SRPs) amplitude can account for over 90% of variance in the behavioral output of eye movement (saccade coordinates in a short 16 position 32 trial calibration task). Furthermore, we provide the first quantitative individual level determination of the spatial origin of the identified ocular components showing over 95% of variance in the scalp projection accounted for by the physical eye movement. These results show that it is now possible to use EEG alone to extract temporally synchronized neural and eye movement signals.


Poster

288. Human Functional Imaging

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 288.16/C70

Topic: B.09. Network interactions

Support: FAU Brain Institute/ FAU OURI Team Science Award

Title: Cortical inactivity evoked by epileptic seizures is mediated by dysregulation of brainstem arousal circuits: Implications for REM and slow-wave sleep
Authors: *C. ISGOR, A. TYULMENKOVA, S. NICOLAS, A. TERHUNE, M. WARD-MOSES;
Biomed. Sci., Florida Atlantic University, Col. of Med., Boca Raton, FL

Abstract: Sudden unexpected death in epilepsy (SUDEP) is a poorly understood neurological disease. SUDEP may result from postictal suppression of brainstem arousal systems induced by a seizure, eventually leading to irreversible inhibition of cardiorespiratory functions. Epileptic seizures are thought to excite the hypothalamus and lateral septum that send inhibitory projections to the subcortical arousal centers, mainly nucleus pontis oralis (NPO) in the brainstem. The NPO appears to be central to cerebral activation during the rapid eye movement (REM) sleep. Postictal generalized electroencephalography suppression (PGES) resulting from inhibition of the NPO, may contribute to the risk of cardiorespiratory failure and death. In parallel with inhibition of NPO and associated decline in cortical “wake” signals with seizures, clinical data showed that slow wave sleep (SWS) power is increased in patients with focal epilepsy, positively correlating with instances of seizures in the 3-5 days preceding recordings. SWS reduces the excitability of the brain and slows the cardiorespiratory functions that is reversed by periodic episodes of REM sleep episodes. Therefore it is not far-fetched to think that increase in duration and/or power of SWS particularly associated with, and possibly driven by incidences of seizure even up to ~5 days prior may predict severely long PGES and consequent risk of death in upcoming epileptic seizures. Therefore tracking changes in sleep architecture as a function of seizures, particularly decrease in duration of REM and accompanying increase in SWS duration may be useful markers for SUDEP risk. Our lab uses transgenic mice that overexpresses the brain-derived neurotrophic factor (BDNF) in the forebrain and develop tonic/clonic seizures at ~5 months of age as a model of adult-onset spontaneous epilepsy. The mice show brief motor seizures to cage shaking/tail lifting stimulation at the onset of disease but seizure episodes get more severe with successive seizures as evidenced by longer durations of PGES. Pilot data showed a negative association between PGES length and REM duration in inactive phase. We will confirm this and additionally determine if PGES and SWS are positively associated as the mice progress from mild to severe seizures. Results of this study will discern the viability of using sleep architectural markers in REM sleep, and SWS stages to map out increased severity of seizure-induced loss of consciousness (cortical inhibition) and associated death risk in epileptic subjects.


Poster

288. Human Functional Imaging

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 288.17/C71
I.07. Data Analysis and Statistics

Support: NSF RII Track-2 FEC 1539068
Oklahoma Science and Technology Research and Development HR16-057

Title: Fluctuations of hemodynamic global signal are correlated with vigilance states in middle-age and older adults: A simultaneous EEG and fNIRS study

Authors: *Y. CHEN¹, J. ROQUE², J. TANG², M. A. CRAFT³, B. W. CARLSON³, H. YUAN²;
¹Univ. Of Oklahoma, Norman, OK; ²Univ. of Oklahoma, Norman, OK; ³Univ. of Oklahoma Healthy Sci. Ctr., Oklahoma City, OK

Abstract: Functional near-infrared spectroscopy (fNIRS) allows for the study of neural activity via tracking the absorption of light by oxygen and deoxygenated hemoglobin. Due to its portability and high-resolution measures, fNIRS has been widely deployed in studies of resting state and sleep. Vigilance states, including the level of alertness, appear to play a critical role in interpreting the study findings; with fNIRS global signal components negatively correlated with the level of vigilance. In this study, 19 healthy subjects (clinician verified) were recruited after giving informed consent (middle-age group: 35.5 ± 7.2 years old, rang 28-46 years old, 4 females and 8 males; old group: 55.7± 6.0 years old, rang 50-63 years old, 6 females and 1 male). All subjects were instructed to nap or rest quietly for 45 minutes while lying in a recliner. Recordings were divided into 30-s epochs and scored by a certified expert (B.W.C.) using standard criteria. We further separated the individuals’ simultaneous fNIRS and EEG recordings into 30-s epochs and investigated the correlation between fNIRS global signal fluctuation and epoch-level vigilance states which were assessed by EEG measurement. One-sample t-test results show the z-transformed correlation coefficients which derived from all subjects’ both HbO and HbR data are significantly different from zero (HbO: t(17)= -2.88, q<0.05, HbR: t(17)= -2.96, q<0.05). We ran a two-sample unpaired t-test on middle-age and older groups, there is no significance between them for both HbO and HbR data (p>0.1 for both). Our results showed fluctuations of fNIRS global signal were negatively correlated with the corresponding fluctuations of vigilance level, suggesting that the neural sources contribute to fNIRS global signal. However, aging did not show a significant effect on the correlations. Vigilance level can be used to interpret the experimental findings of hemodynamic findings.


Poster

288. Human Functional Imaging

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 288.18/C72
Title: Theta power increase in the human visual cortex in response to novelty

Authors: *E. W. TSANG, R. SUN, X. NIU, W. FUNG, A. C. TANG;
Labouratory of Neurosci. for Educ., The Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Novelty detection is an evolutionarily significant and ancient function as well as a relatively stable function whose early life status marks for long-term developmental outcomes and predicts a range of adult functions. While various brain regions have been shown to respond to environmental novelty, how different brain regions coordinate in novelty related information processing remains under-explored. Previously (CNS 2018 abstract) using a combination of high-density EEG, second order blind identification (SOBI), and a standard visual oddball task, we provided evidence for a two-stage novelty processing hypothesis which states that two distinct stages of novelty processing exist, one involves early-occurring domain-specific neural activity in the sensory processing areas of the brain and the other involves later-occurring domain-general neural activity involving brain regions beyond the sensory cortices. Here we report further evidence on the timing of novelty-induced theta power increase. Specifically, the latencies of peak theta increase of the SOBI recovered Early visual components (189 ± 26 ms) were statistical significantly shorter than that of the Late P300 component (310 ± 19 ms) \[t(9)=-8.5, P<0.0001, \text{Cohen's } d_{av}=1.7\] suggesting that there exists cortical processing areas that are capable of detecting novelty approximately 120 ms earlier than the P300 network. Through source localization using BESA, we further ascertained their visual sensory origin and we found that the scalp projections of these Early components can be well explained by equivalent current dipole models consisting of 1 (N=12) pair or 2 (N=1) pairs of symmetrically placed dipole sources within the occipital lobe (goodness of fits: 94% ± 0.7 %; Talairach coordinates: 26±3; 74±4; 6±4; N=13). Together, these results offer new evidence in direct support for an earlier stage rapid computation of novelty signals by the visual cortex prior to the later stage novelty processing by the well-known P300 network.


Poster

288. Human Functional Imaging

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 288.19/C73

Topic: H.02. Human Cognition and Behavior
Title: Resting-state MEG: A versatile marker of long-term brain adverse effects of leukemia treatments

Authors: *V. OSWALD*¹, Y. ZEROUALI², D. MEUNIER³, D. SINNETT⁴, S. LIPPE⁴, C. LAVERDIÈRE⁵, M. KRAJINOVIC⁶, K. JERBI⁷, P. ROBAEY²;
¹Neurosci., Univ. De Montréal, Montréal, QC, Canada; ²Univ. of Montréal, Montréal, QC, Canada; ³Aix-Marseille Univ., Marseille, France; ⁴Univ. de Montréal, Montréal, QC, Canada; ⁵Univ. de Montréal, Montreal, QC, Canada; ⁶Univ. de Montreal, Montréal, QC, Canada; ⁷Lyon Neurosci. Res. Ctr. - Univ. Lyon I - U1028 - UMR5292, Lyon, France

Abstract: Acute lymphoblastic leukemia (ALL) is the most common cancer type in children. The most common treatment-related neurocognitive impairments are attentional and executive functioning difficulties. The goal of this project is to identify how the neural sources power of the resting-state MEG obtained in the different frequency bands can be used to study the long-term effects of leukemia treatment. We recruited 45 ALL survivors (PA) treated on DFCI-ALL 87-01 to 2005-01 protocols, at least five years post-diagnosis who did not require additional treatments. They were all adults diagnosed between 0 and 17 years of age. We also recruited 28 healthy controls (HC). We recorded resting state MEG 5min eyes open and administered the Wechsler Adult Intelligence Scale - 4th edition. Data pre-processing and source reconstruction was performed using Brainstorm. We calculated means Power Spectrum Density for different frequency bands (delta to gamma) and correlated MEG power normalized z-score in each frequency band at the source level with the WAIS-V indices scores. For correlations, we used the function corecoef in MATLAB. Non-parametric cluster mass analyses (p<0.001) were used to determine significant correlations. For the power difference between patients and controls, we used t-tests with permutation and correction with FDR (p<0.01). For between-group difference in correlations between MEG power and cognitive performance, we used Z-fisher transform function (p<0.001). Younger age of diagnostic was related to increased power in alpha and beta bands for bilateral parietal clusters. Higher total corticoid doses received during treatment was related to increased power in alpha and beta bands for bilateral parietal clusters. Higher total dose of methotrexate received during treatment was related to increased power in alpha and beta bands but for frontal and temporal clusters, predominantly in the right hemisphere. In the alpha and beta bands, ALL survivors showed significantly increased power in bilateral frontal and temporal cortex while they showed significant decreased power in bilateral parietal and occipital cortex. Working memory scores correlated with alpha and beta bands clusters in the right frontal and parietal regions, and with beta band clusters in the right frontal and left central regions. The former correlations were significantly stronger in survivors, while the latter ones in controls, as compared to the other group. Resting state MEG appears as a reliable and flexible tool to investigate cognitive performance and treatment toxicity. This is the first MEG study of the chemotherapy-related neurotoxicity may be specific to brain regions.

Poster

288. Human Functional Imaging

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 288.20/C74

Topic: B.09. Network interactions

Support: Defense Advanced Research Projects Agency (DARPA) under Cooperative Agreement Number W911NF-14-2-0045
NIH R01NS062092

Title: Detecting microscale physiological events beyond spikes and slow waves on the human cortical surface with PEDOT: PSS electrodes

Authors: *A. C. PAULK*¹, J. C. YANG², D. R. CLEARY³, D. J. SOPER¹, S. LEE⁷, M. GANJI⁵, H. OH⁴, J. HERMIZ⁷, V. GILJA⁶, D. M. MAUS³, P. S. JONES¹, D. P. CAHILL¹, Z. WILLIAMS⁹, G. COSGROVE¹⁰, S. BEN-HAIM¹¹, E. HALGREN¹², S. DAYEH⁸, S. S. CASH¹³;
¹Massachusetts Gen. Hosp., Boston, MA; ²Neurosurg., ³UCSD, San Diego, CA; ⁴UCSD, La Jolla, CA; ⁵UCSD, Redwood City, CA; ⁶Electrical and Computer Engin., UCSD, La Jolla, CA; ⁷Electrical and Computer Engin., ⁸Dept. of Electrical and Computer Engin., UC San Diego, La Jolla, CA; ⁹Harvard Med. Sch., Boston, MA; ¹⁰Brigham and Women’s Hosp., Boston, MA; ¹¹Univ. of California San Diego, San Diego, CA; ¹²Multimodal Imaging Lab. (MC0841), Univ. of California San Diego, La Jolla, CA; ¹³Dept Neurol, Mass Genl Hosp, Boston, MA

Abstract: Rapid advancements in materials sciences, electronics, and computer technology have led to a revolution in the collection of neurophysiological data. However, these technological innovations have focused almost exclusively on penetrating probes that enhance our ability to collect large numbers of single unit activity. There have been some improvements in surface recording technology as well, but we are in the infancy of understanding the capabilities of these approaches. With advances in microfabrication techniques, we can now sample neural information using hundreds of microscale electrodes which can cover millimeters of the pial surface. These electrodes provide high density sampling over areas on the cortex at lower impedances and smaller contact surface areas than traditional platinum electrodes. Taking advantage of clinically relevant intracranial monitoring performed during surgical brain resections (N=27), we sampled neural activity using a version of these electrodes fabricated from PEDOT: PSS on a parylene substrate. We found that we could observe classifiable events across the data set which varied in size and waveform shape. We confirmed that these signals are unique to brain recordings by comparing tissue recordings to recordings in just saline. Corroborating prior reports, we found waveforms which closely resemble single unit activity across most of the participants (N=23 of 27), some of which were sampled across multiple
electrode contacts. We also found slower events across participants which were modulated by physiological manipulations including epileptiform-inducing medication, cold saline application, and electrical stimulation. These slower events were divided into two types: ones with average rise time constants of 10-15 ms and fall times < 50 ms and others with rise times at 20-50 ms and fall times at 80-100 ms. The slower event type decreased in frequency with the application of cold saline on the surface of the cortex (p<0.0001; N=5) while both event types increased in frequency following local electrical stimulation (N=9). These events traveled across the electrode contacts at ~0.1-0.2 m/sec, the paths spanning a length of 0.200-0.400 mm, suggesting these events may represent axonal action potentials or dendritic calcium spikes. We therefore hypothesize that novel high density, low-impedance electrodes may be able to sample neural dynamics otherwise not possible with other recording methods. We propose further in vitro and in vivo animal studies may reveal the mechanisms underlying these events.


Poster

288. Human Functional Imaging

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 288.21/C75

Topic: H.02. Human Cognition and Behavior

Title: Whether preoperative evaluation of neuropsychological tests can predict the change in visuospatial function following DBS in Parkinson’s disease

Authors: *M. YAKUFUJIANG¹, Y. HIGUCHI¹, Y. OKAHARA², K. AOYAGI², S. IKEGAMI¹, T. YAMAMOTO³, M. IZUMI¹, M. ABE⁴, Y. IWADATE¹;

Abstract: Background: The effects of DBS on non-motor symptoms, such as cognitive function, have been documented. We aimed to investigate whether the preoperative evaluation of neuropsychological function can predict the change in visuospatial function after DBS in patients with Parkinson’s disease. Methods: We included 35 patients with a median age of 60 years (Interquartile range (IQR), 60-68 years) and median disease duration of 11.9 years (IQR, 10.4-15.1) who underwent bilateral STN-DBS. The mean UPDRS-III score was 43.0 ±13.8 / 16.4 ±8.6 (on/off), and the mean L-dopa equivalent dose was 1187 ±326.1 (mean ±standard deviation). Mean MMSE score was 29.1 ±1.5. Cognitive function was evaluated with the
Wechsler Adult Intelligence Scale—Third Edition (WAIS-III) and Rey-Osterrieth Complex Figure Test (ROCFT) preoperatively and one year after the surgery. Block design and matrix reasoning subtests of WAIS-III and ROCFT were categorized as visuospatial function domain. A correlation analysis was performed to investigate the relationship between visuospatial function and preoperative neuropsychological factors. Results: Subthalamic nucleus-DBS significantly improved motor scores, and L-dopa equivalent dose was also significantly reduced one year after the surgery. The patients’ performance on the ROCFT copy test significantly changed after DBS. Factors, such as age, sex, disease duration, or preoperative motor score, were not related to the postoperative change in the ROCFT copy test scores. Decrease in the postoperative ROCFT-copy test scores correlates with the preoperative low score in the block design subtest. Conclusion: The preoperative evaluation of block design subtests might be useful to identify the patients who are susceptible to developing a decline in visuospatial functioning after DBS.


Poster

288. Human Functional Imaging

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 288.22/C76

Topic: I.07. Data Analysis and Statistics

Title: Imaging of human brain using wireless and portable EEG during natural human activities outside of the laboratory

Authors: *A. C. TANG, R. SUN, E. TSANG, G. WONG; Lab. of Neurosci. for Educ., The Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Rapid development in neuroimaging techniques have advanced our understanding of how human brain supports a wide range of human cognitive functions. Yet, much of the research findings are made under a number of well-known experimental paradigms in well-controlled experimental settings. It remains to be explored to what extent the general principles governing the workings of the brain thus discovered can be generalized beyond the confines of the laboratories and how the rich and varying context of the natural and real world will complicate the relatively simple pictures emerged from the highly simplified laboratory environment. Recent development in portable and wireless systems of electroencephalography and advances in blind source separation techniques made it feasible for researchers to explore the possibility of extracting functionally interpretable neuronal source signals from EEG. Building upon our past works on the application of SOBI to MEG and EEG data, in this study, we collected data using a wireless 32 channel systems (Brain Vision Products) during a variety of natural human activities,
previously considered not feasible for making measurement of underlying generators of neural activity using EEG. We include activities that are known to generate ocular, articulation, and body movement artifact, as in the case of (1) reading naturally with free eye movement and aloud; (2) walking around naturally while speaking and during natural head turning; (3) full body physical exercise. We also include activities that involve practicing of being in different mental states, such as different meditation states. Application of SOBI to each set of data of a few minute long resulted in recovery of certain SOBI components similar to those recovered in laboratory settings, indicating some underlying neural networks observable in highly constrained laboratory conditions can also be tracked combining EEG with SOBI in the artifact ridden natural world. More importantly, SOBI components unique to natural activities and certain meditation states were also found and localizable with high values of goodness of fit (Gof).

Disclosures: A.C. Tang: None. R. Sun: None. E. Tsang: None. G. Wong: None.

Poster

288. Human Functional Imaging

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant SC3 GM121192

Title: Metamemory monitoring and control: A high definition transcranial direct current stimulation study

Authors: *C. WILLIAMSON¹, E. CHUA¹,²;
¹CUNY The Grad. Ctr., New York, NY; ²Brooklyn Col., Brooklyn, NY

Abstract: Accurately monitoring one’s own memory, and adopting strategies to improve memory is crucial in daily life. It is assumed that individuals use the outputs of memory monitoring to strategically control memory, and improve performance. Few studies have tested the relationship between monitoring and control at retrieval, with most studies focusing on monitoring only. Previous research showed that high definitional transcranial direct current stimulation (HD-tDCS) to the left dorsolateral prefrontal cortex (DLPFC) increased monitoring accuracy, using a feeling-of-knowing (FOK) task, in which participants predicted their ability to later recognize the answer to non-recallable items. However, it is unclear if HD-tDCS improvements to metamemory monitoring will lead to strategic control of memory. We tested if metamemory monitoring at retrieval would lead to strategic control of memory and increased memory performance, and the role of the DLPFC in this relationship. In Part 1 of the task, participants assessed if they had ever known the answer to a general knowledge question (“Once knew it” judgment), attempted to recall the answer, and then gave an FOK judgment (i.e., a
metamemory monitoring task). In Part 2, participants chose a subset of un-recallable questions to attempt to recall again with a hint (i.e., a metamemory control task). Participants re-answered their chosen subset of questions, along with a subset chosen by the researcher. Third, participants took a recognition test. Active HD-tDCS was applied to the DLPFC during Part 1, right after Part 1 and during a filler task, or sham HD-tDCS was applied. Preliminary data showed that DLPFC stimulation during Part 1 increased the number of questions given “Once knew it” judgments, suggesting an increased sense of familiarity for the question. FOK ratings did not differ by stimulation, suggesting that stimulation did not increase familiarity for the answer. Participants had higher “Once knew it” and FOK judgments for questions that they chose to re-answer compared to researcher chosen items, showing that familiarity with both the question and answer were factors in choice. This effect did not interact with stimulation type. Strategic control did affect memory performance; participants had greater re-answer recall and recognition for items that they chose compared to researchers chosen items. There was a marginal effect of stimulation type on re-answer recall performance, such that DLPFC stimulation after Part 1 showed greater re-answer recall compared to sham, but this did not extend to recognition. In summary, metamemory monitoring at retrieval allows for strategic control of memory, improving performance.

Disclosures: C. Williamson: None. E. Chua: None.

Poster

288. Human Functional Imaging

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 288.24/C78

Topic: H.02. Human Cognition and Behavior

Support: NSF Graduate Research Fellowship
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Title: Scalp and intracranial EEG reveal intermingled surprise and error signals in performance monitoring networks

Authors: *C. W. HOY¹, S. C. STEINER³, D. KING-STEPHENS⁴, K. D. LAXER⁴, P. WEBER⁴, J. LIN⁵, R. T. KNIGHT²;
¹Helen Wills Neurosci. Inst., ²Dept. of Psychology, Univ. of California Berkeley, Berkeley, CA; ³Univ. of California, Berkeley, Berkeley, CA; ⁴California Pacific Med. Ctr., San Francisco, CA; ⁵Dept. of Neurol., Univ. of California, Irvine, Irvine, CA
Abstract: Many studies have identified neural responses following negative task feedback in performance monitoring regions such as medial prefrontal cortex (MPFC) using human scalp and non-human primate electrophysiology. Classically, these responses were interpreted as error signals indicating unsuccessful outcomes. However, more recent observations of scalp EEG responses following surprising positive task feedback have inspired an alternative interpretation of these signals as unsigned prediction errors for unexpected outcomes. It is possible that both error and surprise signals occur in different circuits within performance monitoring networks, but previous work has not used task designs that dissociate these hypotheses with sufficient spatiotemporal resolution.

To address this issue, we measured both scalp EEG in normal participants (n = 10) and intracranial EEG (iEEG) in patients undergoing monitoring for epilepsy treatment (n = 4) while they performed an interval timing task. By manipulating the tolerance around the target interval to create easy and hard conditions, we controlled whether positive and negative feedback is expected, thereby dissociating outcome valence and expectancy. EEG data were referenced to ear channels, demeaned, band-pass filtered from 0.1-30 Hz, cleaned with ICA (eye and muscle artifacts), and visually inspected for noisy trials. ERPs were computed after baseline correction for wins vs. losses separately in easy and hard blocks (cluster-based permutations). iEEG data preprocessing included removing epileptic and excessively noisy channels and epochs, bipolar re-referencing, and band-stop filtering line noise. The data were then filtered to 70-150 Hz to isolate high frequency activity (HFA) known to correspond to local population spiking. A sliding window ANOVA with factors for difficulty (easy, hard), outcome (win, loss), and timing (early, late) was computed using HFA averaged over 200 ms windows, sliding by 50 ms steps from feedback onset to feedback offset 1 s later (FDR corrected).

Feedback-locked scalp ERPs revealed enhanced feedback-related negativity at central electrodes for unexpected outcomes regardless of valence, supporting a surprise signal interpretation. However, iEEG HFA in medial prefrontal electrodes shows a mixture of responses, with some sites showing significant main effects for only outcome valence while others showed significant interactions between outcome valence and difficulty (i.e., outcome expectancy, or surprise). Collectively, these results indicate both error and surprise signals may be computed in different circuits within the same performance monitoring regions.


Poster

289. Networks and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 289.01/C79

Topic: B.09. Network interactions
Local perturbations of cortical excitability propagate differentially through large-scale functional networks


Brain networks exhibit coherent spatiotemporal patterns of activity that can be readily observed in electrophysiology, BOLD fMRI, and optical neuroimaging signals. Importantly, the correlation structure of these networks (functional connectivity, or FC) is sensitive to changes due to both focal injury (e.g. stroke, seizure, TBI) and targeted stimulation (e.g. TMS). Here we mechanistically examine how local perturbations are integrated into global FC networks. We test the hypothesis that excitation/inhibition (E/I) balance maintained by parvalbumin interneurons (PV-INs) helps to modulate local connectivity with larger networks. We take advantage of transgenic mouse models to specifically and bidirectionally manipulate PV-IN activity in the left whisker barrel cortex (S1w) using virally-delivered chemogenetic constructs (DREADDs; ↑PV n = 12, ↓PV n = 12, Control n = 11). We then survey how this focal change in S1w impacts global networks using whole-cortex optical neuroimaging of both calcium dynamics (GCaMP fluorescence) and hemoglobin dynamics (optical intrinsic signal, analogous to BOLD), as well as measuring local field potential (LFP) within S1w. All recordings were conducted in awake animals, to best capture physiological brain dynamics. Strikingly, we show that chemogenetic manipulation of S1w PV-INs alters local excitability, and that this E/I imbalance propagates differentially through intra- and interhemispheric sensorimotor connections. Changes were statistically verified within and between groups by 2-way ANOVA with multiple comparison testing. We observed significant power spectral density changes locally in S1w and remotely in the right and left whisker motor cortices (M1w). Network-level changes were further analyzed using event-triggered average wave dynamics across the cortex, as well as FC mapping of long-range connections between homotopic S1w nodes and between S1w and M1w. In addition, we reveal that prolonged overexpression of DREADDs in S1w PV-INs induces plasticity in sensorimotor connectivity, in the absence of the DREADD-activating ligand, CNO. These findings may provide valuable mechanistic context for understanding how brain networks respond to changes in E/I balance induced by focal injury, as well as how these changes may be counteracted with neuromodulatory therapy.

Pharmacological modulation of whole-brain networks of functional connectivity in preclinical models

The development of novel psychiatric drugs has been slow and particularly challenging. This is partially because, in spite of knowing the molecular mechanisms of action of a given drug, it is harder to assess its effects in multiple circuits across the whole brain. Therefore, it is important to develop tools in pre-clinical models that allow for the assessment of functional connectivity across the whole brain. Here, we combined cFOS whole-brain imaging in mice, as a proxy of neuronal activation, with graph theory to evaluate networks of functional connectivity upon treatment with two compounds with opposite effects on the dopaminergic system: the psychostimulant Modafinil (10, 30, 50 and 100 mg/kg) and the VMAT-2 inhibitor Tetrabenazine (0.25, 0.5, 1 and 5 mg/kg). Two hours after administration, the brains were prepared for whole-brain cFOS staining. While Modafinil induced a dose-dependent increase in the number of cFOS positive neurons in multiple brain regions across the whole brain, Tetrabenazine affected only a modest fraction of brain regions. To assess functional connectivity, we compute correlations across brain regions to identify regions that co-vary across mice (Wheeler et al 2013). Interestingly, in spite of having different pattern of cFOS activation, Modafinil and Tetrabenazine decreased functional connectivity in the cortex and the hippocampus. Moreover, Modafinil increased average thalamic and hypothalamic functional connectivity, while Tetrabenazine decreased hypothalamic and midbrain functional connectivity. To assess hub reorganization, we used graph theory and computed degree and betweenness. Modafinil induced the pre-limbic cortex, medial habenula and the central amygdala to emerge as new hubs. Remarkably, with Tetrabenazine the pre-limbic cortex, infra-limbic cortex, medial habenula and ventral pallidum displaced the striatum as central hub. Further steps will include testing these neuronal networks with opto- or chemogenetic approaches, followed by the identification of selectively expressed targets in these networks. In conclusion, our approach allows the identification and isolation of novel brain regions that form networks of functional connectivity, and will help to develop and refine novel and more selective therapeutic concepts.
Optical manipulation of parvalbumin and somatostatin inhibitory neurons reveal a differential effect on cortical up-state dynamics

Authors: *J. L. ROMERO-SOSA, H. MOTANIS, *D. V. BUONOMANO; Dept Neurobio., UCLA, Los Angeles, CA

Abstract: Many computations the brain performs emerge from the neural dynamics generated by recurrently connected neurons within local cortical circuits. One of the most studied forms of locally generated neural dynamics are Up-states, which refer to network-wide patterns of activity that rely on appropriately balanced excitation and inhibition. Here we examine the contribution of parvalbumin (PV) and somatostatin (SST) interneurons to Up-state dynamics in cortical organotypic slices by optical excitation and inhibition.

Consistent with previous work (Neske et al, 2015) whole-cell recordings revealed that the firing rate of PV (18 ± 2.5 Hz) was higher than pyramidal (Pyr; 7 ± 2.2 Hz) neurons. Paired recordings between Pyr and PV, and Pyr and SST neurons revealed strong but not significantly different correlation during Up-states (Pyr-PV: 0.72 ± 0.02; Pyr-SST: 0.66 ± 0.21 p >0.05). Of 30 Pyr-PV pairs, 12 exhibited Pyr→PV connections, 8 PV→Pyr connections, and 7 were reciprocally connected—thus reciprocity was present at higher than chance levels. Interestingly, the correlation between Pyr and PV activity during Up states was higher in reciprocally connected pairs (0.67 ± 0.01 vs 0.82 ± 0.008, p <10^{-10})—suggesting that connected pairs may share common input. To date few connections were observed between Pyr and SST neurons. We observed that Up-state onset occurred earlier (16.9 ± 5.71ms) in PV neurons than Pyr neurons—indicating that events driving Up states may preferentially activate PV neurons. In contrast, Up-state onset of SST and Pyr neurons was not significantly different.

To examine the causal relationship between inhibitory neuron activity and Up-state dynamics, we studied the effects of activation and inactivation of PV and SST neurons. When optical simulation (ChETA) of PV neurons was triggered 250 ms after an Up-state onset the length of Up-states decreased significantly (1.88 ± 0.30 s vs 0.69 ± 0.29 s, p<0.01). Although Up-state duration decreased on average when SST was activated it did not reach significance (1.41 ± 0.47 s vs 1.06 ± 0.56 s p>0.05). Optical inactivation of PV and SST neurons using halorhodopsin did
not alter in Up-state duration. These results suggest a differential contribution of PV and SST to Up-states, in which PV neurons exert more powerful control over network dynamics and the ability to turn off Up-states. Furthermore, the finding that PV activation shortened Up-states, but PV inactivation did not alter Up-state duration, suggests that Up-Down transitions do not rely on PV neurons, and support models in which Up-Down transition are produced by synaptic depression of Pyr→Pyr connections.


Poster

289. Networks and Connectivity

Location:  Hall A

Time:  Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:/Poster #:  289.04/C82

Topic:  B.09. Network interactions

Support:  HL137094
          NS101596
          NS108874

Title:  Loss of KCNQ2 from interneurons leads to augmented NMDA receptor driven neuronal synchrony in the neonatal forebrain

Authors:  *B. HOU\(^1\), S. SANTANIELLO\(^2\), A. TZINGOUNIS\(^1\);
\(^1\)Physiol. and Neurobio.,  \(^2\)Biomed. Engin., Univ. of Connecticut, Storrs, CT

Abstract:  KCNQ2 potassium channels are essential for normal brain activity as KCNQ2 dysfunction could lead to severe neurodevelopmental disorders. In the neonatal brain KCNQ2 channels are expressed in GABAergic and glutamatergic neurons, but how KCNQ2 loss-of-function in GABAergic neurons alters neuronal population activity in the neonatal brain is not known. To address this question, we monitored synchronized GABAergic population activity across the forebrain using mesoscale calcium imaging combined with local field potential recordings from the hippocampus. For our study, we used acutely isolated horizontal mouse slices from P4-P6 mice. To increase our signal to noise ratio slices were bathed in 8mM extracellular potassium and maintained at near physiological temperature. Following cell-type specific deletion of KCNQ2 channels from GABAergic neurons, we observed a large increased in synchronized activity in the hippocampal formation and to a lesser extent in the neocortex. In hippocampus, we found that loss of KCNQ2 channels leads to greater frequency of synchronized long-lasting population events (>6s). Mechanistically, these prolonged events were due to increased glutamatergic NMDA receptor transmission as the increased extended activity persisted in the presence of a GABAA receptor blocker, but not in the presence of a NMDA...
receptor antagonist. This data suggested that loss of KCNQ2 channels from GABAergic cells leads to augmented glutamatergic transmission in the neonatal forebrain and dampening NMDA receptor activity might be a viable therapeutic target for KCNQ2 encephalopathy.

Disclosures:  B. Hou: None. S. Santaniello: None. A. Tzingounis: None.

Poster

289. Networks and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 289.05/C83

Topic: B.09. Network interactions

Support: CNRS, INSERM, ANR-ERMUNDY
IDEX ANR-10-IDEX-0001-02 PSL*
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Title: Coherence states and signal transfer of communicating gamma oscillatory neural networks

Authors: *G. D. DUMONT¹, B. S. GUTKIN²;
¹Ecole Normale Superieure, Paris, France; ²Group For Neural Theory, LNC INSERM U960, Ecole Normale Superieure, Paris, France

Abstract: Macroscopic oscillations of different brain regions show multiple phase relationships that are persistent across time [6]. Such phase locking is believed to be implicated in a number of cognitive functions and is key to the so-called Communication Through Coherence theory for neural information transfer [8]. Multiple cellular level mechanisms influence the network dynamic and structure the macroscopic firing patterns. Key question is to identify the synaptic properties that permit such motifs to arise and how the different coherence states determine the communication between circuits.

We use a semi-analytic approach to investigate the emergence of phase locking within two bidirectionally delayed-coupled spiking circuits with emergent gamma oscillations. Internally the circuits consist of excitatory and inhibitory quadratic integrate-and-fire neurons coupled synaptically in an all-to-all fashion [7]. The circuits can show global pyramidal-interneuron or interneuron gamma rhythms. Using a mean-field approach and an exact reduction method [3,9], we break down each gamma network into a low dimensional nonlinear system. We then derive the macroscopic phase resetting-curves [2,4] that determine how the phase of the global oscillation responds to incoming perturbations.

We then study the emergence of macroscopic coherence states of two weakly synaptically-coupled gamma-networks [1]. We derive a phase equation that links the synaptic mechanisms to the coherence state of the system. We show that the delay is a necessary condition for symmetry breaking, i.e. a non-symmetric phase lag between the macroscopic oscillations. We find that a
whole host of phase-locking relationships exist, depending on the coupling strength and delay, potentially giving an explanation to the experimentally observations [8]. Our analysis further allows us to understand how signal transfer between the gamma circuits may depend on the nature of their mutual coherence states [5].

References

Disclosures: G.D. Dumont: None. B.S. Gutkin: None.

Poster

289. Networks and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 289.06/C84

Topic: B.09. Network interactions

Support: German Research Foundation (DFG): SFB 1233, Robust Vision: Inference Principles and Neural Mechanisms, TP 14
German Ministry for education and Research (BMBF, FKZ: 031L0059A)

Title: Human cerebrospinal fluid enhances network function of human organotypic brain slice cultures as revealed by micro-electrode array recordings

Authors: *A. Corna1,3, J. Wickham3, N. Schwarz4, T. V. Wuttke2, H. Koch4, G. Zeck3;
1Ctr. for Ophthalmology, 2Dept. of Neurosurg., Univ. of Tübingen, Tübingen, Germany; 3Natural and Med. Sci. Inst. (NMI) at the Univ. of Tübingen, Reutlingen, Germany; 4Dept. of Neurol. and Epileptology, Hertie-Institute for Clin. Brain Res., Tübingen, Germany

Abstract: Ex vivo human tissue from resection surgery represents a unique opportunity to study neural network activity in human cortex and hippocampus. One of the main challenges of this technique is to achieve long term survival of the tissue in culture. Recent studies have proven
that it is possible to overcome this limitation with the use of human cerebrospinal fluid (hCSF) during the culturing steps. The goal of the presented study is to evaluate the effect of hCSF on the functionality of the slices evaluated at cellular and small network level. Human hippocampal and cortical organotypic slice cultures were prepared from spare access tissue, obtained from patients undergoing epilepsy surgery. The tissue was sliced with a vibratome and cultured as organotypic cultures using hCSF as culturing medium. After 5-17 days in-vitro extracellular recordings were performed using two different types of Micro-electrode arrays (MEA): a standard 256 channel MEA and a high-density CMOS-based MEA with 4225 channels.

Spontaneous activity was recorded under three different experimental conditions: first aCSF was used, then hCSF followed by aCSF again. Spontaneous activity recordings proved long term survival of human organotypic slices. The change to hCSF perfusion generated an increased general excitability in almost all the samples recorded. This increase in activity returned to previous levels when aCSF was washed in again. Activity changes were measured in terms of number of active neurons, firing rate, burst and synchronised events. The perfusion with hCSF also caused activation of network regions otherwise silent in aCSF. The long culture time with sustained viability of the slices allowed the use of viral vector based tools, such as optogenetics. We successfully expressed the light-sensitive ion channel channelrhodopsin under the human synapsin promoter in our cultured slices. We performed simultaneous MEA recordings and light stimulation and successfully demonstrate single cell and network modulation due to optogenetic activation. This approach opens the possibility for dissection of human CNS circuitry.


Poster

289. Networks and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 289.07/C85

Topic: B.09. Network interactions

Support: Singapore Ministry of Education MOE2015-T2-2-095
Singapore Ministry of Education MOE2017-T3-1-002

Title: Inhibitory microcircuit function in the claustrum

Authors: *M. GRAF, G. J. AUGUSTINE;
Lee Kong Chian Sch. of Med., Nanyang Technological Univ., Singapore, Singapore
Abstract: The claustrum is highly interconnected with almost all cortical regions and claustrocortical connections have been well-described. However, almost nothing is known about the local network architecture of the claustrum. We have characterized the inhibitory microcircuitry within the claustrum in acute brain slices. 3 different types of INs - parvalbumin (PV), somatostatin (SST), and vasoactive-intestinal peptide (VIP) – were focally photostimulated via Channelrhodopsin-2 (ChR2), while whole-cell patch recordings were used to measure light-evoked responses in their postsynaptic target cells (N=262). For each postsynaptic neuron recording, 3 network parameters were measured: (1) probability of evoking inhibitory postsynaptic currents (IPSCs); (2) area and location where IPSCs were evoked (input map); and (3) IPSC mean amplitude.

- PV-INs inhibited all neuron types, with a slight bias toward inhibiting PNs. The mean area of PV input maps for PNs was 2.3x larger than for INs. The mean amplitude of light-evoked GABA_A-receptor mediated IPSCs was larger in PNs (41.3 ± 2.5 pA) than in INs (18.4 ± 1.5 pA).
- SST-IN connection probability was uniformly high across all neuron types; the mean area of SST-IN input maps for PNs was 2.6x larger than for other claustral INs and the largest among all IN connections. The mean amplitude of IPSCs evoked in postsynaptic PNs (32.2 ± 1.9 pA) was larger than in postsynaptic INs (21.3 ± 1.3 pA).
- VIP-INs preferentially connected to other claustral INs. As a result, maps of VIP inputs onto PNs were 40% smaller than those onto INs; the mean amplitude of IPSCs were similar for PNs (19 ± 1.7 pA) and INs (20.3 ± 1.9 pA). Photostimulation of presynaptic VIP-INs could inhibit AP firing in postsynaptic INs but had little effect in PNs; this differed from PV-INs and SST-INs, which efficiently blocked AP firing in all postsynaptic target cells.

Our results indicate that both PV-INs and SST-INs efficiently inhibit PN activity and, thereby, reduce claustral output. Conversely, VIP-INs can increase PN activity via disynaptic disinhibition, so that VIP-IN activity will increase claustrum output. Thus, local interneurons bidirectionally control claustrum input onto its downstream targets.

Disclosures: M. Graf: None. G.J. Augustine: None.

Poster

289. Networks and Connectivity

Location: Hall A

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Program #: Poster #: 289.08/C86

Topic: B.09. Network interactions

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        NIH NIA Grant AG047667

Title: The effects of altered norepinephrine transmission on functional connectivity in rats
Abstract: The locus coeruleus (LC) densely innervates various cortical and subcortical regions, and is the main source of norepinephrine (NE) in the brain. LC hyperactivity is associated with neuropsychiatric disorders such as depression and anxiety, while LC neurodegeneration is observed in Alzheimer’s and Parkinson’s disease. However, the impact of LC-NE dysfunction and degeneration on its downstream targets remains understudied. The LC has the potential to regulate many brain networks due to its widespread pattern of innervation, and functional magnetic resonance imaging (fMRI) offers a non-invasive approach to studying alterations in these networks. Importantly, fMRI can be combined with pharmacological or genetic manipulations that perturb various systems, providing insight into disease-like states. To investigate the consequences of aberrant NE transmission on brain-wide functional connectivity, adult male wild-type Sprague-Dawley rats were administered vehicle, the selective LC neurotoxin DSP-4 (50 mg/kg, i.p.), or the selective NE reuptake inhibitor atomoxetine (1 mg/kg, i.p.) prior to combined isoflurane-dexmedetomidine anesthesia and structural and functional MRI scans. Preliminary results indicate that perturbations of LC-NE transmission alter brain-wide functional connectivity. Specifically, seed-based analysis indicates consistent decreases in functional connectivity of the medial prefrontal cortex, primary motor cortex, primary somatosensory cortex, and cingulate in animals administered DSP-4 compared to controls. Analysis of functional connectivity in animals administered atomoxetine is ongoing. We are currently leveraging optogenetics during fMRI to selectively drive phasic bursts or tonic LC firing in a rat model of Alzheimer’s disease in order to better understand the consequences of LC pathology on functional connectivity in the forebrain.

Disclosures: M. Kelberman: None. S.D. Keilholz: None. D. Weinshenker: None. W. Pan: None. M. Nezafati: None. A. Abbas: None.
Title: Synaptic organization of cortico-cortical communication in primate somatosensory cortex

Authors: *M. M. ASHABER*¹, L. ZALÁNYI², E. PÁLFI³, I. STUBER⁴, T. KOVÁCS⁵, R. M. FRIEDMAN⁶, A. ROE⁷, L. NEGYESSY⁸;

Abstract: Synaptic communication is based on two types of axon terminals in hierarchical cortical circuits: small and large, with modulatory and driving roles, respectively. Interestingly, feedforward pathways exert driver-like functions, whereas feedback pathways have modulatory role. However, the synaptic organization subserving the functions of feedforward and feedback pathways is not well understood. The goal of this study was to clarify the existence and functionally relevant structural features of large, driver-like cortico-cortical axon terminals in somatosensory cortical areas 3b and 1. Anterograde tract tracing was used both at the light and electron microscopic levels to reconstruct the 3D morphology of large axon terminals labeled after injections in area 3b and area 1 of the squirrel monkey. At the light microscopic level both small and large boutons were formed by intrinsic, feedforward and feedback pathways. However, large boutons labeled by area 3b injections tended to distribute inter-areally, and in supragranular layers at a higher proportion than those labeled by area 1 injections. Our 3D ultrastructural comparisons showed that surface and volume are highly correlated and provide a powerful tool for classifying cortical endings. Principal component analysis highlighted the significance of the size of mitochondria as a distinguishing feature of bouton type, suggesting that activity shapes the size of boutons. However, the size of the postsynaptic density appeared invariant across the bouton types. Interestingly, perforated postsynaptic density, which signify synaptic plasticity, was associated with a higher degree with the large than to small boutons. Similar to the visual cortex, these findings indicate the existence of driver-like and modulator-like axonal endings in the somatosensory cortex in primates. It is suggested that hierarchically organized large driving-like synapses play a role in the dissemination of tactile information across the intrinsic, feedforward and feedback pathways of area 3b and area 1.

Title: Hemispheric differences in functional interaction between dorsal lateral prefrontal cortex and ipsilateral motor cortex

Authors: *Y. WANG¹, N. CAO¹, J. ZHANG¹, R. CHEN²;
¹Sch. of Kinesiology, Shanghai Univ. of Sports, Shanghai, China; ²Div. of Brain, Imaging and Behaviour – Systems Neurosci., Krembil Brain Inst., Toronto, ON, Canada

Abstract: Objective: To test and compare the interactions between the ipsilateral dorsal lateral prefrontal cortex (DLPFC) and the motor cortex (M1) of both hemispheres. Methods: Fourteen right-handed subjects participated in the experiment. We used a paired-pulse stimulation technique with two high-power Magstim 200 machines (Magstim). In the control condition, the intensity of test stimulus (TS) was adjusted to evoke a motor-evoked potential (MEP) of 1 mV peak to peak in the relaxed first dorsal interosseous (FDI) muscle using a 50mm figure-of-eight-shaped coil. In the paired-pulse condition, the conditioning stimulus was set at 110% resting motor threshold (RMT) at 5 cm anterior to the FDI hotspot. Interstimulus intervals (ISIs) between CS and TS were 2, 4, 6, 8, 10, 15, 20, 25 and 30 ms. Ten conditions were randomly intermingled: TS alone (MEP) and CS plus TS (conditioned MEP for each nine different ISIs). Twenty responses were collected for the test stimulus alone and 10 responses for conditioned MEPs at each ISI. In half of the participants, left hemisphere was tested first and the other half vice versa. Results: The two factor ANOVA showed significant interaction between hemisphere and ISI (F=2.007, p<0.05). Post-hoc analysis revealed significant difference between hemispheres at ISIs of 2 (p<0.05), 10 (p<0.01), 15 (p<0.05) and 20 (p<0.05) ms. There was inhibition when CS was applied 2 ms before TS in the right hemisphere but not in the left hemisphere. There was facilitation at 10, 15 and 20 ms ISI in the left hemisphere and inhibition in the right hemisphere. In the right hemisphere, an inhibitory effect was found at 10 ms compared to the control condition. Conclusions: The data suggests that the left DLPFC has a facilitatory influence on motor cortical excitability while the right DLPFC has an inhibitory effect. These effects could be mediated by the superior longitudinal fasciculus between the ipsilateral DLPFC and M1. The hemispheric differences may reflect the different cognitive roles the right and left DLPFC may play in movement tasks.
Disclosures: Y. Wang: None. N. Cao: None. J. Zhang: None. R. Chen: None.

Poster

289. Networks and Connectivity

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Program #/Poster #: 289.11/C89

Topic: B.09. Network interactions

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Title: Three-dimensional in vitro neural tissue culture model to investigate network formation
Abstract: There is a growing need for in vitro platforms to study neural circuitry, as impairments in these networks are involved with numerous neurodevelopmental and neurodegenerative disorders. Three-dimensional (3D) in vitro neural tissue models provide an improved system to gain insights into neural network formation and dysfunction because they recapitulate the 3D in vivo microenvironment and function of the brain more closely than 2D culture. Here we used our unique bioengineering technology to generate a novel 3D brain-like tissue model for investigating neural network development and activity. We fabricated a 3D, biocompatible scaffold composed of porous silk fibroin sponges filled with collagen type I hydrogels. With mouse embryonic (E16) neurons, we used these scaffolds to generate 3D neural tissue models, which resembled the mechanical properties of brain tissue and supported 3D neurite outgrowth and long-term culture stability. Upon culturing the 3D neural tissue model for different times in vitro, the formation of mature 3D networks was observed as evidenced by immunofluorescence imaging of Tuj1-positive and MAP2-positive axons and dendrites. Using GCaMP6, a genetically encoded indicator of intracellular calcium (Ca$^{2+}$) levels, we monitored the activity of the developing neural networks. Employing a custom developed pipeline based on Matlab algorithms, we quantified dynamic changes in the activity of neurons at the single cell level and on the level of the larger 3D networks. Spontaneous but irregular Ca$^{2+}$ transient network-level events were observed at two weeks with varying intervals. At three weeks of culture, network-level Ca$^{2+}$ transients became more frequent with more regular intervals. The increase of network-level events suggested the dynamic maturation process of the 3D neural network, providing a window to investigate potential intrinsic and extrinsic factors that can disrupt this developmental process. To further demonstrate the utility of our 3D neural tissue model to study neural network activity, we treated our 3D brain-like tissues with neurotransmitter receptor antagonists. Treating with GABA receptor antagonists picrotoxin and bicuculline to block inhibitory inputs resulted in increased network-level activity. Treating with AMPA or NMDA receptor antagonist NBQX and AP5 to block excitatory inputs resulted in a reduction of network-level activity. In summary, our 3D bioengineering system presents a powerful tool for investigating neural network structure, function, and defects.


Poster

289. Networks and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

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**Topic:** B.09. Network interactions

**Support:** BRAIN initiative Grant U19 NS107464-01
Division of Intramural Research Program NIMH

**Title:** Avalanches in prefrontal cortex of the awake mouse

**Authors:** *P. A. KELLS, D. PLENZ;*
Sect Critical Brain Dynamics, Natl. Inst. of Mental Hlth., Bethesda, MD

**Abstract:** The prefrontal cortex (PFC) is crucial for many higher-level cognitive functions such as working memory, rule-based decision making, and goal-directed behavior. It is generally accepted that in order to maintain such high-level function, PFC must communicate with many other cortical areas. Here we address this challenge of communication between cortical areas by identifying cortical dynamics suggested by theory to optimize information transfer between networks. Cortical networks which exhibit population dynamics in the form of neuronal avalanches have been shown to maximize dynamic range and information capacity in local networks while simultaneously maximizing information transmission between distant cortical regions. Our hypothesis is that PFC maintains neuronal avalanches thereby establishing critical brain dynamics which theory and experiments have shown to optimize numerous aspects of information transfer. To test this hypothesis, we transfected adult mice (>P35;C57BL/6) with a nonspecific viral vector to express jGCaMP7s in PFC neurons. A dorsal cranial window was placed to study dorsal anterior cingulate cortex (ACC). In a second set of animals, the dorsal cranial window was combined with a microprism to record from the contralateral prelimbic cortex (PLC). After 2 weeks of recovery, mice were head-fixed on a running wheel and we recorded ongoing population activity in ACC or PLC with 2-photon imaging. Simultaneous recordings of >200 neurons were obtained during quiet resting and free locomotion for periods of 11 min over multiple recording sessions and many days. The arousal state of the animal was tracked using pupillometry. Raw data were motion corrected and ROIs automatically detected using Suite2P (Pachitariu et al. 2016). Spike probabilities were obtained using MLspikes (Deneux et al. 2016). Spike rates were distributed lognormally across animals and periods of increased spiking were typically associated with locomotion (0.2372±0.1734 vs. 0.1832±0.1107 Hz). Animals spent the majority of recordings quietly resting (7.0788±0.1476 min). Ongoing population activity during quiet resting was characterized by intermittent spike clusters. Size and duration of clusters distributed according to power laws with exponents -1.5 and -2 respectively. Despite changes in firing rates between locomotion and rest, size and duration distributions were robust. Our results identify neuronal avalanches as the dominant organization of population activity in PFC in line with expectations for a critical state. We suggest that the maintenance of avalanche dynamics optimizes information transmission between PFC and other brain areas.

**Disclosures:** P.A. Kells: None. D. Plenz: None.
Title: Modeling network effects of dendritic plateau potentials in cortical pyramidal neurons

Abstract: It has been demonstrated in brain slices that releasing glutamate near the basal dendrites of cortical pyramidal neurons can generate dendritic plateau potentials—long-lasting depolarizations of the dendritic membrane mediated by activation of synaptic NMDA and AMPA receptors and extrasynaptic NMDA receptors. Depending on the location and strength of the glutamate stimulus, as well as on local dendritic morphology and activity, the depolarization from dendritic plateaus can spread into the soma, reducing membrane time constant and bringing the cell closer to the spiking threshold. Using data from voltage-sensitive dye imaging in dendrites and whole-cell patch measurements in somata of prefrontal cortex pyramidal neurons from rat brain slices, we developed a morphologically-detailed cortical pyramidal neuron model with active dendrites that reproduced experimental observations: a threshold for activation of the plateau, saturation of plateau amplitude but increasing plateau duration with increasing glutamate application, depolarization of the soma by approximately 20 mV, and back-propagating action potential amplitude attenuation and time delay. For use in network modeling, this cell model was then simplified morphologically while maintaining overall electrophysiological and plateau behavior. Network simulations demonstrated increased synchrony between cells during induced dendritic plateaus. These results support our hypothesis that dendritic plateaus provide a 200-500 ms time window during which a neuron is particularly excitable. At the network level, this predicts that sets of cells with simultaneous plateaus would provide an activated ensemble of responsive cells with increased firing. Synchronously spiking subsets of these cells would then create an embedded ensemble. This embedded ensemble would demonstrate a temporal code, at the same time as the activated (embedded) ensemble showed rate coding. This line of research may help to understand the implications of dendritic plateaus at the cellular and network level,
and may lead to a better understanding of ensemble synchronization and multimodal cortical information processing.


Poster

289. Networks and Connectivity

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Title: Large-scale model of human cortex captures LSD-induced connectivity changes via HTR2A-mediated gain modulation

Authors: *J. B. BURT*1, K. H. PRELLER5, M. DEMIRTAS2, F. VOLLENWEIDER6, A. ANTICEVIC3, J. D. MURRAY4;
1Physics, 2Psychiatry Dept., 3Psychiatry, 4Dept. of Psychiatry, Yale Univ., New Haven, CT; 5Dept. of Psychiatry, Psychotherapy and Psychosomatics, Psychiatric Univ. Hosp. Zürich, Zurich, Switzerland; 6Univ. of Zurich, Zurich, Switzerland

Abstract: Biophysically-based modeling of neural circuit dynamics is a powerful tool for linking molecular and neurophysiological changes to systems-level disturbances in brain function. Neuroimaging and modeling of pharmacological manipulations, which can alter brain physiology while preserving brain structure, provide the ideal framework for probing the causal mechanisms through which molecular perturbations produce large-scale functional disruption. We recently showed experimentally that the neural and subjective effects of the psychoactive drug LSD are primarily mediated by the serotonin-2A receptor (5-HT2AR), yet the spatial topography, cell-type and receptor specificity of these effects remain unclear. Here, we combined structural and pharmacological fMRI (ph-fMRI) with transcriptional data from the Allen Human Brain Atlas to model the neuromodulatory impact of LSD in a large-scale model of human cortex. Gain modulation—a consequence of 5-HT2AR agonism—in the model was
parametrized by regional expression of HTR2A, the gene which codes for the 5-HT2AR. Our model of HTR2A-mediated gain modulation captured the spatial topography of LSD-induced global brain connectivity (GBC) changes, including hyper- and hypo-connectivity of sensory and association areas, respectively. Moreover, our model captured the change in GBC when LSD was administered with vs. without a 5-HT2AR antagonist, implicating the 5-HT2AR specifically. Finally, empirical GBC changes were not as well captured by: models of neurotransmitter-mediated gain modulation at other synaptic receptor sites; null models in which the strength of gain modulation was parametrized by spatial autocorrelation-preserving surrogate maps; or a static linear structural model incorporating diffusion MRI data and the HTR2A map. Thus, our findings collectively link LSD-induced functional alterations to the unique spatial topography and neurophysiological properties of the 5-HT2AR. More generally, our study demonstrates the potential for biophysical modeling, integrated with transcriptomics and ph-fMRI, to causally link molecular manipulations to systems-level disruptions and thereby guide the development of novel therapeutics for psychiatric disorders.

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Poster

289. Networks and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 289.15/D1

Topic: B.09. Network interactions

Title: Inference of cortical network connectivity using maximum entropy models

Authors: *S. MAHALLATI¹,², M. R. POPOVIC¹,³, T. A. VALIANTE⁴,²;
¹Univ. of Toronto, Toronto, ON, Canada; ²Krembil Res. Inst., Toronto, ON, Canada; ³Kite, Univ. Hlth. Network, Toronto, ON, Canada; ⁴Dept. of Surgery, Div. of Neurosurgery, Univ. of Toronto, Toronto, ON, Canada

Abstract: Microcircuit level investigations of brain networks aim to link cellular measure of activity (ie. spikes) to collective dynamics (ie. brain oscillations). With our current ability to record from thousands of neurons, computational methods to understand collective neuronal activity (ie. as a network) are required. Maximum entropy models (MEM), are statistical models to describe such collective activity¹ where neuronal spiking (spike vs. no spike) is analogous to the spin states (up vs. down) of atoms in a ferromagnetic lattice. ‘Connectivity’ can then be inferred by solving the inverse Ising model for the coupling between neurons, which is a MEM constrained only by pairwise correlations². There is an ongoing debate on whether pairwise-based models are generalizable to neural systems at various spatial and temporal scales³,⁴. We
thus assessed the reliability of the inverse Ising couplings as a proxy for connectivity to
differentiate between different systems or network states. We tested the model on in silico
inhibitory-excitatory spiking cortical networks using a range of synaptic delays, firing rates, and
synchrony levels (ie. different types of collective behavior like oscillations vs. asynchronous
firing). In addition, we evaluated the effect of two factors: spike train bin size, and spatial
subsampling which affect the synaptic and propagation delays captured by the model, and the
practical limits in real recordings respectively. This work thus benchmarks the network
characteristics for which a connectivity reconstruction using inverse Ising models are reliable.
interaction network underlies a highly correlated neural population code, PNAS. [4]
Ohiorhenuan, I.E. et al., 2010. Sparse coding and high-order correlations in fine-scale cortical
networks, Nature.


Poster

289. Networks and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 289.16/D2

Topic: B.09. Network interactions

Support: NIH 1PO1 GM118629-01A1

Title: Computational modeling of cortical transition to slow wave oscillations modulated by K-
ATP current

Authors: *B. SETZER*¹, M. M. KOWALSKI², M. M. MCCARTHY¹, N. J. KOPELL¹; ²Dept. of Mathematics and Statistics, ¹Boston Univ., Boston, MA

Abstract: The driving mechanisms of arousal-state transitions from wake to unconsciousness
remain unknown. During many types of unconsciousness, networks of neurons in the brain
demonstrate periods of high-frequency activity interspersed with periods of quiescence, which
gives rise to slow wave oscillations (SWO), a pattern commonly observed in
electroencephalogram recordings. Previous in-vitro slice experiments and mathematical
simulations implicate the K-ATP current in the generation of SWO. However, it is not known
what changes to cortical dynamics could allow for the expression of this slow rhythm. Here, we
propose a Hodgkin-Huxley-based biophysical model that builds upon previous work to
demonstrate that K-ATP driven SWO may arise as a result of the interaction between neuronal
dynamics, ATP-concentration, and altered input strength to either excitatory or inhibitory
neurons. The slow timescale of ATP concentration dynamics enables an awake to SWO transition given only a change in sub-cortical input to excitatory cells from the brainstem or, alternatively, sufficient disinhibition of pyramidal cells via suppression of interneuron activity. These findings make our model generalizable to several states of altered consciousness, including general anesthesia, sleep, and coma, and point to the K-ATP current as an important contender for the source of SWO in these states.

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**Poster**

**289. Networks and Connectivity**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 289.17/D3

**Topic:** B.09. Network interactions

**Support:** Boston University

**Title:** Entrainment of heterogeneous network with excitatory and inhibitory cells

**Authors:** *J. WEI*¹, M. M. MCCARTHY², N. J. KOPELL²;
¹Physics Dept., ²Math and Statistics Dept., Boston Univ., Boston, MA

**Abstract:** Networks of excitatory (E) and inhibitory (I) neurons can produce rhythms and react to input with spectral content in multiple ways. Here we investigate the response of E-I networks to external periodic input in the presence of multiple forms of heterogeneity. Our focus is on understanding which features of the target network and the input allow for a large frequency range of entrainment by the periodic input. The target network consists of single compartment neurons modeled with the spiking currents (Na+, K+, leak) with Hodgkin-Huxley-type conductances. The target network heterogeneity investigated included: network connectivity, coupling strength, applied current to both E and I cells. In addition, we investigated heterogeneity in the inputs including Poisson noise amplitude. We also considered changes in time scales of synapses. Surprisingly, most forms of heterogeneity did not expand the frequency range over which the target was entrained. An exception was heterogeneity in the strength of the AMPA synapse from E to I cells. This allowed the target network to recruit an appropriate number of I cells so that the resulting time-scale of effective inhibition could match the periodic input. In particular, increasing frequency of input lead to few I cells participating in the rhythm. This comes about because a higher frequency of input leads to a faster I-cell rhythm which interacts with the Poisson heterogeneity to the E-cells to suppress some of the latter. With heterogeneity from E to I cells, this smaller amount of excitation picks out only the I-cells most excited by the E cells. The response of the I-cells to a change in local E cells excitability is very
rapid (within one cycle) allowing this mechanism to describe cycle by cycle changes in the balancing of excitation by inhibition as in Atallah et al., 2009. This suggests that there is a relationship between ability to balance excitation with inhibition and ability to entrain to a large range of input frequencies.

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Poster

289. Networks and Connectivity

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Title: Cortical UP DOWN state synchrony drives propofol phase amplitude coupling in slow waves

Authors: *A. E. SOPLATA¹, M. M. MCCARTHY¹, E. A. ROBERTS¹, E. N. BROWN², P. L. PURDON³, N. J. KOPELL¹;
¹Boston Univ., Boston, MA; ²MIT, Cambridge, MA; ³Anesthesia, Critical Care, and Pain Mgmt., Massachusetts Gen. Hosp., Charlestown, MA

Abstract: In adult humans, the level of propofol-induced unconsciousness can be inferred from the type of phase-amplitude coupling (PAC) between slow wave oscillations (SWO, 0.1-1.5 Hz) and alpha oscillations (8-14 Hz) seen on the electroencephalogram (EEG). Low-dose propofol (corresponding to partial responsiveness) shows "trough-max" PAC where alpha power is maximal in the trough of the SWO phase. High-dose propofol (corresponding to complete unresponsiveness) shows "peak-max" PAC where alpha power is maximal in the peak of the SWO phase. How these PAC regimes are related to propofol oscillation interactions and behavioral changes is still unknown. The thalamus, cortex, and their interaction are critical to natural sleep oscillations which have frequencies similar to those of propofol. Therefore, we combined previous thalamic and cortical Hodgkin-Huxley computational models, enabling us to model the EEG signal directly via thalamocortical and intracortical AMPA currents onto pyramidal dendrites. Applying direct propofol effects on the network via increasing GABA-A inhibition and decreasing H-current conductance produced thalamic alpha oscillations, but was insufficient for enabling cortical SWO generation. In contrast, applying indirect propofol effects
via decreasing acetylcholine (ACh) cortical tone enabled cortical SWO generation by decreasing pyramidal K(\textit{Na})-current conductance. By combining direct and indirect effects of propofol, we found that cortical SWO UP/DOWN state synchrony could control whether our synaptic EEG model expressed trough-max (low synchrony) or peak-max PAC (high synchrony). Additionally, in the thalamus, trough-max behavior corresponded to persistent thalamic alpha oscillations, while peak-max behavior showed discontinuous thalamic alpha bursting only near cortical UP states. Our simulated EEG model not only exhibits both PAC states, but also corresponded correctly to the relative power differences in alpha and SWO between coupling states seen in experimental data. By analyzing critical factors for these PAC regimes, we have shed light on the mechanisms powering these profound behavioral differences between anesthetic states.

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**Poster**

289. Networks and Connectivity

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**Topic:** B.09. Network interactions

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**Title:** Abnormal cortical excitability in schizophrenia-like mouse models leads to impaired evoked gamma band activity during behavioral task performance

**Authors:** D. D. AGUILAR\textsuperscript{1}, L. K. RADZIK\textsuperscript{2}, R. E. STRECKER\textsuperscript{1}, *J. M. MCNALLY\textsuperscript{1}; \textsuperscript{1}VABHS, Harvard Med. Sch., West Roxbury, MA; \textsuperscript{2}Stonehill Col., Easton, MA

**Abstract:** Gamma band (25-80 Hz) oscillations (GBO) serve as a fundamental mechanism for the coordination of function across different brain regions and have been strongly linked to cognition. Abnormal GBO, particularly elevated spontaneous GBO and reduced task-evoked GBO, have been linked with all symptom classes of schizophrenia (Sz). Thus, investigating GBO may provide insights into novel therapeutic approaches. Cortical GABAergic interneurons which
express parvalbumin (PV) are centrally involved in regulating GBO and are impaired in Sz. PV impairment disrupts the balance of excitatory and inhibitory neuron activity (E/I balance) in the cortex, which likely impairs GBO. Here, we test this hypothesis using two models that mimic Sz-like cortical PV impairment. First, an optogenetic model utilizing PV-expressing neurons in the basal forebrain (BF), a subcortical brain region involved in cortical activation/arousal. Previous work from our group, has shown that stimulation of BF-PV neurons can modulate cortical GBO. Here, we examine how selectively stimulating BF-PV neuron populations effects GBO in response to novel-object investigation. We also use a transgenic model of NMDAR hypofunction, which also plays a role in impaired PV activity, to examine GBO evoked by social investigation.

EEG activity was recorded during task performance in adult mice via skull screw electrodes above the frontal and parietal cortices, referenced to cerebellum. For novel object we used transgenic mice expressing Cre recombinase in PV neurons (n=10). Channelrhodopsin-2 was virally expressed, and optical fibers implanted bilaterally into the BF. Tonic low wattage stimulation (5mW, 2-5 min) of BF PV neurons was used to elicit cortical E/I imbalance and psychosis-like phenotypes. For social investigation, homozygous serine racemase knockout mice (SR KO, n=4) and wild-type (WT, n=3) littermates were used. Online tracking was employed to tag periods of EEG associated with investigation.

Our results demonstrate an impairment in both task performance as well as a significant reduction in evoked low frequency GBO (25-60Hz) response to novel object/mouse investigation in both models. Thus, altered cortical E/I balance may be causally linked to impairments in working memory and the social domains of cognition. Abnormal E/I and GBO are associated with numerous severe neuropsychiatric disorders including schizophrenia, autism, Alzheimer’s disease, and Parkinson’s disease. Thus, this research provides valuable insight into the pathogenesis of such disorders and lays the groundwork for the development of therapeutic interventions to modulate GBO and improve cognitive function.


Poster

289. Networks and Connectivity

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Title: Altered lateral cortical spread of sensory-evoked signals in YAC128 mice

Brain Res. Ctr., Psychiatry, Dept Psychiatry and Ctr. for Brain Hlth., Univ. of British Columbia, Vancouver, BC, Canada; Dept. of Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada; Dept. of Psychiatry, UBC, Vancouver, BC, Canada; Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Huntington’s disease (HD) is a fatal, monogenic inherited neurodegenerative disorder, which typically manifests in middle age and is characterized by progressively disordered cognition and movement. HD symptoms are largely due to the profound dysfunction and later degeneration of striatal and cortical neurons. Although less extensive than striatal degeneration, cortical degeneration likely underlies much of the troubling cognitive dysfunction experienced by HD patients. Furthermore, mounting evidence suggests cortical pathology at least partly mediates striatal degeneration by virtue of altered cortex to striatum signalling. Despite this, effects of the mutant huntingtin protein on cortical neuron activity are poorly understood. In particular it is largely unclear if, and how, information processing is altered within the intact HD cortex. We have performed *in vivo* wide-field voltage-sensitive dye imaging (VSDI), in lightly anaesthetized YAC128 HD-model and wildtype (WT) mice, to measure neuronal activity across large swaths of dorsal neocortex. Compared to WT littermates, YAC128 mice showed a more extensive spread of neuronal signals across the cortical surface evoked by limb sensory stimulation. Despite this, spontaneous brain activity revealed reduced functional cortical connectivity in YAC128 mice. Data from pilot *in vivo* recordings, using electrode arrays placed at various cortical areas, is consistent with the above VSD findings. We hypothesize the above cortical activity alterations are mediated by one of the following mechanisms: (1) Deficient feedforward inhibition from cortical interneurons; or (2) Enhanced recurrent excitation due to increased cortical pyramidal neuron expression of extrasynaptic NMDA receptors. *Ex vivo* brain slice patch clamp electrophysiology experiments are currently underway to determine whether YAC128 mice cortical pyramidal neurons show reduced GABA-mediated inhibition or increased extrasynaptic NMDA receptor currents. Ultimately, we hope to clarify details of cortical circuit dysfunction in HD and to identify potential therapeutic drug targets.


Poster 289. Networks and Connectivity

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Title: A study of the resting state brain network dynamics between different arousal levels of awake mice

Authors: *Z. HAN*¹, G. LI², Z. LIANG³;
¹Inst. of Neurosci., Shanghai, China; ²Dept. of Biomed. Engin., Peking Univ., Beijing City, China; ³Inst. of Neuroscience, CAS, Shanghai, China

Abstract: Converging evidence of PET (positron emission tomography) and fMRI (functional magnetic resonance imaging) studies have revealed the existence of default mode network (DMN) in the human and non-human primate brains. DMN exhibits higher metabolic activity during the rest, and lower activity during active tasks. Till now, the neural mechanisms underlying the DMN are still unclear. To solve these questions, techniques of combing brain-wide fMRI and electrophysiological recording will be useful. By combining fMRI and LFP (local field potential) recording methods, previous studies performed on non-human primates showed cross-correlations between the rCBV (cerebral blood volume) signal and LFP γ-band power (Duyn, J. H., et al., 2010). But their results lacked evidence to explain the regional specificity of DMN.

Comparing to non-human primates, rodents are much more accessible for neuroscience research. Based on our previously published work (Han et. al., Neuroimage 2019), we aimed to study the neural mechanisms underlying the DMN in awake mice. In the current study, we developed the simultaneous fMRI and electrocorticogram (ECoG) recording paradigm for awake mice. The ECOG electrodes were specifically designed for a large coverage of the brain (roughly 1/3 of the cortex), and also excellent MRI compatibility (e.g., very low degree of distortion in fMRI images). We collected the BOLD (blood oxygenation level dependent), CBF (cerebral blood flow) and the ECOG signals on awake and resting mice. The ECOG signals were denoised to eliminate the artifacts caused by the fast switching of gradient coils. Based on the ECOG signals recorded from the primary somatosensory area (S1), we categorized the mouse arousal states as alert or drowsy. Our preliminary results indicated different spatiotemporal dynamics of DMN in both ECoG and fMRI signals. Further analysis is being conducted to examine the relationship between ECOG signal dynamics and DMN dynamics.

Disclosures: Z. Han: None. G. Li: None. Z. Liang: None.

Poster

289. Networks and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 289.22/D8
Causal role of the P25 TMS-evoked potential in modulation of motor cortex excitability: A TMS-EEG study

Authors: *S. AHN*¹,², F. FROHLICH¹,²;
¹Dept. of Psychiatry, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; ²Carolina Ctr. for Neurostimulation, Chapel Hill, NC

Abstract: Corticospinal excitability measured by motor-evoked potentials (MEPs) is modulated by polarity-dependent transcranial direct current stimulation (tDCS). Specifically, anodal- and cathodal-tDCS applied to the motor cortex (return electrode: supraorbital cortex) increases and decreases the amplitude of MEPs elicited by transcranial magnetic stimulation (TMS), respectively. Yet, it remains unknown how tDCS polarity modulates cortical reactivity and its relationship with corticospinal excitability. The recent development of combined TMS and electroencephalography (EEG) enables us to probe altered brain network dynamics. Here we performed a crossover, double-blind, sham-controlled TMS-EEG study with three tDCS conditions (anodal/cathodal tDCS, and sham) to examine the causal role of cortical reactivity in corticospinal excitability. We recruited 18 healthy right-handed participants (male, 18-35 years old, free of neurological or psychiatric illness) and applied TMS pulses on the hand area of the left precentral gyrus based on individual magnetic resonance imaging (3 Tesla, T1-weighted). We first identified resting motor threshold (RMT) and then applied 100 TMS pulses with 120% intensity relative to RMT over a period of 5 minutes. We collected MEPs and 128-channel EEG data before and after tDCS administration for 10 minutes. We found anodal and cathodal tDCS significantly increased and decreased MEP ratio (post/pre) in “stimulation condition” ($F_{2,28}=255, p<0.0001$) using a linear-mixed effect model with factors “stimulation condition” and “session”. This result replicates and confirms previous findings of bidirectional modulation of corticospinal excitability with tDCS. To investigate cortical reactivity in the EEG data, we removed the TMS-induced artifact from -10 to 20ms relative to stimulation onset and calculated the TMS-evoked potential (TEP) components (P25, N45, P60, N100, P180, N280) with respect to the TMS onset. We found that the P25, N45, P60 TEP components are significantly modulated by tDCS. No other TEP components exhibited such modulation by stimulation condition (all $p>0.05$). Importantly, the modulation of the MEPS and the P25 TEP component by tDCS were correlated for anodal tDCS ($r=0.54$, $p=0.022$), cathodal tDCS ($r=0.51$, $p=0.032$), but not for sham tDCS ($r=-0.15$, $p=0.54$). Together, our findings demonstrate (1) a clear effect of tDCS on corticospinal excitability, (2) the positive cortical reactivity at 25ms (P25) drives the change in corticospinal excitability induced by tDCS. These results provide a strong foundation for future neurophysiology-based examination of tDCS and its effect of motor excitability.

Disclosures: S. Ahn: None. F. Frohlich: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pulvinar Neuro LLC.
Poster

289. Networks and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 289.23/D9

Topic: B.09. Network interactions

Support: NIH Grant R01NS092474

Title: Prediction of peptidergic intracortical modulation networks from single-cell transcriptomic data

Authors: *S. J. SMITH1, U. SÜMBÜL1, L. GRAYBUCK1, F. COLLMAN1, S. SESHAMANI1, R. GALA1, O. GLIKO1, L. ELABBADY1, J. A. MILLER1, T. BAKKEN1, J. SCHARDT1, J. ROSSIER2, Z. YAO1, E. LEIN1, H. ZENG1, B. TASIC1, M. HAWRYLYCZ1;
1Allen Inst. for Brain Sci., Seattle, WA; 2Neurosci. Paris Seine, Sorbonne Univ., Paris, France

Abstract: Seeking insight into the homeostasis, modulation and plasticity of cortical synaptic networks, we have analyzed single-cell mRNA-Seq data on expression of neuropeptide (NP) signaling genes in large numbers of mouse and human cortical neurons. Our results suggest that virtually all cortical neurons in both mouse and human strongly express genes encoding at least one neuropeptide precursor protein (NPP) and at least one neuropeptide-selective G-protein-coupled receptor (NP-GPCR). It is also evident that most individual cortical neurons express more than one NPP and more than one NP-GPCR. In both species, expressed NPP and NP-GPCR genes are drawn from large palettes (18 to 29 genes), with unique signatures of NPP and NP-GPCR gene expression highly specific to particular transcriptomic neuron types. Intracortical NP signaling is most likely predominantly paracrine, with soluble NP products diffusing locally from NPP-expressing source neurons to numerous nearby neurons, while type-specific NP-GPCR expression determines which individual neurons respond. The sets of NPP and NP-GPCR genes expressed in cortex contain many “cognate” pairs (37 for mouse; 36 for human), where an expressed NP-GPCR is selective for the predicted product of an expressed NPP. NP-GPCRs are known to act via second messengers, including cyclic AMP and calcium ions, to powerfully regulate ion channel and synaptic function. Neuron-type-specific NPP and NP-GPCR gene expression patterns therefore allow for prediction of type-specific intracortical NP signaling networks likely to participate in synaptic network homeostasis, modulation and plasticity. We have generated more than 100 predictions of neuron-type-specific NP signaling networks for two areas of mouse neocortex (primary visual VISp and anterior lateral motor ALM) and two areas of human cortex (primary visual V1 and middle temporal gyrus MTG). Here we’ll present our predictions in the form neuron-type-specific coupling matrices and outline experimental strategies for empirical test of such predictions.
Disclosures:  

Poster

289. Networks and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: 289.24/D10

Topic: B.09. Network interactions

Support:  
R01MH101547
T32NS007431

Title: Causal role of the higher-order visual thalamus in cortico-cortical synchronization during sustained attention

Authors: *W. A. HUANG¹,²,³, Z. C. ZHOU¹,²,³, I. STITT¹, S. RADTKE-SCHULLER¹, F. FROHLICH¹,²,³,⁴,⁵,  
¹Psychiatry, ²Neurosci. Curriculum, ³Carolina Ctr. for Neurostimulation, ⁴Cell Biol. and Physiol., ⁵Neurol., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Sustained attention, the continuous allocation of processing resources to respond to infrequent but behaviorally relevant stimuli, is impaired in many psychiatric disorders and represents an important aspect of cognitive control. It has been shown that sustained attention is related to top-down signaling from the frontoparietal network as well as thalamo-cortical modulation. However, the causal role of these two circuits - the fronto-parietal network and the posterior visual thalamo-cortical network - in sustained attention remains unknown. Here, we studied this question by combining rhythmic optogenetics and simultaneous recording from four nodes in the frontoparietal and posterior visual thalamo-cortical visual circuit in ferrets. The four nodes are prefrontal cortex (PFC), pulvinar complex (lateral aspect of lateral posterior nucleus, LPI), posterior parietal cortex (PPC), and primary visual cortex (V1). To probe visual sustained attention, we employed a widely used paradigm: the five-choice serial reaction time task, in ferrets, as ferrets have a relatively well-developed higher-order visual thalamus and have a brain size suitable for optogenetics manipulation. In this task, animals need to pay attention to a computer screen during a delay period with random length until a visual stimulus is presented, which the animal touches for reward. We found that in both animals: (1) theta-band functional connectivity (eg. coherence) between LPI, PPC and V1 but not PFC increases during the delay
period before the animal touched the correct stimulus location, (2) theta power/functional connectivity increase is coupled with gamma power/functional connectivity increase and alpha power/functional connectivity decrease in all regions, (3) optogenetic stimulation in LPl in alpha frequency band during delay period increases corresponding functional connectivity between LPl, PPC and V1, but not PFC, (4) alpha-band stimulation increases number of omission trials by 33% (N=2 animals) compared to sham condition and increases reaction time by 0.14s on average (1-way ANOVA, p=0.055, N=136 trials/condition). This alpha stimulation effect is in agreement with human studies that suggest a causal role of alpha oscillation in attentional gating. As a whole, our study provides causal evidence that LPI coordinates cortical functional connectivity of the LPI-PPC-V1 during sustained attention in a frequency specific way, with increasing alpha power in LPI increases omission trials and reaction time. Our work will ultimately provide a circuit-level mechanistic explanation on how a higher-order visual thalamic structure modulates cortical communication.

Disclosures:  W.A. Huang: None. Z.C. Zhou: None. I. Stitt: None. S. Radtke-schuller: None. F. Frohlich: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pulvinar Neuro LLC.

Poster

289. Networks and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 289.25/D11

Topic: B.09. Network interactions

Support:  DE-AC52-07NA27344
LDRD award 17-SI-002

Title: Optimizing cell encapsulation conditions in ECM-collagen hydrogels to support long-term maintenance of 3D neuronal cultures

Authors:  *D. LAM¹, H. A. ENRIGHT¹, S. K. G. PETERS¹, J. CADENA², M. L. MOYA², D. A. SOSCIA², J. A. ALVARADO², K. S. KULP¹, E. K. WHEELER², N. O. FISCHER¹;
¹Physical and Life Sci. Directorate, ²Engin. Directorate, Lawrence Livermore Natl. Lab., Livermore, CA

Abstract: The emergence of three-dimensional (3D) cell culture platforms in neural tissue engineering has significantly elevated the complexity of in vitro systems. While strategies for tissue engineering have identified a number of biomaterials for cell encapsulation in hydrogels, it will be important to standardize the methodology for neuron encapsulation in 3D and assess their health and function in long-term cultures. In the present study, we investigated the effects of tuning collagen gel to include a heterogenous mixture of ECM called MaxGel, and encapsulated
neurons at three cell concentrations (i.e., 2×10⁶ cells/ml, 4×10⁶ cells/ml, and 1×10⁷ cells/ml). Then, we evaluated the influence of a CO₂ environment during fibrillogenesis on cell health and distribution within the 3D hydrogel at 14 DIV. Our findings demonstrate that increasing the concentration of MaxGel (up to 500 µg/ml) or the initial cell concentration (up to 1×10⁷ cells/ml) did not alter the fibrillogenesis kinetics or the mechanical properties of collagen at a concentration of 1 mg/ml. The distribution of cells within the 3D hydrogel, however, was dependent on the initial cell concentration during encapsulation and the presence of CO₂ during fibrillogenesis of collagen. Uniform distribution of cells was observed in cultures that were initially encapsulated with 2×10⁶ cells/ml and 4×10⁶ cells/ml and in the presence of 5% CO₂ during fibrillogenesis. More heterogeneous cell distributions (i.e. higher cell density at the bottom of the gel relative to the top) occurred when the cell concentration increased to 1×10⁷ cells/ml or in the absence of CO₂ during fibrillogenesis. While the majority of cells encapsulated were viable in the 3D ECM-collagen hydrogel at 14 DIV, a linear relationship between increased cell concentration encapsulated and percentage of cell death was observed. Nevertheless, cells remain viable (assessed by viability staining and calcium imaging) in long term cultures >30 DIV. Furthermore, the ECM-collagen hydrogel was adapted to human primary cells. Collectively, this study demonstrates a framework that systematically evaluates cell viability and the distribution of cells in 3D as a result of tuning the collagen gel with a heterogenous ECM mixture and CO₂ environment, which can have implications on the neurophysiological responses (i.e., electrophysiology and response to drugs) of these 3D neuronal cultures. This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344 through LDRD award 17-SI-002. (LLNL-ABS-771597)


Poster

289. Networks and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 289.26/D12

Topic: B.09. Network interactions

Support: NIH grant 1R56NS109916-01

Title: Biophysics of emergence and irreversibility of epileptiform activity in organotypic cultures of mouse cortex following homeostatic adjustment to activity deprivation

Authors: *D. WISE, S. VAN HOOSER, S. NELSON; Neurosci., Brandeis Univ., Waltham, MA
Abstract: Cells deprived of activity become hyperactive when their activity is restored. This homeostatic adjustment has been linked to spontaneous seizures in vivo following blockade with the sodium channel blocker tetrodotoxin (TTX). This work seeks screen and understand the conditions necessary to induce a dangerous firing paradigm using calcium imaging, synaptic staining and whole cell patch clamp of organotypic slice cultures of mouse cortex. We find that cortex is reliably driven to hyperexcitation upon release from TTX and while slices will recover if deprived for 5 days they will be irreversibly hyperactive if deprived for 10 days. Additionally, preliminary data indicates that the second week of development in mice, concordant to studies on human epileptogenesis and previous work in the rodent, is a vulnerable period for this sort of homeostatic response to activity deprivation. We hope to understand the principles by which homeostatic plasticity might contribute to seizures in humans when neuronal activity is altered by injury or when homeostatic actors in the cell are misapplied.

Disclosures: D. Wise: None. S. Nelson: None. S. Van Hooser: None.

Poster

289. Networks and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 289.27/D13

Topic: B.09. Network interactions

Title: Eminent increase in EEG gamma oscillation in the Wistar Kyoto rat model of treatment-resistant depression

Authors: *N. UPTON, M. S. DUXON, S. KANTOR; Transpharmation LTD, London, United Kingdom

Abstract: Introduction: Wistar–Kyoto (WKY) rats exhibit abnormal behavioural, hormonal, neurochemical as well as sleep-wake characteristics that are often associated with depression. Since WKY rats show decreased sensitivity to conventional monoamine-based antidepressant treatment, they are used as a model of treatment-resistant depression (TRD). The N-methyl-D-aspartate-receptor antagonist ketamine has emerged recently as a rapidly acting antidepressant with high efficacy in TRD patients. The aim of this study was to determine whether a subanaesthetic dose of ketamine has differential effects on sleep-wake behaviour and brain oscillations in WKY rats in comparison with Sprague-Dawley (SD) rats.

Methods: 8 SD and 8 WKY adult (200-250 g) male rats were surgically implanted with telemetry transmitters (F40-EET; DSI, USA) for EMG and fronto-parietal EEG recordings. Following post-surgical recovery, baseline sleep-wake behaviour was recorded in the rats for 72 h. Then the rats were treated with a single dose of ketamine (10 mg/kg, s.c.) or its vehicle, and EEG, EMG, locomotor activity and body temperature were recorded for 24 h. Vigilance states were automatically scored using a customized algorithm (SleepSign, Kissei Comtec, Japan).
EEG power spectra were computed for 2 s epochs (FFT routine, Hanning window) between 1-50 Hz.

Results: WKY rats showed about twice as much rapid eye movement (REM) sleep as SD rats during the passive (light) phase of the 24 h cycle. Although ketamine had similar effect on REM sleep amount in both strains, its effect on high-gamma (>40 Hz) EEG oscillations was different in SD and WKY rats. Specifically, ketamine induced an almost twice as high increase in high-gamma EEG power in WKY rats as in SD rats.

Conclusions: Our data suggest that ketamine is more effective in modulating cortical activity in the WKY rat model of depression than in control SD rats. An eminent increase in EEG gamma oscillation after treatment may indicate a therapeutic potential in depression, although this notion needs to be further investigated. Since the sleep and EEG abnormalities in WKY rats largely recapitulate the changes seen in TRD patients, these neurophysiological measures may serve as key translational tools in an effort to discover novel therapeutics against TRD.

Disclosures: N. Upton: A. Employment/Salary (full or part-time):; TRANSPHARMATION LTD. M.S. Duxon: A. Employment/Salary (full or part-time):; TRANSPHARMATION LTD. S. Kantor: A. Employment/Salary (full or part-time):; TRANSPHARMATION LTD.

Poster

289. Networks and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 289.28/D14

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

Title: Decreased delta oscillations after administration of TSPO ligands Pk11195, Ro5-4864, and FG1N1-27

Authors: *M. I. KHUMNARK\textsuperscript{1}, R. M. HINES\textsuperscript{2}, D. J. HINES\textsuperscript{2};
\textsuperscript{1}Univ. of Las Vegas , Nevada, Las Vegas, NV; \textsuperscript{2}Psychology, Univ. of Nevada Las Vegas, Las Vegas, NV

Abstract: Network communication in the CNS relies upon multiple neuronal and glial signaling pathways. In addition to chemical transmission between cells, mitochondria are able to directly and indirectly affect cellular communications by either providing energy for vesicular release or though production of neurosteroids respectively. One highly conserved mitochondrial signaling mechanism involves an 18kDa outer mitochondrial membrane protein called translocator protein (TSPO). Originally, the function of TSPO was thought to be as a binding site for benzodiazepines in the periphery, while GABA\textsubscript{A} receptors served as the binding site centrally. It was later discovered that TSPO is implicated in multiple cellular processes, with its role in translocation of cholesterol and steroidogenesis being the most well-known and debated. TSPO has also been implicated in porphyrin transport, cellular response to
stress, inflammation and tumor progression. Although many of these roles have been well defined in the periphery, how TSPO signaling plays a role in the CNS has not been fully clarified. In the present study we assessed the effects of TSPO ligands Pk11195 (antagonist), Ro5-4864 (inverse agonist), and FGIN1-27 (agonist) in adult mice. EEG recordings of the frontal cortex revealed a decreased power in the δ frequency band (0.4Hz - 4Hz) after administration any of the compounds described above. Ro5-4864 and PK11195 had similar levels of suppression of δ, while FGIN 1-27 treated animals had the highest δ suppression. FGIN 1-27 also suppressed the θ frequency band (4Hz -12.0Hz), while Ro5-4864 and Pk11195 did not. These data suggest that modulation of TSPO causes changes in circuit level signaling.

Disclosures: M.I. Khumnark: None. R.M. Hines: None. D.J. Hines: None.

Poster

289. Networks and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 289.29/D15

Topic: H.02. Human Cognition and Behavior

Title: Pathological mechanisms of DSCR3 mediating APP trafficking in cognitive dysfunction of Down syndrome

Authors: *Q. CUI;
The Inst. for Brain Research, Collaborative In, Wuhan, China

Abstract: Down's syndrome (DS) is one of the most common chromosomal abnormalities. Most patients have cognitive impairment, which seriously affect the quality of life. However, there is no effective treatment and the pathological mechanism is not clear. Therefore, it is of great significance to study the mechanism of DS cognitive impairment. Previous studies showed that Down syndrome critical region protein DSCR3 increased significantly in DS mice brain, and downregulated DSCR3 rescued the cognitive dysfunction phenotype in DS mouse model. It is suggested that the overexpression of DSCR3 may be involved in the occurrence of DS cognitive dysfunction, but the specific mechanism is not clear. Further studies have shown that overexpression of DSCR3 decreased the expression of vesicle transport related protein SNX17 and the content of amyloid precursor protein (APP) on the cell surface. So, we hypothesize that overexpression of DSCR3 induces abnormal APP transport by reducing SNX17 and resulting in cognitive dysfunction of DS. This project will study the role of DSCR3 in cognitive impairment of DS on the cellular and animal level, and elucidate its molecular mechanism, so as to provide new targets for the treatment of DS cognitive dysfunction.

Disclosures: Q. Cui: None.
Mossy cells in the rostral and caudal dentate gyrus differ in their patterns of axonal projections

**Authors:** C. R. HOUSER¹, Z. PENG¹, C. S. HUANG¹, X. WEI², *I. MODY²; ¹Dept. Neurobiol, ²Physiol., David Geffen Sch. of Med. at UCLA, Los Angeles, CA

**Abstract:** Mossy cells (MC) of the dentate gyrus (DG) are a major group of excitatory hilar neurons that are important for regulating activity of DG granule cells. MC are particularly intriguing because of their extensive commissural and longitudinal association connections within the DG. While it generally has been assumed that MC in the rostral and caudal DG have similar patterns of termination in the inner one-third of the dentate molecular layer, this has not been demonstrated directly. We compared the projection patterns of MC in the rostral and caudal DG. Two different transgenic mouse lines that express Cre-recombinase relatively selectively in dentate MC (Drd2-Cre and CalCrl-Cre mice) were used in this study. The results were similar in the two mouse lines, with MC exhibiting characteristic morphological features and expression of GluA2 throughout the longitudinal extent of the DG. To label MC axons from rostral and caudal regions of the DG differentially in the same mouse, Cre-dependent eYFP and tdTomato were transfected caudally and rostrally respectively. At 3-4 weeks following unilateral labeling of MC in the caudal DG, a dense band of labeled fibers was present in the inner third of the molecular layer and extended bilaterally throughout the rostral-caudal extent of the DG, thus replicating the expected distribution of MC axons. In contrast, following unilateral labeling of MC in the rostral dentate gyrus, a narrower band of fibers was evident and was strongest on the contralateral side. At the level of the transfection, this band was present in the inner molecular layer. However, at progressively more caudal levels, the fibers expanded into the middle molecular layer until, most caudally, the MC axons formed a distinct band in the middle to outer molecular layer and no longer overlapped with the local MC projections of the inner molecular layer. Optogenetic stimulation of these caudal fibers in slices with Cre-dependent expression of ChR2/eYFP or ChIEF/tdTomato in MC demonstrated robust EPSCs, particularly in ipsilateral granule cells, but only sporadic IPSCs. These findings suggest that MC in the rostral and caudal dentate gyrus differ in the distribution of the axonal projections and possibly their function.
Disclosures:  C.R. Houser: None. Z. Peng: None. C.S. Huang: None. X. Wei: None. I. Mody: None.

Poster

290. Epilepsy: Animal Models and Network Dynamics

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 290.02/D17

Topic: B.10. Epilepsy

Support:  NIH NINDS F32NS106732
        VA Merit Review Grant I01-BX002949
        DoD CDMRP W81XWH-18-1-0598
        NIH P30 NS061800

Title: Circuit reorganization of hippocampal mossy cell outputs in epilepsy

Authors:  *C. R. BUTLER*¹, G. L. WESTBROOK², E. SCHNELL³;

Abstract: The hippocampal mossy cell, a glutamatergic neuron in the dentate hilus, projects to local GABAergic interneurons and dentate granule cells (DGCs). Mossy cells are lost in neurologic diseases such as epilepsy; however, relatively little is understood about how residual mossy cells drive hippocampal network function. To address this issue, we used a transgenic mouse model, the calcitonin receptor-like receptor Cre mouse (Crlr-Cre), which selectively expresses Cre recombinase in mossy cells. In preliminary studies, we evaluated the specificity of mossy cell labeling in these mice using both Cre-dependent reporter mouse lines and intracerebral Cre-dependent viral injections. We then used optogenetics to functionally characterize mossy cell projections onto DGCs in both healthy controls and in epileptic mice, using the pilocarpine model of temporal lobe epilepsy. In acutely prepared hippocampal slices from healthy mice, mossy cell activation evoked both a direct glutamatergic (AMPAR/NMDAR-mediated) and a di-synaptic GABAergic response in DGCs. In epileptic mice, similar stimulation evoked a significantly reduced direct AMPAR-mediated current, coincident with a dramatic reduction in mossy cell number. However, the amplitudes of di-synaptic GABA-mediated responses were relatively maintained, suggesting network compensation. This observation was not associated with changes in short term plasticity of the direct glutamatergic mossy cell-DGC connection. We are currently working to better characterize whether this maintenance of inhibitory connectivity results from changes at the mossy cell-to GABAergic interneuron connection, or alternatively, a change in interneuron function. Overall, these results suggest that surviving mossy cells preferentially provide feedback
inhibition onto DGCs in epilepsy, which could serve to maintain inhibitory tone following the development of hippocampal hyperexcitability.

Disclosures:  C.R. Butler: None. G.L. Westbrook: None. E. Schnell: None.

Poster

290. Epilepsy: Animal Models and Network Dynamics

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: 290.03/D18

Topic: B.10. Epilepsy

Support: DFG (SFB 1089, FOR 2715)
       BONFOR

Title: Identification of zinc-sensitive metal-regulatory transcription factor 1 positive neurons to study the hippocampal epileptic network activity

Authors: *A. MECONI, S. SCHOCH, A. J. BECKER, K. M. J. VAN LOO; Dept. of Epileptology, Inst. of Neuropathology, Bonn, Germany

Abstract: The emergence of a hyperexcitable hippocampal network characterizes temporal lobe epilepsy (TLE), the most common focal epilepsy in adults. Previously, we have shown that the metal-regulatory transcription factor 1 (MTF1) can increase the expression of the T-type calcium channel CaV3.2 after a rise in intracellular Zn\(^2+\), resulting in an enlarged density of T-type Ca\(^2+\)-currents, an augmented propensity for burst discharges, as well as spontaneous seizures. To date, little is known about MTF1-induced signalling cascades and their functional relevance in the development of neuronal microcircuits to generate seizures.

A better understanding of the molecular and functional network recruited by Zn\(^2+\)-sensitive MTF1-signaling can provide fundamental new insights into key and selective molecular signalling pathways inducing pathological hippocampal microcircuit plasticity. Here, we developed different MTF1-transcriptional units to genetically label MTF1-responsive cells. Analysis of these units in NG108-15 cells and primary neurons led us to the identification of one promising MTF1-transcriptional unit which will be now used to study how the Zn\(^2+\)-sensitive immediate early gene MTF1 can convert a hippocampal micronetwork hyperexcitable.

Poster

290. Epilepsy: Animal Models and Network Dynamics

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 290.04/D19

Topic: B.10. Epilepsy

Support: NIH/NINDS R01 NS095842

Title: The effect of serotonergic neurotransmission and vigilance state on apnea during and after kindled seizures in mice

Authors: *K. G. JOYAL, B. S. PURNELL, G. F. BUCHANAN; Neurol., Univ. of Iowa, Iowa City, IA

Abstract: Sudden unexpected death in epilepsy (SUDEP) is the leading cause of death in patients with refractory epilepsy. SUDEP is second only to stroke in terms of years of potential life lost to disease. While the exact etiology of SUDEP remains unclear, it is believed apnea is a major precipitant of death. A large body of evidence has implicated serotonin (5-HT) in SUDEP. 5-HT is important in the regulation of breathing and plays a role in regulating seizure threshold and severity. Since seizures can alter 5-HT signaling, we hypothesized that enhancing 5-HT neurotransmission prior to seizure onset would decrease the incidence of ictal and postictal apnea. As SUDEP more often occurs at night, and 5-HT tone is sleep-state dependent, seizures were induced during both wake and sleep. Due to a putative role for the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors in setting seizure threshold and severity, agonists for these receptors were also utilized. Adult (8-12 weeks) male and female C57BL/6 mice were instrumented for EEG/EMG recording and stereotaxically implanted with a bipolar stimulating/recording electrode into the right amygdala [AP: -1.3; ML: -2.8; DV: -4.7] for amygdala kindling. After recovery from surgery, afterdischarge threshold determination, kindling, and acclimation to the recording apparatus, seizures were induced during wake and non-rapid eye movement (NREM) sleep following treatment with selective 5-HT reuptake inhibitors (SSRI) or 5-HT receptor agonists. We found that the SSRI citalopram (20 mg/kg, i.p.) eliminated ictal and postictal apneas that were observed in animals only receiving saline. Another SSRI (fluoxetine, 10 mg/kg, i.p.) significantly decreased the incidence of ictal and postictal apnea. Alternatively, there was no decrease in apneas when animals received the 5-HT$_{2C}$ receptor agonist MK-212 (10 mg/kg, i.p.) or the 5-HT$_{2A}$ receptor agonist TCB-2 (10 mg/kg i.p.). No effect of sleep state was observed. To further investigate this circuit, a series of 5-HT$_{2}$ antagonists were administered along with citalopram. These results indicate that increasing endogenous 5-HT before a seizure reduces ictal and postictal apnea. Ongoing work is aimed at better understanding receptor and circuit mechanisms in regulation of post-ictal breathing.

Disclosures: K.G. Joyal: None. B.S. Purnell: None. G.F. Buchanan: None.
Effects of serotonin replacement on seizure profile in genetically serotonin neuron deficient mice subjected to the pilocarpine model of temporal lobe epilepsy

**Authors:** *R. Li*\(^1\), K. Claycomb\(^2\), M. Matkovich\(^3\), G. F. Buchanan\(^3\);
\(^1\)Neurol., Univ. of Iowa Carver Col. of Med., Iowa City, IA; \(^2\)Neurol., Yale Sch. of Medicine, Dept. of Neurol., New Haven, CT; \(^3\)Neurol., Univ. of Iowa, Iowa City, IA

**Abstract:** Approximately 4% of Americans will develop epilepsy in their lifetime. Despite the existence of numerous therapies for epilepsy, about a third of patients remain refractory, which at its worst could threaten their lives. It is notable that seizures are more likely to happen during wakefulness and non-rapid eye movement sleep instead of during rapid eye movement sleep in epilepsy patients and in animal models of epilepsy, suggesting a naturally occurring preventive mechanism that has yet to be fully understood. The neuromodulator serotonin (5-HT) regulates sleep as well as seizures, and its concentration fluctuates in a sleep-dependent manner.

Therefore, we hypothesized that genetic elimination of 5-HT neurons in the brain alters the sleep-dependent pattern of seizures and can be partially restored by 5-HT brain infusion. To test our hypothesis, *Lmx1b*\(^{ff/p}\) (n =14) mice of which 5-HT neurons have been genetically deleted and their littermates *Lmx1b*\(^{ff}\) (n =13) mice of both sexes were used in the current study. Mice were injected intraperitoneally with pilocarpine (250-300 mg/kg) 20-30 min after injection of scopolamine methyl nitrate (1 mg/kg). Status epilepticus induced by pilocarpine was stopped by diazepam (1 mg/kg) 60 min later. Mice were then implanted with EEG/EMG headmounts along with a cannula (Brain Infusion Kit 2, Alzet) targeting the lateral ventricle. Osmotic pumps (Model 2002, Alzet) containing either aCSF or 5-HT at different concentrations were switched between each recording session. EEG and EMG were recorded using Sirenia Acquisition (Pinnacle Technology) at a sampling rate of 1000 Hz with a 300-Hz low pass filter and seizures were detected offline using Sirenia Seizure Pro (Pinnacle Technology). Preliminary analyses showed that seizures tended to occur more frequently in *Lmx1b*\(^{ff/p}\) mice with slightly higher duration and a lower coefficient of variance compared with *Lmx1b*\(^{ff}\) mice under aCSF condition. 5-HT at 1 mM was likely to suppress seizures in *Lmx1b*\(^{ff/p}\) mice but not in *Lmx1b*\(^{ff}\) mice. The interaction between vigilant state and seizure frequency, duration, and dynamics will also be examined. The source of 5-HT within the brain is located at the midline raphe nuclei of the...
midbrain, pons, and medulla. Future studies will focus on the midbrain raphe nucleus to identify receptor and neuronal circuitry mechanisms of such a phenomenon. Epilepsy patients also have a high prevalence of depression, sleep disorders, and sudden unexpected death. Therefore, understanding the role of serotonin in epilepsy will aid development of new therapies to reduce the burden of epilepsy and its comorbidities.

**Disclosures:** R. Li: None. K. Claycomb: None. M. Matkovich: None. G.F. Buchanan: None.

**Poster**

290. Epilepsy: Animal Models and Network Dynamics

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 290.06/D21

**Topic:** B.10. Epilepsy

**Support:** University of Virginia Medical Scientist Training Program
NIH R01-NS099586

**Title:** A heterogeneous thalamic network model that recapitulates oscillations modulated by GABA transporter blockade

**Authors:** *A. C. LU¹, C. K. LEE², B. TRUONG¹, K. S. SILIS¹, J. R. HUGUENARD², M. P. BEENHAKKER¹;

**Abstract:** Absence seizures are thought to result from 3~5 Hz generalized thalamocortical oscillations. Understanding how such oscillations persist could lead to novel treatments for the most common form of pediatric epilepsy. In acute thalamic slices, bicuculline-induced GABA$_B$ receptor-dependent oscillations were prolonged upon pharmacological blockade of GABA transporters GAT1 or GAT3 individually, but were surprisingly suppressed upon dual blockade of GAT1 and GAT3. Using pharmacological manipulations, distinct temporal profiles of inhibitory post-synaptic currents (IPSCs) from GABA$_B$ receptors of thalamocortical (TC) relay neurons could be recorded for each of 4 pharmacological conditions: (1) control, (2) GAT1 blockade, (3) GAT3 blockade, (4) dual blockade. We propose that the differential effects of GAT blockade on thalamic oscillations could be accounted for by the differential activation of GABA$_B$ receptors in TC neurons. To this end, we developed a biophysical thalamic network model that can recapitulate differences in oscillations when GABA$_B$ activation profiles were varied. We first established 3-compartment single TC neuron models that could recapitulate differences in GABA$_B$ IPSC responses. Using dynamic clamp, voltage responses to each of the 4 GABA$_B$ IPSC conductance profiles, at 3 different levels of maximal amplitude, as well as voltage responses to a current impulse, was recorded for 36 TC neurons. In general, TC neurons had an increase in
low-threshold spike (LTS) probability with GAT1 or GAT3 blockade but a significant decrease with dual blockade. However, there was also high IPSC response heterogeneity among the 36 neurons. By fitting simulated current impulse responses and simulated IPSC responses for each individual cell, we sought to infer geometric and conductance parameters for all 36 neurons. Finally, we incorporated the heterogeneous model TC neurons into an established thalamic network model. A homogeneous network model was sufficient to recapitulate the exacerbation of oscillations with GAT1 or GAT3 blockade and the abolishment of oscillations with dual blockade. Nevertheless, network heterogeneity seemed to be necessary for modelling seizure termination. In summary, we have developed a biophysically-based computational model that recapitulates most effects of GABA transporter antagonists on thalamic oscillations. These results suggest that modulation of GABA transporters is a potential novel treatment for absence epilepsy.


Poster

290. Epilepsy: Animal Models and Network Dynamics

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 290.07/D22

Topic: B.10. Epilepsy

Support:  5R01NS099586
          T32 Gm007055

Title: Low glucose modulation of thalamocortical neuron excitability

Authors:  *K. A. SALVATI1,2, A. C. LU1,3,2, M. L. RITGER1,3,2, M. P. BEENHAKKER1;  
1Pharmacol., 2Neurosci. Grad. Program, 3Med. Scientist Training Program, Univ. of Virginia, Charlottesville, VA

Abstract: The link between diet and epilepsy remains unresolved, particularly for epilepsies characterized by spike-and-wave discharges (SWD). Herein, we provide evidence demonstrating that thalamocortical circuits implicated in SWD generation alter activity during acute neuroglycopenia. Using video-EEG, we first demonstrate that low glucose intensifies SWDs in two rodent models [DBA/2J mouse (D) and WAG/Rij rat (W)]. SWDs increase following a 16 hour fast (p<0.05, n=12D; 13W animals). Also, seizure exacerbation generally correlates with decreased blood glucose levels (p<0.05; p<0.001, n=12D; 13W animals). To disambiguate the effects of hypoglycemia and hyperketonemia on SWDs, we monitored seizures following insulin injection, a manipulation that results only in the former. Insulin injection increased SWD occurrence (p<0.05, n=11D; p <0.05; n=9W). These data suggest that low glucose, not elevated
ketone bodies, modulates SWD occurrence. To support the above stated hypothesis, we directly inhibited glycolysis within somatosensory thalamus using 2-deoxyglucose (2-DG). SWD count increased during 2-DG infusion (p<0.05; n=6W), thus demonstrating that thalamocortical (TC) SWDs are sensitive to glucose metabolism. Next, we performed cell-attached voltage-clamp recordings in acutely prepared thalamic brain slices to determine whether brief exposure to low glucose alters the intrinsic excitability of TC neurons. We observed a significant increase in the number of spikes and mean spike frequency (p<0.05; n=12W cells). We hypothesize that changes in TC neuron excitability are attributable to modulation of GABA\textsubscript{B} receptor activity in low glucose. Indeed, thalamocortical oscillations in sleep and epilepsy are dependent on GABA\textsubscript{B} receptor activity. Previous work demonstrates that p-AMPK interacts with the GABA\textsubscript{B} receptor, resulting in a potentiation of GABA\textsubscript{B}-mediated IPSCs. To establish that this interaction is present in TC neurons, we performed voltage-clamp recordings and added AMPK activators to the internal solution. Baclofen (100µM) was puffed adjacent to the dendrites of the recorded neuron to evoke GABA\textsubscript{B}-mediated currents. AMPK activators caused an attenuation of GABA\textsubscript{B}-mediated current run-down (p < 0.05; n = 18W cells). Additionally, fasting increased p-AMPK expression in the thalamus, suggesting an increase in p-AMPK likely correlates with an increase in SWDs. Our future \textit{in vitro} and \textit{in vivo} experiments will further elucidate this mechanism. In sum, we demonstrate that glucose modulates thalamocortical excitability and SWDs. Our data support the hypothesis that diet can influence the occurrence of epileptic activity in the brain.


Poster

290. Epilepsy: Animal Models and Network Dynamics

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 290.08/D23

Topic: B.10. Epilepsy

Support: NIH grant NS096092

Title: Local synaptic connectivity of subicular pyramidal neurons sorted by multivariate analysis and its impact on population activity

Authors: *M. P. FISKE\textsuperscript{1}, M. ANSTÖTZ\textsuperscript{1}, G. MACCAFERRI\textsuperscript{2}; \textsuperscript{1}Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; \textsuperscript{2}Dept Physiol, Northwestern Univ., Chicago, IL

Abstract: The subiculum is a key area involved in temporal lobe epilepsy, and its local excitatory circuits have been suggested to be involved in the generation of various forms of epileptiform activity. Although it has now established by many studies that subicular pyramidal
cells exhibit distinct intrinsic excitability, to our knowledge only one study has examined the local connectivity between the distinct types of subicular pyramidal neurons classified as “regular firing” or “bursters” (Böhm et al., 2015). We have re-addressed pyramidal cell diversity with direct patch clamp recordings from subicular slices, classified by a combination of principal component analysis and Gaussian mixture models. Out of n=995 recorded cells, we observed variable proportions of what we term “type 1” and “type 2” cells (51.1% and 48.9%, respectively). When the intercellular relationships were examined, we found functional evidence for both homotypic (type 1 to type 1, n=29 and type 2 to type 2, n=34 synapses) and heterotypic synaptic connectivity (type 1 to type 2, n=25, and type 2 to type 1, n=7 synapses). Overall, the probability of finding a connection was roughly symmetrical for homotypic connections (type 1 to type 1, p=0.046, type 2 to type 2, p=0.052), whereas it was highly asymmetrical in heterotypical connections (type 1 to type 2, p=0.088, type 2 to type 1, p=0.025). The properties of the unitary excitatory postsynaptic potentials were also similar across connections (amplitude 0.54 mV), without obvious differences. In n=6 experiments we verified that uEPSPs were glutamatergic and fully blocked by the AMPA receptor antagonist NBQX (20 µM). When the anatomy of the connected pairs was examined at the light microscopy level, we observed putative contact sites that ranged in numbers between 1 and 9, and appeared consistently biased towards the basal dendrites of the postsynaptic cell (85.3% basal vs 14.7% apical). Lastly, we tested the influence of these local excitatory connections on population activity by recording from pairs of cells in slices surgically isolated from extra-subicular inputs and exposed to gabazine (12.5 µM) and CGP55845 (5 µM). In n=11 recordings, we found that blockade of GABAergic circuits allowed the development of synchronous epileptiform bursts (number of action potentials/burst=29.5, n=27 cells). In conclusion, our data suggest a complex cell type-specific connectivity of intrinsically diverse pyramidal neurons that can sustain, in the absence of GABAergic inhibition, epileptic-like hypersynchronous activity. Supported by NIH grant NS096092

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Poster

290. Epilepsy: Animal Models and Network Dynamics

Location: Hall A

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Topic: B.10. Epilepsy

Support: NIH Grant NS093510
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Brown-Coxe Postdoctoral Fellowship
American Epilepsy Society Postdoctoral Fellowship
Title: Dysplastic neurons mediate epileptogenesis in tuberous sclerosis complex

Authors: *L. S. HSIEH1, L. NGUYEN1, A. F. BORDEY2;
1Neurosurg., Yale Sch. of Med., New Haven, CT; 2Dept Neurosurg, Yale Sch. Med., New Haven, CT

Abstract: Disorders caused by mutations in the mTOR pathway genes, exemplified by Tuberous Sclerosis Complex (TSC), lead to mTOR hyperactivity, brain malformations, and life-long epilepsy in the majority of patients. While partially effective, surgical resection of seizure foci or pharmacotherapy with mTOR inhibitors are the only available treatments for epilepsy in TSC. A better understanding of epileptogenic mechanisms in the disease is therefore needed. Two modern hypotheses compete to explain the underlying mechanism of epileptogenesis in TSC. 1) the neuronal hypothesis implicates dysplastic neurons and their abnormal activity as the source of seizure activity. 2) the neuro-glial network hypothesis implicates a pathophysiological network of neuro-glial interplay that occurs outside of cortical tubers. To test whether dysplastic neurons mediate epileptogenesis, we located the seizure focus, measured excitability of dysplastic neurons, and tested the tunability of dysplastic neurons alone to modulate seizure severity in a mouse model of cortical tubers with spontaneous epileptogenesis. We found, after analyzing independent electroencephalographic recordings from each hemisphere that high frequency oscillations (HFOs), which denotes cortical seizure initiation sites, occurs in the tuber containing hemisphere before traversing to the contralateral hemisphere. We also found that dysplastic neurons express more sodium currents, can sustain longer bursts of regenerative firing, and are more depolarized than normal neurons in and outside of cortical tubers as well as in control animals. Furthermore, suppressing activity in the dysplastic neurons alone, with the expression of an exogenous inward-rectifier potassium channel Kir2.1, mitigates seizure activity. Finally, the escalation of neuronal dysplasia severity, i.e. larger cells and greater migration deficits, leads to greater seizure activity. Together these evidences suggest that dysplastic neurons mediate epileptogenesis in TSC.


Poster

290. Epilepsy: Animal Models and Network Dynamics

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 290.10/D25

Topic: B.10. Epilepsy

Support: NIH grant R01 NS104368
CIHR

Title: Sustained network activity prevents development of post-traumatic epilepsy
**Abstract:** It has long been appreciated that traumatic brain injury is a common cause of acquired epilepsy. Post-traumatic epilepsy (PTE) remains to be a difficult disorder to treat as there can be a prolonged period of time during which epileptogenesis can arise following the initial brain insult. Indeed, it has been reported that epilepsy can develop up to 15 years after the occurrence of the brain trauma. The likelihood of developing epilepsy increases with age at the time of the trauma. Recent *in vivo* studies have shown that older animals were more susceptible to the development of epilepsy following cortical undercut as compared to younger animals. The mechanism that gives rise to PTE remains to be fully understood but may involve mis-regulation of synaptic weights through homeostatic synaptic scaling. In response to brain trauma, there is a reduction of network activity within and near the traumatized brain area. This reduction of activity triggers homeostatic up regulation of synaptic and intrinsic excitability in an attempt to recover normal levels of network activity. If trauma is severe, homeostatic scaling may overcompensate and increase synaptic weights such that the network is primed for transitions to hypersynchronized seizure states. In this new study, we tested the hypothesis that preventing homeostatic up-scaling of synaptic weights following cortical deafferentation could prevent epileptogenesis. Using a detailed biophysical model of the neocortex, we found that a sustained depolarization of the traumatized network was capable of preventing up-scaling of synaptic weights to a pathological state and thereby preventing occurrence of spontaneous recurrent seizures found in the model without depolarization mechanism. In contrast, a sustained hyperpolarization of the traumatized network resulted in increased homeostatic up-scaling, triggering a severe pathological state characterized by the occurrence of frequent spontaneous recurrent seizures. These findings from the computational model are in agreement with *in vivo* experiments in mice where cortical undercut was followed by activation of DREADDs (hM3DQ or hM4Di) to alter baseline network activity around the undercut area. Together, these results provide evidence for the role of homeostatic synaptic scaling in the development of post-traumatic epilepsy and may provide new insights into novel treatments or preventative measures for trauma-induced epilepsy.

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**Poster**

290. Epilepsy: Animal Models and Network Dynamics

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 290.11/D26
Abstract: Cortical spreading depressions (CSDs) are characterized by a wave of neuronal and glial depolarization that results in a loss of neuronal membrane resistance. Generally, this leads to neuronal silencing that lasts for minutes following a CSD as this migrates through the brain at rates of around 4 mm per minute. These events occur in the brain following injury and are associated with migraines and seizures in experimental conditions. While we have a general understanding of the mechanisms contributing to the spread of CSDs, very little is known about the underlying cellular triggers. Experimentally, CSDs can be induced through an increase in extracellular K\(^+\), electrical stimulation, and, more recently, with Channelrhodopsin to activate neurons. Importantly, the mechanism thought to be a key trigger to the induction of the CSDs in these manipulations is a pathological elevation in extracellular K\(^+\). Using the optogenetic chloride-pump Halorhodopsin, we demonstrate that loading chloride into neurons can reliably trigger a CSD in acute slices (prepared from both male and female mice expressing Halorhodopsin either under the EMX or CaMKII promoter; CSDs arose \(~33\%\) of the time following a 90 sec activation of Halorhodopsin from 60 slices). Remarkably, these CSDs start before any increase in extracellular K\(^+\), monitored using an ion-selective microelectrode. Notably, we do not see the same effect following activation of the proton-pump Archaerhodopsin, which achieves similar levels of neuronal hyperpolarisation (\(n = 12\)). In addition, Halorhodopsin can trigger CSDs in the presence of TTX, glutamate and GABA receptor blockers, and during blockade of the potassium chloride cotransporter KCC2. This leads us to hypothesise that the chloride-loading effect of Halorhodopsin is the critical trigger. These data suggest a dynamic role of chloride in the induction of CSDs, and we are actively investigating secondary consequences of this increased neuronal chloride. A complete understanding of the critical ionic and cellular triggers of a CSD could have enormous therapeutic benefits for various neurological disorders.

**Topic:** B.10. Epilepsy

**Support:** European Union’s Horizon 2020 research and innovation programme: Graphene Flagship.

**Title:** Concurrent infraslow and high frequency recordings of pathological brain activity using arrays of graphene transistors in awake mice

**Authors:** *R. C. WYKES*¹, E. MASVIDAL², T. M. SMITH¹, A. BONACCINI³, X. ILLA⁴, J. GARRIDO³, A. GUIMERA-BRUNET²;
¹UCL Inst. of Neurol., London, United Kingdom; ²Inst. de Microelectronica de Barcelona (IMB-CNM, CSIC), Barcelona, Spain; ³Catalan Inst. of Nanoscience and Nanotechnology - ICN2, Barcelona, Spain; ⁴Ctr. de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Barcelona, Spain

**Abstract:** DC shifts and infraslow activity (ISA), frequencies < 0.1 Hz, are associated with pathological brain states including cortical spreading depression (CSD) and seizures. Recording ISA with microelectrodes is hampered by current electrode materials and technology due to limitations resulting from high electrode impedance and voltage drift. Few studies have attempted to investigate CSD or the involvement of ISA in seizure initiation and propagation in awake brain. A major limiting factor has been the absence of experimental tools that allow concurrent recordings of both ISA and high frequency activity associated with seizures. Graphene transistor arrays (gSGFETs) have demonstrated full-band recording capabilities due to the direct signal coupling on the transistor and from the electrochemical inertness of graphene (Masvidal-Codina, E et al Nature Materials, 2019). We report the ability of epicortical and flexible intracortical gSGFETs arrays for studying two types of pathological brain activities, CSD and seizures, in awake head-fixed mice. To study CSD we virally transduced cortical neurons in the motor cortex (M1/M2) of adult male c57bl/6J mice with channelrodopsin-2. An epidural 16 channel transistor array (1.6 x 1.6mm) was placed over somatosensory cortex. Continuous blue light stimulation (5-20s, 12mW), administered through the skull, induced in a wave of depolarisation propagating across the cortex at a speed of ~4 mm/min. With non-invasive and reproducible properties, optogenetic induction of CSD is highly suited for evaluating pharmacological agents capable of reducing CSD propagation. Chemoconvulsants (Picrotoxin 10 mM and 4-AP 50 mM) induced epileptiform activity and seizures and electrographic recordings made using either epidural or penetrating depth gSGFETs. DC shifts were frequently observed just before, at onset, or during seizures. Around 20% of seizures were followed by a post-ictal CSD. Additionally gSGFETs recorded cortical high frequency oscillations (HFO) ~200-300Hz. These novel devices permit recording concurrently ictal baseline shifts and HFOs, markers identified as useful in localising seizure onset zones. They may also aid in preclinical investigations examining the link between post-ictal CSD and sudden unexplained death in epilepsy (SUDEP).

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Poster

290. Epilepsy: Animal Models and Network Dynamics

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 290.13/D28

Topic: B.10. Epilepsy

Title: In vivo approaches to study seizure generation and epileptogenesis in SCN1A knock-in mouse models


Abstract: Genetic epilepsies show a large variability in their clinical features, even if the same mutation segregates within a single family. One well-studied epilepsy gene is SCN1A which encodes the voltage-dependent sodium channel Nav 1.1, which is mainly expressed in GABAergic interneurons in the whole central nervous system (CNS). Mutations in this gene can cause the Dravet syndrome (DS), a pharmaco-resistant severe developmental and epileptic encephalopathy (DEE) with febrile seizures starting during early childhood, a high seizure frequency and a delayed development. Additionally, mutations in SCN1A cause the milder generalized epilepsy with febrile seizures plus (GEFS+). The most frequently detected genetic causes of these syndromes are loss-of-function (LOF) mutations in the SCN1A gene. Recent publications including from our own group, suggest that LOF is leading to epilepsy due to hypoexcitability of inhibitory neurons. Here, we tried to unravel the consequences of two known SCN1A mutations, one causing the mild GEFS+ and the other one the severe DS, on the network function in vivo using a knock-in mouse model for GEFS+ (Hedrich et al., 2014) as well as a conditional LOF knock-in mouse line expressing a DS-causing mutation in the presence of Cre. By using these mouse models, we were able to study (i) the appearance of thermally-induced generalized seizures by using ultra-flexible surface grids with high recording resolution and (ii) the critical time window and brain region for the development of specific phenotypes by injecting a Cre-carrying virus at a specific time point during development into particular brain regions for locally restricted expression of the mutation. Our results thus gain insight into the spreading of temperature-induced generalized seizures as well as underlying mechanisms during epileptogenesis. To experimentally elicit the beginning of febrile seizures in these mice, we tested different temperature ramps to heat up the surrounding of the animals. We did not find any differences in the velocity of the temperature increase, only in the maximum temperature needed to elicit the beginning of a febrile seizure. Since generalized seizures appear to start in all parts of the brain simultaneously and have no identifiable onset, we are currently working on the identification of the origin of the experimentally induced febrile seizures with the GEFS+ mouse strain. This study will help to shed light on the mechanisms underlying ictogenesis and cortical spreading depression to identify new treatment options for the affected patients.
Disclosures:  

Poster

290. Epilepsy: Animal Models and Network Dynamics

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 290.14/D29

Topic: B.10. Epilepsy

Support: HHMI Gilliam Fellowship for Advanced Studies
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Title: Seizure genesis broad spectrum dynamics in rodent epilepsy models

Authors: *D. E. EHRENS¹, F. ASSAF², N. J. COWAN³, S. V. SARMA⁴, Y. SCHILLER⁵;
  ¹Johns Hopkins Sch. of Med., Baltimore, MD; ²Fac. of Med., Technion Israel Inst. of Technol.,
  Haifa, Israel; ³Dept. of Mechanical Engin., ⁴Biomed. Engin., Johns Hopkins Univ., Baltimore,
  MD; ⁵Technion Med. Sch., Haifa, Israel

Abstract: A third of epilepsy patients are pharmacologically intractable and must consider
invasive alternatives such as resective surgery and neural electrical stimulation. Current
stimulation strategies attempt to suppress seizures once they have initiated, but none aim to
prevent the seizure from initiating altogether. For this, reliable detection of a neurophysiological
state leading to seizure initiation (preictal state) is needed in order to apply the proper electrical
stimulation waveform and drive the epileptic network away from entering a seizure state. Using
two different electrographical seizure models in rodents (4-AP, Kainic Acid), LFP and MUA
activity was recorded to characterize the progression in neural activity leading to seizure onset in
the spectral domain. Spectral analysis was performed on a broad power spectrum (4-1000 Hz)
divided into 15 different bands. Showing increased activity in frequencies above 500 Hz before
seizure onset. Singular values from correlation matrices were computed to track the degree of
correlation as the seizure onset approached. Results indicate a progressively increasing
correlation between higher frequency bands (>500 Hz) preceding seizure initiation that drops at
seizure onset. This results demonstrate that correlation in the high frequencies can be used by
closed-loop systems for detection of a preictal state and prevention of seizure genesis.

Disclosures:  
Title: All or nothing change in network excitability immediately before the transition into an ictal event

Authors: R. GRAHAM, L. ALBERIO, R. PARRISH, *A. J. TREVELYAN; Newcastle Univ., Newcastle upon Tyne, United Kingdom

Abstract: Understanding the nature of epileptic state transitions remains a major goal for epilepsy research. Simple in vitro models offer unique experimental opportunities for exploring these issues. Bathing brain slices in 0Mg$^{2+}$ artificial cerebrospinal fluid (aCSF) induces an evolving pattern of epileptiform activity, including short interictal bursts of activity, and sustained discharges that replicate many features of tonic-clonic seizures in vivo. Removing Mg$^{2+}$ ions from the bath provides a rapid enhancement of glutamatergic excitatory synaptic effects, and yet the evolution of epileptic activity occurs over a far slower time frame. These network changes therefore, cannot be ascribed simply to the NMDA effect, but rather, involve dynamic reorganisation of the network. We used an optogenetic approach, using cell-class specific expression of Channelrhodopsin to selectively stimulate one of three different subclasses of neurons, namely pyramidal cells, and parvalbumin-positive (PV+) and somatostatin-positive (SOM+) interneurons. We then monitored progressive changes in the postsynaptic effect downstream of the activated neuronal population, and related that to the network excitability. Brief optogenetic activation (10ms flashes, delivered at <0.1Hz, close to the recording location through an optic fibre connected to an LED. The postsynaptic effects of pyramidal activation was monitored using parallel extracellular electrodes in layers 2/3 of neocortex, and stratum radiatum of CA1 in hippocampus. Postsynaptic interneuronal efficacy was monitored by voltage clamp recordings of pyramidal cells downstream of activated interneurons. We found clear changes in all three synaptic pathways associated with the evolving activity. Critically, we demonstrate a sudden, irreversible transformation of the excitatory post-synaptic potential minutes before the onset of an ictal event in the local tissue. This provides a key insight into a sudden, all-or-nothing transformation in network excitability, that is a key stage in ictogenesis.

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Corticocortical evoked potentials reveal epileptogenic network and locate epileptogenic zone

**Authors:** *K. SUN*\(^1\), L. WANG\(^2\), W. WANG\(^1\);
\(^1\)Sch. of Syst. Science, Beijing Normal Univ., Beijing, China; \(^2\)Inst. of Psychology, Chinese Acad. of Sci., Beijing, China

**Abstract:** It is gradually recognized that epilepsy is a neural network disease involving both epileptogenic brain areas and normal brain areas. Despite many approaches based on time series analysis of seizure signals, the network structure and mechanisms underlying the process of seizure propagation is elusive. Here study epileptogenic networks based on cortico-cortical evoked potentials (CCEP) that capture direct electrical responses among Intracranial electrodes and map an incentive-response neural network. Our purpose is to reveal the relationship between CCEP and epileptogenic propagation networks, and explore the potential function of CCEP on locating seizure zone. Specifically, we recorded seizure signals and construct CCEP networks of 12 patients by stereotactic-EEG. We found (i) when exclusively stimulating epileptogenic zone, the responses of epileptogenic zone have significantly higher amplitudes and root-mean-square (RMS) than those in outside zones, i.e., propagation zone and non-involved zone; (ii) when stimulating all electrodes in different zones, the overall responses of epileptogenic zone have significantly higher amplitudes and RMS than the other two zones. The finding (i) and (ii) can help to recover epileptogenic networks and locate epileptogenic zone, respectively. Our findings have potential to support better diagnosis and treatment of epilepsy.

**Disclosures:** K. Sun: None. L. Wang: None. W. Wang: None.
Title: Functional mapping of frontal motor seizures

Authors: *A. BRODOVSKAYA*, S. SHIONO, J. WILLIAMSON, J. KAPUR;  
¹Neurol., Univ. of Virginia, Charlottesville, VA; ²Dept Neurol., Univ. Virginia Hlth. Sci.Ctr., Charlottesville, VA

Abstract: Motor seizures are dangerous because they increase the risk of injury and sudden unexpected death in epilepsy (SUDEP). However, circuits that underlie these seizures have not been fully described.

We used TRAP (Targeted Recombination in Active Populations) technology that utilizes the promoter region of immediate early genes (IEGs) to drive the expression of tamoxifen-dependent CreER², so that only neurons that express IEGs are labeled with tdTomato. To initiate frontal motor seizures, we implanted 1.7 mg of cobalt wire in the secondary motor cortex of TRAP mice. For track tracing, we injected AAV9-GFP virus two weeks prior to Co insertion. For temporal resolution of the seizure spread, we recorded local field potentials (LFP) with microelectrodes.

We hypothesized that seizure spread follows the anatomical connections from the focus. Seizures spread rapidly to the contralateral premotor cortex, faster than ipsilateral somatosensory cortex (5 sec delay, 0-10.5 sec, n=8, p<0.001). Callosal pyramidal neurons were activated during seizures as demonstrated by colocalization of GFP and tdTomato in the corpus callosum and layer 2/3 pyramidal neurons. To characterize spread from motor to somatosensory cortex, we quantified tdTomato expression using cortical layer markers (n=6). Focal seizures consistently engaged layer 2/3 of posterior somatosensory cortex (100%, 50-100%, p=0.002) but not layer 5/6 (71.4%, 0-85.7%, p=0.002). Posterior layer 5/6 was engaged later than 2/3 during generalized seizures (5 sec delay, 0-10.5 sec, n=8, p<0.001). Premotor onset seizures also traveled through striatum, globus pallidus, and substantia nigra reticulata as indicated by the tdTomato expression and LFP recordings. Electrophysiological recordings also indicate that seizure spread to the contralateral cortex was faster than to contralateral thalamus.

Our results delineate a more complex neuronal network activated by motor seizures than previously proposed thalamocortical circuit.

Disclosures: A. Brodovskaya: None. S. Shiono: None. J. Williamson: None. J. Kapur: None.
Title: Evoked assay of neural circuit function with microelectrode array (MEA) technology and in vitro cell culture models

Authors: *D. C. MILLARD, A. M. NICOLINI, C. A. ARROWOOD, H. B. HAYES, S. A. CHVATAL;
Axion Biosystems, Atlanta, GA

Abstract: Microelectrode arrays (MEAs) monitor and manipulate cultured cell activity in vitro, providing insight into neuronal network interactions to inform “disease-in-a-dish” models, stem cell characterization, toxicology screening, and drug safety and development. Recently-developed multiwell MEA systems enable high-throughput assessment of functional endpoints at greatly reduced time and cost. Activity, Synchrony, and Oscillation endpoints quantify the functional behavior of neurons, synapses, and networks. However, spontaneous neural network activity may vary across wells within a plate or across model systems. Here, we describe an evoked assay of neural circuit function, providing complementary information to spontaneous endpoints, using pairs of stimuli with varying interstimulus interval via electrical or optogenetic stimulation. The paired stimulation quantified the balance of excitation and inhibition in the network, by comparing the response to a second stimulus relative to the first as a function of the interstimulus interval. Measures of evoked neural response for single and paired stimulation protocols were used to quantify changes in activity induced by pharmacological manipulation with 12 compounds, including proconvulsants, anti-epileptic drugs, and negative controls. These findings demonstrate the potential of evoked assays to characterize neural circuit function in vitro for applications in safety pharmacology, toxicology, stem cell model development and validation, and “disease-in-a-dish” models.


Poster

290. Epilepsy: Animal Models and Network Dynamics

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 290.19/D34

Topic: B.10. Epilepsy

Support:  DFG Grant KO-4877/2-1
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Title: Human cortical brain slice cultures derived from spare access tissue of epilepsy surgery: Implementation of a model system to study pathophysiological mechanisms of human disease

Authors: *T. V. WUTTKE*¹, N. SCHWARZ², B. UYSAL², J. BAHR², N. LAYER², H. LÖFFLER², K. STANAITIS², Y. G. WEBER², U. B. HEDRICH², J. HONEGGER³, A. SKODRAS⁴, H. KOCH²;
¹Dept. of Neurosurg. and Dept. of Neurol. and Epileptology, ²Dept. of Neurol. and Epileptology, Hertie Inst. for Clin. Brain Res., Tübingen, Germany; ³Dept. of Neurosurg., Univ. Hosp. Tübingen, Tübingen, Germany; ⁴Cell Biol. of Neurolog. Dis., German Ctr. for Neurodegenerative Dis. (DZNE), Tübingen, Germany

Abstract: Investigation of pathophysiological mechanisms of human CNS disease, such as epilepsy or neurodegenerative disorders, mostly relies on studies based on human post mortem or biopsy/surgery tissue and on studies using animal models, cell culture or heterologous expression systems. However, it frequently remains elusive whether such data can be translated to the human brain. Development of strategies relying on live human CNS tissue could offer a promising step toward bridging this gap. We present the development of a model system based on human organotypic cortical slice cultures derived from spare access tissue of epilepsy surgery. Extensive studies were performed to quantify the stability of function and morphology of human pyramidal neurons within human cortical slice cultures. Proof-of-concept experiments involving viral transduction demonstrate the feasibility of genetic manipulation of human neurons within cultures. Our data reveal sustained cortical neuronal survival up to several weeks, both on single neuron and on network level, including maintenance of action potential generation, synaptic connectivity and presence of tonic and phasic network activity. Biocytin fillings and virus driven overexpression of GFP were applied toward high-resolution 3D morphological assessment of human pyramidal neurons demonstrating robust preservation of characteristic complex neuronal cytoarchitecture. Comparative analysis of both approaches validates genetic labeling as an efficient tool toward detailed morphological studies of relatively large quantities of adult human neurons per tissue sample, providing a valuable alternative to classic single cell biocytin fillings. Combinations of the described model system with state-of-the-art technology including viral transduction of human neurons, high-resolution confocal and two-photon microscopy together with electrophysiological approaches are applied toward the goal of validating data from non human model systems and to translate such data to the human brain. The focus of interest of ongoing and future studies includes investigation of plasticity mechanisms of human neurons, the impact of epilepsy-causing mutations on the excitability of human neurons and neuronal networks as well as dissection of human cortical circuitry.

290. Epilepsy: Animal Models and Network Dynamics

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 290.20/D35

Topic: B.10. Epilepsy

Support: Epilepsy Canada
Medicine by Design

Title: Characterization of WWOX-deficient cerebral organoids treated with 4-aminopyridine

Authors: *A. SALEEM1,2, D. STEINBERG3, M. AQUILINO1,2, S. MYLVAGANAM2, S. REPUDI3, J. HANNA4, R. AQUEILAN3, P. CARLEN1,2;


Abstract: Objective Approximately 1% of the world population suffers from epilepsy, a neurological disorder characterized by repeated seizures. The degree and symptoms of the disease differ significantly among patients, resulting in the need for further characterization of the specific patient-centered epileptic pathophysiology. Recent advances in tissue engineering enable the development and use of genetically modified cerebral organoids as a unique platform for investigating these patient-specific analogues. Point mutations and deletions in the WW domain-containing oxidoreductase (WWOX) gene result in one such analogue, associated with severe neurological disorders and epilepsy in both rodents and humans. By exploring the phenotypic electrophysiological changes in WWOX-deficient organoids, this research sheds light onto the WWOX-related epileptic encephalopathy (WOREE) and other epilepsy disorders.

Methods Cerebral organoids were generated using both hEPC and iPSC lines, matured to 70-80 days, and prepared for electrophysiological recordings. Organoids were sliced into 300-micron thick slices and incubated in artificial cerebrospinal fluid at 37 degrees. A recording electrode was inserted into various layers of the organoid structure to record local field potentials (LFPs). A stimulating electrode was used to assess excitatory responses, while 4-aminopyridine (4-AP) was added to the incubating media to enhance spontaneous-epileptiform currents.

Results Early results from both WWOX-KO (generated by CRISPR/CAS9) and control organoids show evoked and spontaneous extracellular activity, with WWOX-KO being more active than control. Both WWOX-KO and control organoids showed increased slow wave activity upon application of 4-AP. Several metrics of excitability, including spectral power and frequency of seizure-like events were evaluated and contrasted between all groups of organoids, highlighting WWOX-dependent changes in both spontaneous and chemically-evoked
Significance Acute recordings of LFPs from cerebral organoids validate their electrical connectivity and viability for assaying their physiological function. Observed differences in the electrophysiological activity of organoids with WWOX deletions present a unique opportunity to study the developmental effects of WWOX on epileptogenesis. We are now developing an organoid-based high-throughput drug screening platform for assessing various putative treatments of epilepsy syndromes in personalized, patient-derived cerebral tissue to target intractable epilepsy.

This work is supported by Epilepsy Canada and Medicine by Design UofT.


Poster

291. Aging: Molecular Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 291.01/D36

Topic: C.01. Brain Wellness and Aging

Support: Ono Pharmaceutical Co., Ltd
Stanley Center for Psychiatric Research
Klarman Cell Observatory

Title: Single-cell transcriptomic profiling of the aging mouse brain


Abstract: The mammalian brain is complex, with multiple cell types performing a variety of diverse functions, but exactly how the brain is affected with aging remains largely unknown. Here we performed a single-cell transcriptomic analysis of young and old mouse brains. We provide a comprehensive dataset of aging-related genes, pathways and ligand-receptor interactions in nearly all brain cell types. Our analysis identified gene signatures that vary in a coordinated manner across cell types and gene sets that are regulated in a cell type specific manner, even at times in opposite directions. Thus, our data reveal that aging, rather than
inducing a universal program, drives a distinct transcriptional course in each cell population. These data provide an important resource for the aging community and highlight key molecular processes, including ribosome biogenesis, underlying aging. We believe that this large-scale dataset will facilitate additional discoveries directed towards understanding and modifying the aging process.


Poster

291. Aging: Molecular Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 291.02/D37

Topic: C.01. Brain Wellness and Aging

Support: NIA/NIH Grant 1K99AG055683
       NIA/NIH Grant AG019719

Title: Using novel mouse models to study the role of epigenetic alterations in age-related cognitive decline

Authors: *J. M. ROSS1,2,3, G. COPPOTELLI1,3, E. POTTS1, J. AMORIM1,4, L. E. HARMON1, E. HILLSTEDT1,2, P. GRIFFIN1, A. KANE1,5, D. SINCLAIR1;
1Genet., Harvard Med. Sch., Boston, MA; 2Neurosci., Karolinska Institutet, Stockholm, Sweden; 3Ryan Inst. for Neuroscience, Col. of Pharmacy, Dept of Biomed. and Pharmaceut. Sci., Univ. of Rhode Island, Kingston, RI; 4IIUC – Inst. of Interdisciplinary Res., Univ. of Coimbra, Coimbra, Portugal; 5Charles Perkins Ctr., The Univ. of Sydney, Sydney, Australia

Abstract: Aging of the brain is a cause of cognitive decline and the major risk factor for neurodegenerative diseases, such as Alzheimer’s disease. Epigenetic noise has been highly implicated in both aging per se and cognitive impairment. In order to delineate the role of epigenetics in brain aging, we are investigating three mouse models, ICE, NICE, and NASA, to evaluate age-related cognitive decline due to different DNA damage responses, as compared to normally aged C57/B6J wild-type mice. The novel ICE mouse has whole-body inducible changes in the epigenome, whereas the NICE mouse has neuronal-specific inducible changes in the epigenome, with both ICE and NICE models using the homing endonuclease I-Ppol to induce tissue- and time-specific non-mutagenic, site-specific double-strand DNA breaks. The NASA mouse has been irradiated with 30 cGy over a sequential exposure to 6 different beams to
mimic the deep-space cosmic radiation damage that astronauts are exposed to during spaceflight. Using a battery of physiological and cognitive tests, our results thus far demonstrate that 15 month-old ICE and NICE mice show cognitive impairments similar to that found in 24 month-old C57 mice. Specifically, Barnes maze results demonstrate that ICE and NICE mice underperform with regards to memory recall, compared to age-matched Cre controls, and more closely resemble memory deficits in aged C57 animals. In the elevated plus maze, both ICE and NICE mice spent more time in and actively sought out the open arms suggesting a decrease in thigmotaxis and anxiety. Open-field testing in ICE and NICE mice showed an increase in time spent in the center of the arena, a finding confirmed also in aged C57 mice. Interestingly, although no major impairments in spatial working memory were detected with the Y-maze in ICE mice, NICE mice did present with deficits similar to that found in aged C57 mice. Additionally, using frailty index testing, we found that ICE, but not NICE mice, present with advanced aging phenotypes more akin to those found in aged C57 mice. Immunohistochemical studies thus far indicate a change in gliosis with an increase in activated microglia, but no increase in neurodegeneration in ICE and NICE mice. Preliminary studies using the NASA mice suggest an increase in aging phenotypes as well as changes in thigmotaxic behavior that mirror ICE, NICE, and aged C57 mice. Ongoing studies aim to elucidate how different types of DNA damage can elicit chromatin remodeling and neuronal functional changes, which may identify treatment strategies for age-related diseases and disorders of the brain.


Poster

291. Aging: Molecular Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:/Poster #: 291.03/D38

Topic: C.01. Brain Wellness and Aging

Support: Paul G. Allen Frontiers Group

Title: A novel pan-mammalian epigenetic clock to study disease-accelerated brain aging

Authors: N. Wang1, A. C. Yang2, A. T. Lu2, M. Stricos1, M. J. Thompson3, M. Pellegrini3, A. Sperlea4, J. Ernst4, P. Langfelder1, *X. Yang1, S. Horvath2; 1Ctr. for Neurobehavioral Genetics, Semel Inst. of Neurosci. and Human Behavior, 2Dept. of Human Genetics, David Geffen Sch. of Med., 3Dept. Mol, Cell & Devmt Biol, 4Dept. of Biol. Chem., UCLA, Los Angeles, CA

Abstract: Human DNA-methylation data have been used to develop highly accurate biomarkers of aging ("epigenetic clocks"). Recent studies demonstrate that epigenetic clocks for mice (Mus
Musculus) can be developed. Epigenetic clock studies in mice have revealed that epigenetic aging can be slowed by gold standard anti-aging interventions such as calorie restriction and growth hormone receptor knock-outs. Here we present a novel epigenetic clock based on over 600 mouse tissue samples from across the entire lifespan (3 weeks old to 30 months old). Methylation levels were assessed using a highly robust Infinium array platform: 38k probes from the HorvathMammalMethyl40 chip. The resulting pan tissue mouse clock stands out in terms of its high reproducibility across different data sets and mouse strains. Moreover, we begin to correlate the DNA methylation changes to transcriptomic changes in a specific brain region (i.e. the striatum). Huntington's disease (HD) has been found to be associated with epigenetic age acceleration in human brain samples. It is not yet known whether epigenetic age acceleration can also be observed in tissues from mouse models of Huntington's disease. We apply our new mammalian epigenetic clock to address the question whether HD accelerates epigenetic aging in different tissues (striatum, cortex, liver, blood, cerebellum) of the Q175 knock-in model of HD. We compared age- and sex-matched HD mice to wildtype controls and observed that HD is associated with significant age acceleration in mouse cerebellum (p=0.002), striatum (p=0.016), and liver (p=0.01). In summary, our study demonstrates that Huntington disease is associated with accelerated epigenetic age acceleration in mice.


Poster

291. Aging: Molecular Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 291.04/D39

Topic: C.01. Brain Wellness and Aging

Support: NIH R01MH11197101A1 NIH R00DA037028 NIH R21MH11197101A1 NIH R01MH102377 NIH K24MH110807 NIH R03MH110745 NIH R01MH108574

Title: Neuroepigenetic alterations in white matter in the living human brain across the lifespan

Authors: T. M. GILBERT1, N. R. ZURCHER1, *M. C. CATANESE1, C.-E. J. TSENG1, M. A. DI BIASE2, A. E. LYALL2,3, B. G. HIGHTOWER1, A. J. PARMAR1, A. BHANOT1, C. WU1, M. L. HIBERT1, M. KIM1, U. MAHMOOD1, S. M. STUFFLEBEAM1, F. A. SCHROEDER1, C.
Abstract: In the past century, life expectancy has increased dramatically, and we do not yet understand how this may impact the brain, including at the level of gene expression. Aging is the main risk factor for neurodegenerative disease, yet the molecular underpinnings of resiliency and decline are unknown. Epigenetic mechanisms may play important roles in the aging brain. Histone deacetylases (HDACs) are epigenetic enzymes that regulate gene expression via chromatin modification. Preclinical and postmortem studies have demonstrated HDAC-related alterations with age in brain regions relevant for cognition and neurodegeneration, however, until now, nothing has been known about HDAC levels in the living human brain across the lifespan. Using $[^{11}C]$Martinostat, for positron emission tomography (PET) to measure HDACs in the living human brain, we observed an increase in $[^{11}C]$Martinostat uptake in healthy individuals with age. The increase localized to white matter ($n=41$, age 18-79; 20 females, 21 males; voxel-wise analysis correlating standard uptake value normalized to whole brain mean with age, controlling for sex and brain volume; $P_{FWE} < 0.05$; Gilbert and Zurcher et al., in submission). $[^{11}C]$Martinostat uptake negatively correlated with white matter microstructure measured by general fractional anisotropy, derived from simultaneously acquired diffusion imaging (Spearman’s $r = -0.37$, $P = 0.018$). HDAC expression also localized to specific white matter tracts negatively correlated with social cognition in a subset of individuals assessed with the Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT) ($n=23$, age:23-79,12 females, 11 males, $P_{cluster} < 0.05$) Using imaging findings to guide biochemical analyses, we identified increases in HDAC1 and HDAC2 protein levels as drivers of the increase in $[^{11}C]$Martinostat uptake in older compared to younger donor periventricular white matter ($n=9$ each group, mean ages 85±8 and 18±1 years; sex matched; three replicates; two-tailed unpaired $t$-test, false discovery rate (FDR) corrected for multiple comparisons; HDAC1: $P = 0.022$ and $P_{FDR} = 0.031$, HDAC2: $P = 0.018$ and $P_{FDR} = 0.29$). HDACs 1 and 2 are critical for oligodendrocyte differentiation and in rodents become dysregulated with age, resulting in failed re-myelination. We initiated a program to identify cell-types related to increased HDACs and will ultimately measure age-associated transcriptional change. Our work suggests that HDACs may become altered across the lifespan in relation to decreased white matter structural integrity. These data carry implications for neurodegenerative disease and will help further stratify HDACs as therapeutic targets.

Recurrent herpes simplex virus type 1 infection modulates aging-related epigenetic markers in mouse brains

Authors: *G. NAPOLETANI*¹, M. E. MARCOCCI¹, V. PROTTO², A. T. PALAMARA¹,³, G. DE CHIARA²;
¹Dept. of Publ. Hlth. and Infectious Dis. Lab. affiliated to Inst. Pasteur Italia, Sapienza Univ. of Rome, Rome, Italy; ²Inst. of Translational Pharmacol., Natl. Res. Council, Roma, Italy; ³San Raffaele Pisana, IRCCS, Telematic Univ., Rome, Italy

Abstract: Aging is one of the major risk factors for Alzheimer’s disease (AD). At the brain level, this process is characterized by a slow, time-dependent change of multiple physiological functions and cognitive decline. Genome instability, together with changes in gene expression, driven by epigenetic imbalance, are the main features of neuronal senescence. Recent data from our group showed that recurrent Herpes Simplex Virus 1 (HSV-1) infection in mice induces an accumulation of AD hallmarks, including amyloid-β and tau hyperphosphorylation, paralleled by irreversible cognitive deficits. In this scenario, we hypothesized that recurrent HSV-1 infection may also accelerate the normal brain aging, by affecting epigenetic mechanisms. To verify this hypothesis, we evaluated the levels of specific aging hallmarks, such as histone 3 deacetylation (i.e., at H3K56), in mouse experimental models of acute and recurrent virus infection. We also analysed the expression of two key epigenetic regulators, such as Sin3/HDAC1 complex, and the histone chaperone HIRA, both involved also in the regulation of HSV-1 life-cycle and viral-host interaction. To these aims, entorhinal cortex homogenates from HSV-1- and Mock-infected BALB/c female mice were analysed in western blot for H3K56 acetylation, and HIRA and
Sin3/HDAC1 expression. A group of mice was analysed 4 days post primary infection (dpi), whereas the others were subjected to multiple thermal stress (TSs) every 6 weeks, to induce repeated virus reactivations. 5 mice for group were sacrificed after the 3rd and the 7th TS. A group of 4 mice were sacrificed just before the 7th TS (during latent infection). The virus presence in the brain was assessed by molecular analysis of viral gene/protein expression as well as by virological methods. We found that TS-induced virus replication caused in mouse brain a significant decrease in H3K56 acetylation (p<0.05 post-3TS; p<0.01 post-7TS) as compared to those observed in Mock-infected mice, suggesting that the infection impairs the histone acetylation process. Accordingly, we found a significant increase in Sin3/HDAC1 protein expression levels with respect to matched Mock-infected mice, starting from 4 dpi (p<0.05). A similar increase was observed following 7 TSs (p<0.05). Notably, these effects, together with a significant increase in HIRA protein levels, were found also in those mice sacrificed before the 7th TS, indicating that epigenetic imbalance may persist over time. Overall these data strongly suggest that recurrent HSV-1 infection enhances cellular epigenetic aging and may widely affect the epigenetic landscape in mice.

Disclosures: G. Napoletani: None. M.E. Marcocci: None. V. Protto: None. A.T. Palamara: None. G. De Chiara: None.

Poster

291. Aging: Molecular Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 291.06/D41

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant F32NS106812
R01 AG061787

Title: Exceptionally long-lived proteins as pillars of mitochondrial architecture in mammalian brains

Authors: *E. BOMBA-WARCZAK, J. N. SAVAS;
Northwestern Univ., Chicago, IL

Abstract: Aging is a major risk factor for a host of diseases, including cancer, cardiovascular disorders, and neurodegenerative diseases. Currently available data suggests that an accumulation of mitochondrial damage together with an age-dependent decline in protein degradation activities are likely to play a key role in aging. In fact, mitochondrial dysfunction represents a point of convergence for a number of adult-onset neurological disorders. Yet, the precise mechanism behind mitochondrial contribution to the pathology of these conditions remains unknown. In our previous research, we identified proteins with exceptionally long life
spans that we termed extremely long-lived proteins, or ELLPs. Due to their persistence, ELLPs likely accumulate damage and are thus a potential point of vulnerability in an organism’s fight against aging. This is especially vital for post-mitotic cells, such as neurons, which cannot dilute the macromolecular damage through cell division. Interestingly, we found that a small fraction of the brain mitochondrial proteome lasts the entire lifetime of a mouse. We propose to investigate the role that these newly identified mitochondrial ELLPs (mito-ELLPs) play in the processes of aging in the brain. We will characterize the mitochondrial long-lived proteome by integrating metabolic stable isotope pulse-chase labeling, advanced proteomic analysis, live cell fluorescent imaging, and in vivo mouse aging experiments. This research provides a rare opportunity to obtain a previously inaccessible understanding of mitochondrial contribution to the process of aging and the mechanisms causing age-related neurological disorders. Insights from this research could lead to novel targets for potential therapeutic interventions for a myriad of age-related disorders.

Disclosures:  E. Bomba-Warczak: None. J.N. Savas: None.

Poster

291. Aging: Molecular Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: 291.07/D42

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant P01 AG017617

Title: Extracellular vesicles in the adult murine brain: When heterogeneity reveals a different neuropathological potential

Authors: *P. D'ACUNZO1,2, T. HARGASH1, C. N. GOULBOURNE1, M. PAWLIK1, R. PEREZ-GONZALEZ1,2,5, E. LEVY1,2,3,4, 1Ctr. for Dementia Res., Nathan S. Kline Inst., Orangeburg, NY; 2Dept. of Psychiatry, 3Dept. of Biochem. & Mol. Pharmacol., 4Neurosci. Inst., New York Univ. Langone Hlth., New York, NY; 5Dept. of Neurol., Hosp. de la Santa Creu i Sant Pau, Ctr. for Networked Biomed. Res. on Neurodegenerative Dis. (CIBERNED), Barcelona, Spain

Abstract: Extracellular vesicles (EVs) are nanoscale-sized vesicles that are released by cells and are thought to be key factors in intercellular communication. Historically, two main EVs subtypes have been characterized: microvesicles, which bud directly from the plasma membrane, and exosomes, which have an endosomal origin and are released upon fusion of the late endosomes/multivesicular bodies with the plasma membrane. More recently, it was postulated that different subsets of microvesicles and exosomes might exist. We hypothesized that these subpopulations carry different cargos and likely have specialized functions, giving new
perspective into cell-to-cell communication both in physiology and pathology of the brain. However, the lack of valuable methods and markers to diversify these subtypes impinged on their characterization. Herein, we describe a new high-resolution, density-based method to fractionate the small EVs isolated from 12-month-old murine brains. Combining cryogenic electron microscopy, morphometric properties, and biochemical markers, we provide an extensive characterization of eight different EVs fractions. While microvesicles appear electron-lucent, bare polarized membrane micro-domains and are preferentially restricted to low-density fractions, exosomes show electron-dense granularity and are mostly found in average-to-high density sections. We demonstrate that at least two different exosome subclasses are present in the murine brain and that they differ in their content of the full-length amyloid precursor protein and of its neurotoxic metabolites implicated in Alzheimer’s disease pathology. These data suggest the existence of brain exosomal-subtypes with different neuropathological roles. We also demonstrate for the first time the presence of a previously unidentified high-density EVs subset bearing no known microvesicles or exosomes markers. In summary, here we show an extensive characterization of the murine brain EVs subpopulations and provide evidence that different exosome subgroups have specialized roles in vivo. We also show that cryogenic electron microscopy is a valuable (yet not fully explored) method to shed light on EVs heterogeneity.


Poster

291. Aging: Molecular Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 291.08/D43

Topic: C.01. Brain Wellness and Aging

Support: This research was supported entirely by the Intramural Research Program of the NIH, National Institute on Aging.

Title: Arc content in blood circulating extracellular vesicles as a biomarker of memory-related plasticity in a rat model of aging

Authors: P. MORENO-CASTILLA, E. L. RIVERA, L. M. WANGLER, J. M. LONG, *P. R. RAPP;
Neurocognitive Aging Section, NIH, Natl. Inst. on Aging, Baltimore, MD

Abstract: The activity-regulated cytoskeleton-associated protein (Arc) is an essential coordinator of synaptic plasticity important for cognition that has a highly dynamic expression in
the brain. Arc expression in brain peaks rapidly at sites of local synaptic activity and is cleared in a short time course. Interestingly, we previously found that aged rats with spatial memory impairment have increased basal Arc protein levels in hippocampus and fail to induce Arc expression following behavioral training. These findings point to dysregulation of Arc protein homeostasis as a potentially important contributor to age-related cognitive decline. Other studies have revealed that Arc protein and mRNA are intercellularly transferred by self-assembling into capsid-like structures that are released from neurons in extracellular vesicles (EVs). Brain-produced EVs readily cross the blood brain barrier, and accordingly we hypothesized that blood circulating Arc-EVs might be coupled to the Arc synthesis and turnover in active neuronal circuits and thus predict memory dysfunction in aging. To test this hypothesis, we used an established model of cognitive aging in which aged Long-Evans rats are categorized as impaired (AI) or unimpaired (AU) relative to young, based on their spatial learning and memory capacities. Plasma EVs were isolated under basal conditions and after memory-induced activation of relevant neural networks using a task in which animals distinguished a novel odor from a familiar one, after a short (30 min) or a long delay (24 h). Young rats with good memory showed increased levels of Arc-EVs after the 90 min long-term memory test compared to baseline, but not after the short-term memory test. We also found increased basal levels of Arc-EVs in aged rats with memory impairment, together with a loss of the memory-dependent increase in Arc-EVs observed in young rats. Our results suggest that the activation of neural circuits can be detected in plasma Arc-EVs and serve as a peripheral biomarker of memory dysfunction in aging.


Poster

291. Aging: Molecular Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 291.09/D44

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant NS094171
        NIH Grant NS105638

Title: Age-dependent expression of neurodegeneration-causing antimicrobial peptides

Authors: *L. E¹, N. PUJOL³, D. YAN²;
         ¹Duke Univ., Durham, NC; ²Duke Univ., Chapel Hill, NC; ³Ctr. d’Immunologie de Marseille-Luminy, CIML, Aix Marseille Univ., Marseille, France
Abstract: Antimicrobial peptides (AMPs) are part of the innate immune defense mechanism in response to infections. Recent studies have suggested a link between AMPs and neurodegenerative diseases, such as Alzheimer’s disease. Previously, using *C. elegans* as a model we revealed a causative role of an epidermally-expressed AMP, neuropeptide-like protein 29 (NLP-29), in triggering aging-associated neurodegeneration, by binding its specific receptor on neighboring neurons to transduce autophagy-mediated degeneration signals. We also discover that NLP-29 expression level significantly increases with age in wild-type animals. Finding the mechanisms underlying this age-dependent increase will therefore be crucial in identifying the root causes of aging-associated neurodegeneration mediated by AMPs. Here, we show that in *C. elegans* the age-dependent expression of NLP-29/AMP requires the DCAR-1/GPCR - TIR-1/SARM - PMK-1/p38 MAPK innate immune signaling, partially sharing the same pathway in response to infections. In addition, NIPI-3, whose mammalian homolog Tribbles has been implicated in Parkinson’s disease, plays a critical role in the upstream regulation of TIR-1 - PMK-1 signaling and subsequent production of NLP-29 during aging. Epidermis-specific overexpression of NIPI-3 can induce an early-onset degeneration of epidermis-neighboring neurons by overproducing NL-29. Interestingly, NIPI-3 expression level also significantly increases with age in wild-type animals. Surprisingly, the DAF-2/IGF-1R - DAF-16/FOXO1/2/4 classic aging signaling is not involved in regulating the age-dependent expression of NLP-29 or NIPI-3. DAPK-1/death-associated protein kinase 1 is a negative regulator of NLP-29 expression via interaction with TIR-1 - PMK-1 signaling. We find that in wild-type animals the ratio of NIPI-3 and DAPK-1 expression levels strongly and positively correlates with NLP-29 expression level during aging. Moreover, epidermal overexpression of DAPK-1 provides neuroprotective effects by substantially delaying the onset of aging-associated neurodegeneration. Our data suggest that the aging-associated imbalance of non-neuronal immune signaling networks functions as a trigger for AMP-mediated neurodegeneration, and provide exciting insights for the root causes of aging-related neurodegenerative diseases.

Disclosures: L. E: None. N. Pujol: None. D. Yan: None.

Poster

291. Aging: Molecular Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 291.10/D45

Topic: C.01. Brain Wellness and Aging

Title: Contribution of chaperone-mediated autophagy malfunctioning to aging and neurodegeneration

Authors: *M. BOURDENX¹, J. A. RODRIGUEZ¹, H. R. MONDAY², I. TASSET¹, A. DIAZ¹, P. E. CASTILLO³, A. CUERVO¹;
Abstract: Neuronal protein quality control decreases with age increasing the risk of neurodegenerative diseases. The contribution of different types of autophagy in the clearance of toxic neurodegeneration-related proteins is well documented. We are interested in the dual interplay of a selective form of autophagy, chaperone-mediated autophagy (CMA), with neurodegeneration-related proteins. Previous results have shown a direct toxic effect of these proteins on CMA. In addition, during physiological aging, lysosomal levels of LAMP-2A, the rate limiting component for CMA, decrease, leading to a slower activity of the pathway. However, the contribution that reduced neuronal CMA with age may have in the progression of neurodegenerative diseases remains unknown. In this work we have investigated: (i) first, if LAMP-2A knockout in neurons induces changes in neuronal proteostasis similar to those observed in aging, thus accelerating disease progression in a neurodegeneration disease context; (ii) second, if maintaining LAMP-2A levels throughout life-span slows down the progression of age-related neuronal impairments. Using a combination of mouse lines with knock-out or knock-in of LAMP-2A specifically in neurons, we have found that blockage of neuronal CMA induces behavioral impairments, alterations of neuronal physiology, changes in the neuronal proteome and protein aggregation reminiscent of brain aging. Conversely, maintaining LAMP-2A levels throughout life reduces age-associated behavioral impairments and protects neurons from age-related dysfunction. We are currently investigating the molecular mechanisms underlying the deleterious effect of CMA deficiency as well as the beneficial effect upon restoration of neuronal CMA activity in old mice.


Poster

291. Aging: Molecular Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 291.11/D46

Topic: C.01. Brain Wellness and Aging

Support: NIH T32 T32GM008688
NIH T32 T32AG000213
AFAR Young Investigator Award
DP2 NIH New Innovator Award
Sloan Foundation Fellowship

Title: Vimentin protects neural stem cells from a loss of proteostasis
Abstract: Maintaining proteostasis, the balance between the synthesis and degradation of protein, is essential for neural stem cell (NSC) function and is lost with aging, leading to stem cell dysfunction. In many cell types, the intermediate filament vimentin reacts to a loss of proteostasis by becoming upregulated and collapsing to form cages around aggregated proteins residing in the nuclear bay in a structure called the aggresome. Despite knowledge of vimentin’s presence at the aggresome for over twenty years, vimentin’s role at the aggresome remains unclear. We used CRISPR/Cas9 to generate mouse NSCs that have vimentin tagged with the fluorophore mNeon or vimentin knocked out (KO). Vimentin-mNeon NSCs confirm that NSCs form aggresomes surrounded by vimentin cages. Vimentin KO NSCs are able to make aggresomes, but demonstrate a reduced capacity to recover from a loss of proteostasis. Taken together, our data suggests that vimentin is critical for proper aggresome function and suggests a critical role for vimentin in protecting NSCs during aging.

It has been established that de novo protein synthesis is an integral process in long-term memory formation and synaptic plasticity. Eukaryotic elongation factor 2 (eEF2) is a crucial modulator of mRNA translation, mediating the translocation step of elongation. eEF2 is tightly regulated by its only known kinase, eEF2 kinase (eEF2K), and phosphorylation of eEF2 (by eEF2K) inhibits its activity, hindering de novo protein synthesis. We have previously shown that knockdown of eEF2K in mouse models of Alzheimer’s disease (AD) alleviates AD-associated cognitive deficits. Here, we seek to determine whether cognitive deficits in aged mice (19-22 months old) can be improved by genetically deleting eEF2K (eEF2K KO) and thus reducing eEF2 phosphorylation. Multiple behavioral tasks were applied to evaluate cognition including hidden platform Morris water maze (MWM) and novel object recognition (NOR). We found that performance of eEF2K KO mice in these cognitive tasks was not significantly different from that of WT littermates. Additionally, deletion of eEF2K did not alter de novo protein synthesis in the hippocampus, as assessed by SUnSET assay. Collectively, these results provide preliminary evidence that removal of eEF2K does not improve cognitive deficits or de novo protein synthesis in aged mice.

Disclosures:  S. Gosrani: None.  H. Jester: None.  X. Zhou: None.  T. Ma: None.

Poster

291. Aging: Molecular Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 291.13/E2

Topic: C.01. Brain Wellness and Aging

Support:  NIGMS 1SC3GM086323
CTSC UL1-RR024996
NIH G12 RR003037
NIH 8 G12 MD0075998
NIH G12 MD007599

Title: Temporal reduction in proteasome activity results in irreversible protein aggregation in Drosophila melanogaster

Authors:  *T. SCHMIDT-GLENEWINKEL\(^1\), C.-H. YEH\(^1\), V. VERNACE\(^2\), M. JANSEN\(^1\), J. GAO\(^1\), M. FIGUEREIDO-PEREIRA\(^1\);  
\(^1\)Biol. Sci., Hunter Col. and Grad. Ctr. of CUNY, New York, NY;  \(^2\)Hlth. and Natural Sci. Dept., Goodwin Col., East Hartford, CT

Abstract: The ubiquitin-proteasome system and autophagy have an important role in the control of protein turnover in eukaryotic cells. In a previous study, we have investigated proteasome activity and ATP levels as part of an aging study in Drosophila melanogaster. We found a sharp
decline in both genders of about 50% ATP levels as well as a sharp decrease in the 26S proteasome activity in 43-47 day old flies (Vernace et al., 2007). We have now extended our studies to investigate if a brief temporal decline in proteasome activity results in an accumulation of ubiquitinated proteins. We used an RNAi directed against the dbeta5 subunit of the proteasome in a binary system in which RNAi expression was controlled by an RU486 inducible Act5 promoter. This way proteasome activity could be tuned to any level at any stage of the development. Exposure of flies for 5 days (0-5 days post eclosion) to 200umoles of RU486 resulted in a 23% decrease in proteasome activity and an increase of polyubiquitinated proteins by 74%. Proteasome activity was then allowed to recover in the absence of RU486 and returned to control levels. However, ubiquitinated proteins, accumulating during the temporal disruption of proteasome activity, continue to accumulate after restoration of proteasome activity and are not removed when proteasome activity is restored to control levels. This clearly indicates that ubiquitinated proteins are in a state not accessible for degradation by the proteasome. Immunocytochemistry using an anti–Fk1 antibody against polyubiquitin shows widespread aggregates in the CNS of Drosophila melanogaster after temporary inhibition of proteasome activity. Future experiments using different RU486 inducible promoters will allow us to investigate the effect of reduced proteasome activity at different stages of development and in different models of neurological diseases in Drosophila melanogaster.


Poster

291. Aging: Molecular Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 291.14/E3

Topic: F.03. Neuroendocrine Processes

Support:  Medical Research Council (MRC)

Title: Exploring the role of the endoplasmic reticulum unfolded protein response in *C. elegans* neurons

Authors:  *N. P. OZBEY*¹, S. IMANIKIA¹, C. KRUEGER², M. SHENG¹, M. CASANUEVA², R. C. TAYLOR¹;
¹MRC LMB, Cambridge, United Kingdom; ²Babraham Inst., Cambridge, United Kingdom

Abstract: The nervous system of *C. elegans* plays a role in the orchestration of systemic stress responses. One of these stress responses, the unfolded protein response of the endoplasmic reticulum (UPR<sup>ER</sup>), is activated to re-establish proteostasis upon the detection of ER stress; overexpression of active, spliced XBP-1 (XBP-1s), a transcription factor that acts downstream of
the UPR\textsuperscript{ER} kinase/endoribonuclease IRE-1, in the nervous system of \textit{C. elegans} increases the lifespan and healthspan of worms through UPR\textsuperscript{ER} induction in the intestine. To investigate XBP-1s-dependent changes in the nervous system of these animals, we conducted tissue-specific RNA-Seq in neurons. This approach allowed us to characterise differentially regulated neuronal and synaptic components, which may mediate changes to the nervous system that cause the release of inter-tissue UPR\textsuperscript{ER}-activating signals. We also employed a candidate approach based on our previous finding that neurotransmitter secretion is required for cell non-autonomous UPR\textsuperscript{ER} activation, and identified positive and negative regulators of intestinal UPR\textsuperscript{ER} activation. We find that the neuronal circuitry required to activate the UPR\textsuperscript{ER} within the intestine can also generate behavioural phenotypes following neuronal XBP-1s overexpression. This suggests that inter-tissue UPR\textsuperscript{ER} activation, increased longevity and healthspan can be coordinately regulated with stress-responsive behaviour by the activation of this transcription factor in the nervous system. We are further investigating this circuitry to better understand the mechanistic links between the regulation of behaviour and longevity by neuronal XBP-1s activation.


Poster

291. Aging: Molecular Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 291.15/E4

Topic: C.01. Brain Wellness and Aging

Support: Department Support

Title: Inhibition of Thioredoxin interacting protein ameliorates age associated neuroinflammation and memory decline

Authors: *S. ISMAEL\textsuperscript{1}, L. LI\textsuperscript{2}, A. YOO\textsuperscript{2}, F. WAJIDUNNISA\textsuperscript{2}, M. KHAN\textsuperscript{3}, T. ISHRAT\textsuperscript{2};\textsuperscript{1}Anat. and Neurobio., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN; \textsuperscript{2}Anat. and Neurobio., Memphis, TN; \textsuperscript{3}Neurol., Univ. of Tennessee Hlth. science Ctr., Memphis, TN

Abstract: Inflamm-aging is an independent risk factor for several age associated neurological diseases including Alzheimer’s disease (AD), dementia, stroke and neurodegeneration. Accumulating evidence showed that thioredoxin-interacting protein (TXNIP), an endogenous negative regulator of thioredoxin (TRX, a cellular redox regulator) is known to contribute to the progression of age-associated neorodegeneration. However, the contribution TXNIP in mediating age associated neuroinflammation and cognitive impairment through NOD-like receptor protein (NLRP3)-inflammasome axis remain unclear. In the present study, we have determined influence of temporal and gender specific difference in TXNIP-NLRP3 activation in
C57BL/6 mouse brain of different age group (2, 12 and 18 months) using western blot and immunostaining analysis. To evaluate the role of TXNIP on neuroinflammation, 18 months old C57BL/6 mice were treated with verapamil (1mg/kg/day) in drinking water for one month and. The animals were tested for cognitive function by novel object recognition and Morris water maze. The results demonstrated that there was an age dependent increase in the protein expression of TXNIP-NLRP3 inflammasome components in male and female mouse brain with reciprocal down regulation of TRX. In addition, the age and sex-dependent increase in TXNIP immunoreactivity was also observed in cortex and hippocampus of mouse brain and is localized in the neurons. Moreover, inhibition of TXNIP with a non-selective inhibitor, verapamil significantly attenuated NLRP3 inflammasome activation, oxidative stress and improved cognitive function in aged mice without affecting blood pressure. Supportingly, genetic deletion of TXNIP attenuated neuro inflammation in older animals. Together, these findings shed light on the mechanistic and molecular basis for age-associated changes in the brain. This suggests that these targets might contribute to the higher prevalence and severity of AD in the elderly and other age-related dementias.

<table>
<thead>
<tr>
<th>Protein expression in fold change</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 months</td>
<td>1±0.18</td>
<td>3.85±0.27</td>
</tr>
<tr>
<td>12 months</td>
<td>1.6±0.19</td>
<td>0.40±0.20</td>
</tr>
<tr>
<td>18 months</td>
<td>1.09±0.15</td>
<td>0.89±0.04</td>
</tr>
<tr>
<td>TXNIP</td>
<td>1±0.09</td>
<td>0.90±0.16</td>
</tr>
<tr>
<td>TRX</td>
<td>1±0.17</td>
<td>1.33±0.03</td>
</tr>
<tr>
<td>NLRP3</td>
<td>1±0.22</td>
<td>19.6±1.45</td>
</tr>
<tr>
<td>Cl. Caspase</td>
<td>4.48±1.11</td>
<td>3.45±0.25</td>
</tr>
<tr>
<td>IL-1β</td>
<td>1±0.35</td>
<td>2.4±0.33</td>
</tr>
</tbody>
</table>

| Effect of verapamil on age associated inflammatory makers and memory function |
|-----------------------------|-----------------------------|-----------------------------|
| TXNIP                       | 2 months | 18 months | 18 months + Verapamil |
|                             | 1±0.23 | 2.25±0.10 | 1.14±0.26 |
| TRX                         | 1±0.54 | 0.30±0.04 | 0.54±0.07 |
| NLRP3                       | 1±0.01 | 1.44±0.16 | 0.7±0.11 |
| Cl. Caspase                 | 1±0.45 | 10.35±0.27 | 1.76±0.72 |
| IL-1β                       | 1±0.40 | 7.3±0.19 | 1.71±0.73 |
| Recognition Index (NOR)     | 0.33±0.04 | 0.071±0.02 | 0.36±0.068 |

<table>
<thead>
<tr>
<th>Morris Water maze performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target quadrant time</td>
</tr>
<tr>
<td>Target quadrant distance</td>
</tr>
</tbody>
</table>

Disclosures:  S. Ismael: None. L. Li: None. A. Yoo: None. F. Wajidunnisa: None. M. Khan: None. T. Ishrat: None.
**Poster**

**291. Aging: Molecular Mechanisms I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 291.16/E5

**Topic:** C.01. Brain Wellness and Aging

**Support:** CAPES-FCT  
CNPq  
FAPESC  
PRONEX/NENASC

**Title:** Hippocampal bradykinin B1 receptors blockade mitigates spatial learning and memory deficits in middle-aged rats

**Authors:** R. M. BITENCOURT¹, A. C. GUERRA DE SOUZA¹, *M. A. BICCA¹, F. A. PAMPLONA², N. DE MELLO¹, G. F. PASSOS³, R. MEDEIROS¹, R. N. TAKASHI¹, J. B. CALIXTO¹, R. D. S. PREDIGER¹;  
¹Pharmacol., Univ. Federal de Santa Catarina, Florianopolis, Brazil; ²Inst. D’Or de Pesquisa e Ensino, Rio de Janeiro, Brazil; ³Biotecnologia Farmaceutica, UFRJ, Rio de Janeiro, Brazil

**Abstract:** A variety of studies have demonstrated abnormal bradykinin receptors expression in Alzheimer’s disease (AD) brains from humans and animal models, as well as that targeting bradykinin receptors is an efficient strategy to counteract the cognitive and memory impairment associated with aging and AD. Being the hippocampus critical for cognition, abnormalities in this brain region are linked to memory decline. Yet, the impact of bradykinin signaling on hippocampal function remains poorly understood. Here, we aimed to investigate the role of hippocampal bradykinin receptors B1R and B2R on the cognitive function of twelve month-old rats (middle-aged) in comparison to 3 month-old rats (mature-adult). We used male Wistar rats divided in three cohorts for behavioral (n = 8-10 animals/group), neurochemical (n = 5 animals/group) and immunohistochemical (n = 4 animals/group) studies. Rats were kept in collective cages (2-3 animals/cage) and distributed randomly in between groups. All efforts were made to minimize the number of animals used and their suffering. All procedures performed complied with university IACUC and NIH guidelines. Middle-aged rats exhibited impaired ability to acquire and retrieve spatial information, evaluated using the Morris water maze task. Furthermore, a single intra-hippocampal injection of the selective B1R antagonist des-Arg9-[Leu8]-bradykinin (DALBK, 3 nmol), but not the selective B2R antagonist D-Arg-[Hyp3,Thi5,D-Tic7,Oic8]-BK (Hoe 140, 3 nmol), reversed spatial learning and memory deficits in these animals. Interestingly, the cognitive function of mature-adult rats was not affected by any of the drugs, suggesting absence of nootropic properties. Western blot and immunohistochemistry analysis revealed an up-regulation of B1R expression in the hippocampal...
CA1 sub-region and pre-frontal cortex of middle-aged rats, whereas no changes in B2R expression were observed. Our findings provide new evidence that inappropriate hippocampal B1R expression and activation exert a critical role on the cognitive and memory function of middle-aged rats.


Poster

291. Aging: Molecular Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 291.17/E6

Topic: C.01. Brain Wellness and Aging

Support: HHMI

Title: Perirhinal cortex projection neurons to the dentate gyrus mediate novel object recognition in both young and aged mice

Authors: *S. KOSMIDIS\(^1\), L. R. HARVEY\(^2\), E. R. KANDEL\(^1\);
\(^1\)Neurosci., \(^2\)Columbia Univ., New York, NY

Abstract: Normal aging is often accompanied by mild memory loss, which affects the quality of life and potentially progresses to severe deficits into the dentate gyrus and Alzheimer’s-like phenotypes. We have previously found that direct administration of Osteocalcin (OCN), a bone-derived hormone, in the dentate gyrus (DG) of young and aged mice can enhance discrimination memory for Novel object Recognition (NOR) by increasing the expression of RbAp48 protein. We used rAAV2 viruses to identify the neuronal circuitry mediating NOR in association with the DG. We find that the ventral perirhinal cortex, a region involved in object recognition in humans, and the entorhinal cortex, both project to the DG. In addition, we examine whether activity-dependent inhibition of RbAp48 protein in the perirhinal cortex and DG can affect object recognition preference in young animals. Further, we show that opto-genetic stimulation of c-fos-positive neurons of the DG after a NOR task can restore NOR preference. We also demonstrate restoration of NOR memory in Aged animals upon injection of OCN in the perirhinal cortex. Collectively, our studies identify a DG-perirhinal cortex neuronal circuit as a mediator of NOR discrimination memory and shows that this specific circuit deteriorates with Aging.

Disclosures: S. Kosmidis: None. L.R. Harvey: None. E.R. Kandel: None.
**Title:** Modulation of calcium dysregulation in aged F344 rats by FK506 and FK520

**Authors:** *J. C. GANT*¹, O. THIBAULT², N. M. PORTER³, E. M. BLALOCK⁴, P. W. LANDFIELD⁵;

**Abstract:** Normal aging results in a decline of cognitive performance. During this process there is a progressive alteration in the function of principal neurons within the limbic system. We have shown that FK506 binding protein 1b (FKBP1b/FKBP12.6) expression is also influenced by age. A decline in the FKBP1b protein occurs simultaneously with the decline in cognition and we have shown that increasing FKBP1b protein *in vivo* by genetic therapy can reverse age-related cognitive impairment as well as age- and learning-related neuronal biomarkers in the hippocampus. However, the mechanistic processes underlying their effect are less clear. Here, we tested for such *ex vivo* effects. For the current experiments we used FK506 (tacrolimus) and FK520 (ascomycin; an analog of FK506) in young and aged slices to determine their effect on these biomarkers. Hippocampal slices were prepared from 20 mo and 5 mo old F344 rats and maintained in a holding chamber containing artificial cerebral spinal fluid. FK506 or FK520 was added to the wells of the incubation chamber (final concentration of 5µM) for at least 2 hours before electrophysiological measures were obtained. Neither FK506 nor FK520 influenced passive membrane properties of CA1 pyramidal neurons. However, both compounds reduced the amplitude and duration of the slow afterhyperpolarization in aged slices, but had no effect in young. In a second set of experiments aged and young rats received bilateral injections of AAV expressing GCaMP6s (a calcium sensitive fluorescent protein) and remained in the vivarium for at least 4 weeks before slices were prepared. Again the slices were maintained in the incubation chamber and treated with FK506 or FK520 for 2 hours before imaging. We are currently examining the multi-photon data to determine Fk506 and FK520’s effect on calcium transients. These results demonstrate that FK506 and FK520 might be useful compounds for treating memory dysfunction associated with aging through its interaction with calcium-induced calcium release in the CA1 region of the hippocampus.
Disclosures:  J.C. Gant: None. O. Thibault: None. N.M. Porter: None. E.M. Blalock: None. P.W. Landfield: None.

Poster

291. Aging: Molecular Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 291.19/E8

Topic: C.01. Brain Wellness and Aging

Support: DFG ZI1224/4-1
IZKF Jena

Title: DNMT1 deletion prevents age-related loss of cortical interneurons

Authors: C. BAYER¹, D. PENSOLD¹, A. HAHN², L. BERMUDEZ², T. PIELER³, T. LINGNER⁴, G. SALINAS-RIESTER⁴, L. BLÜMEL², A. URBACH⁵, *G. ZIMMER-BENSCH¹; ¹RWTH Aachen Univ., Aachen, Germany; ²Univ. Hosp. Jena, Jena, Germany; ³Ctr. for Nanoscale Microscopy and Mol. Physiol. of the Brain, Göttingen, Germany; ⁴Transcriptome and Genome Analysis Lab., Göttingen, Germany; ⁵Dept. of Neurol., Jena Univ. Hosp., Jena, Germany

Abstract: The cerebral cortex, the seat of higher cognitive functions, is composed of two main neuronal subtypes: the excitatory principal neurons and the inhibitory gamma-amminobutyric (GABA)-positive interneurons. Although GABAergic interneurons make up only 20% of the overall neuronal population, their inhibitory action is elementary for proper cortical function. Interestingly, different studies reported a selective vulnerability of inhibitory interneurons towards aging in different mammalian species. The aging phenotype, characterized by declined cognitive, motoric and sensory skills, represents a result of complex interactions between genetic, epigenetic and environmental factors. Epigenetic gene regulation by DNA methylation, executed by DNA-methyltransferases (DNMTs), and histone modifications emerge to be critical for the maintenance of neuronal health and function throughout the entire lifespan. As we found that DNMT1 affects cortical interneuron survival during development (Pensold et al. 2017), we next asked for a potential implication of DNMT1 in the long-term survival regulation of cortical interneurons in the aging brain. To this end, we analyzed a transgenic knockout mouse model in which Dnmt1 was specifically deleted in PV-positive interneurons. We found an amelioration of the age-related interneuron loss in motoric and visual cortical areas in Dnmt1 depleted mice, which was accompanied by improved motor performances of aged knockout mice compared to equal aged wild-types. Together, we provide evidence for DNMT1 being implicated in the age-associated vulnerability of inhibitory cortical interneurons.

Poster

291. Aging: Molecular Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 291.20/E9

Topic: C.01. Brain Wellness and Aging

Support: 19-BR-02-06
NRF-2019R1A2C1011083
NRF-2017R1E1A1A01075226

Title: SIRT1 is negatively regulated by CHFR to maintain neuronal integrity

Authors: *M. KIM¹, J. SEO², J. SEOL²;
¹Korea Brain Res. Inst., Daegu, Korea, Republic of; ²Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: SIRT1 is a nicotinamide adenine dinucleotide (NAD+)-dependent protein deacetylase that controls brain development, aging, and neurodegenerative disease. SIRT1 is known to regulate neurite length through its deacetylase activity and control apoptosis by suppressing p53. In addition, SIRT1 is phosphorylated upon oxidative stress and subsequently down-regulated. However, it still remains elusive how SIRT1 stability and activity are controlled. Here, we have demonstrated that CHFR functions as an E3 Ub-ligase of SIRT1, responsible for its proteasomal degradation under oxidative stress conditions. CHFR interacts with and destabilizes SIRT1 by ubiquitylation and subsequent proteolysis. Such CHFR-mediated SIRT1 inhibition leads to the increase of p53 acetylation and its target gene transcription. CHFR facilitates SIRT1 destabilization and prominent apoptotic cell death upon oxidative stress. Meanwhile, JNK inhibitor prevents SIRT1 phosphorylation, leading to elevated SIRT1 protein levels even in the presence of H₂O₂. Taken together, our results indicate that CHFR plays a crucial role in maintaining neuronal integrity during neuronal stress response by controlling the stability and function of SIRT1.

Disclosures: M. Kim: None. J. Seo: None. J. Seol: None.
Title: High-temperature-processed green tea potentiates neuronal differentiation by enhancing the levels of epimerized catechins that inhibit DNA methyltransferase1

Authors: *H.-S. KIM, A. KIM, S.-Y. CHO, W.-S. PARK; AMOREPACIFIC R&D Unit, Yongin-Si, Korea, Republic of

Abstract: Alterations in neurogenesis appear to be a common hallmark in different neurodegenerative diseases including Parkinson's disease (PD), Alzheimer's disease (AD), and Huntington's disease (HD). For neurogenesis, neuronal stem cells undergo appropriate neuron differentiation and differentiates into neurons. DNA methylation plays a central role in epigenetic regulation of neuronal differentiation. Green tea catechins, especially (-)-epigallocatechin (EGCG), have been reported to modulate gene expression by targeting DNA methyltransferases. EGCG can be epimerized during the processing of tea into (-)-gallocatechin gallate (GCG), which is present in tiny amounts in fresh tea leaves. However, the regulatory effect of GCG on DNA methylation is not delineated. In the present study, we characterized the effect of GCG and high-temperature-processed green tea extract (HTP_GTE), which contained about 6 fold increased GCG than fresh green tea leaves extract by heat-induced epimerization of EGCG, on DNA methylation catalyzed by DNA methyltransferase1 (DNMT1) and neuronal differentiation. Remarkably, GCG exhibited a higher inhibitory effect on DNMT1 than its corresponding epimer (EGCG). HTP_GTE also efficiently inhibited DNMT1 in vitro. In addition, to induce neuronal differentiation, simultaneous treatment of HTP_GTE and retinoic acid in the neuroblastoma cell line, SH-SY5Y, downregulated DNMT1 and induced DNA hypomethylation at Synaptophysin promoter. HTP_GTE also increased in the mRNA expression of synaptophysin, which is neuronal differentiation marker. These results suggest that HTP-GTE could facilitate neuronal differentiation by regulating DNA methylation level.

**Poster**

**292. Alzheimer's Disease: Omics Approaches**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 292.01/E11

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Support:** R01 AG057522

**Title:** Bioinformatics strategy to advance the interpretation of Alzheimer’s disease GWAS discoveries

**Authors:** *O. CHIBA-FALEK¹, D. SPRAGUE², M. LUTZ²;
¹Duke Med., Duke University, NC; ²Duke Univ., Durham, NC

**Abstract:** Late-onset Alzheimer’s disease (LOAD) is a genetically heterogenous disease. Genome wide association studies (GWAS) and whole genome/exome sequencing (WGS/WES) discovered about 40 LOAD-associated SNPs, navigating the genetic research of LOAD to specific regions in the genome. While the target genes have been inferred by proximity to the most significantly associated SNPs, the actual causal genes are yet to be identified. Thus, there is an unmet need for post-GWAS research that applies a multifaceted strategy combining *in silico*, *in vitro* and *in vivo* approaches to identify and validate the precise causal gene/s within the associated loci. We propose a bioinformatic strategy built on the hypothesis that dysregulation of gene expression mediated by genetic and epigenetic mechanisms contributes, at least in part, to LOAD pathogenesis. We defined LOAD-GWAS regions by the associated SNP±0.5Mb and extended the loci boundaries such that they are not within a gene. We developed a bioinformatics pipeline that utilizes the hippocampus-specific chromatin state segmentation track available from the Roadmap Epigenomics Project to map active enhancers and data on hippocampus specific frequencies of chromatin interaction using the virtual 4C software to visualize interactions between active enhancers and gene promoters. We augment our pipeline with biomedical and functional information from publications. We applied the bioinformatics strategy using three ~1Mb LOAD-GWAS loci known by the proximate genes: *BIN1, PICALM, CELF1*. These loci contain 10-24 genes, an average of 106 active enhancers and 80 CTCF sites. We integrated the active enhancers from the chromatin state segmentation track with the enhancer-promoter interactions from 4C data and identified all genes corresponding to the promoters that interact with the active enhancer positioned the closest to the LOAD-GWAS-SNP. This strategy generated a shorter list of prioritized candidate LOAD genes, ranging from 5-14 per locus. In conclusion, we suggest that the interpretation of LOAD-GWAS discoveries requires the integration of brain-specific functional genomic and epigenomic datasets and information related to regulatory activity, rather than inference based on proximity to the GWAS-SNPs. We
demonstrated that our bioinformatics pipeline is feasible to identify the highest priority LOAD candidate genes and to guide post-GWAS laboratory experiments to investigate causality.


Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 292.02/E12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: U01AG046139
U01AG046152
U01AG046161
U01AG046170
R01AG046171
R01AG046174

Title: Reproducible bioinformatic tools for analysis of AMP-AD RNA-seq data


Abstract: Background: The systemic failure to develop successful treatments for Alzheimer's disease (AD) is in part driven by an incomplete understanding of underlying heterogeneous biological processes such as gene expression patterns. To help address this challenge in the context of RNA-seq analysis, we develop reproducible bioinformatic tools for reprocessing 2114 RNA-seq samples from postmortem brain tissue. These samples are derived from three AMP-AD studies (ROSMAP, Mayo RNASeq, and MSBB), and are available through the AMP-AD Knowledge portal (ampadportal.org). To perform a comprehensive meta-analysis between these studies, it is necessary to standardize the processing of these datasets such that software tools, versions, and parameters are consistent across samples. The development of an automated workflow that allows processing of RNA-seq datasets will benefit the research community by enabling reproducible execution in diverse computing environments, and re-use of tools for new RNA-seq studies.

Methods: We implement an RNA-seq processing pipeline in common workflow language (CWL). This workflow begins with BAM alignment files that were previously generated using diverse
software tools across research groups. Following conversion to fastq, reads are re-aligned to the hg19 human reference genome using the STAR read aligner and gene counts are quantified. We demonstrate execution of this workflow in a batch computing environment on Amazon Web Services (AWS). The Toil workflow engine is utilized to direct execution of tasks in the workflow.

Results:
We demonstrate that our workflow is scalable to thousands of samples. The results of this study is available through the AMP-AD Knowledge Portal in the rnaSeqReprocessing study: https://www.synapse.org/#!Synapse:syn9702085. We provide documentation on configuring and executing the workflow in github: https://github.com/Sage-Bionetworks/amp-workflows, and provide further resources for CWL workflow execution: https://www.synapse.org/#!Synapse:syn17872025.

Conclusions:
The development of a reproducible RNA-seq reprocessing workflow provides a valuable resource for the research community. By enabling consistent software execution, users can perform similar RNA-seq experiments without implementing new pipelines. Furthermore, as new datasets are produced, they can be processed in a standardized manner that allows for cross-study comparisons. While we have demonstrated execution of this workflow in the cloud, users may also run this workflow on local HPC systems. As these infrastructures evolve, our CWL workflow will remain a stable resource for processing RNA-seq datasets.


Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 292.03/E13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG057443

Title: Agora: A platform for exploration of Alzheimer’s disease evidence

Abstract: Previous clinical trials for Alzheimer’s disease (AD) have failed to identify successful disease modifying therapies. This is due, in part, to a primary focus on a small number of targets and therapeutic hypotheses. One way to de-risk the drug development process is for the research community to collectively share and evaluate evidence in support of new AD targets during early stages of research. To this end, we developed the Agora platform (https://agora.ampadportal.org), which provides open access to molecular evidence derived from a variety of analytical approaches. Agora currently supports two main functions. First, users can evaluate their own hypotheses about the molecular basis for AD by viewing multi-omics analyses relating genes and proteins to AD. These results are visualized through interactive explorers designed to support non-bioinformaticians in the interpretation of multi-omic analytical results. Second, users can explore hypotheses being considered by other researchers in the field by viewing a list of new AD targets developed from molecular assessments of human samples. The initial list of nominated targets was provided by the Accelerating Medicines Partnership in AD (AMP-AD) research consortia who derived their nominations based on systems biology analyses of human multi-omic data. To support the selection of candidate targets for further validation and/or drug discovery, Agora also presents information about the druggability of these targets. In summary, we have built a platform to empower the AD research community to contribute and unite around promising target hypotheses.


Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program#/Poster #: 292.04/E14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG047928
        NIH Grant AG053987

Title: RNA splicing dysfunction exacerbates the progression of Alzheimer’s disease

Authors: *X. HAN*1, P.-C. CHEN2, J. YUN1, J. PENG3;
Abstract: Alzheimer’s disease (AD), the most common form of dementia, is a chronic neurodegenerative disease. AD leads to memory loss and bodily function disorders, but its causative mechanisms are not fully elucidated. Besides Aβ and tau aggregation, the insoluble proteomics data from our group have identified, an early, tangle-like pathology of U1 small nuclear ribonucleoprotein complex (snRNP), including U1-70K and its N-terminal cleavage product (N40K), in sporadic and familial cases. N40K has a dominant effect to down-regulate full-length U1-70K. The mouse model with neuronal expression of N40K (N40K Tg), can recapitulate AD-associated events, such as neuronal loss, memory loss and RNA splicing dysfunction. We also characterized an inducible N40K transgenic mouse model (iTg), in which N40K expression is controlled by tetracycline-controlled transactivator (tTA). iTg displays AD-related phenotypes, like neurodegeneration and memory loss which are similar to the phenotypes observed in N40K Tg. The two mouse models prove that RNA splicing dysfunction are crucial in AD development. As RNA splicing dysfunction and Aβ aggregation are two important pathologies in AD cases, I tested that if these two pathologies play a synergistic role in AD development by crossing N40K Tg mice with 5xFAD to get a new double transgenic mice (dTg) mouse model. It was observed that the performances of dTg were worse than 5xFAD and N40K Tg only in Morris Water Maze and Novel Object Recognition. These studies imply that splicing dysfunction and Aβ aggregation play a synergistic effect on AD progression. These studies represent RNA splicing dysfunction as a novel target for potential pharmaceutical treatment.

Disclosures: X. Han: None. P. Chen: None. J. Yun: None. J. Peng: None.

Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 292.05/E15

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Translational study of biomarkers and potential drug targets for Alzheimer's disease

Authors: *X. WANG, T. XU, X. ZHANG, W. YU, Y. SUN, X. RONG;
Inst. Materia Med., Beijing, China

Abstract: Alzheimer’s disease (AD) is a common neurodegenerative disease among elderly people. So far the pathogenesis of AD is still not clear. Therefore, the diagnosis of the disease at early stage and therapeutic evaluation are needed urgently. To solve these issues, we tried to find AD biomarkers by screening the protein changed in the brain of APP/PS1 transgenic mice using 2DE proteomics and further validated by Elisa and western blot. About 30 differentially expressed proteins were found. Next step, we detected the expression of these differential proteins in AD patient serums. In our study, 68 serums from AD patients were collected and 6 proteins were validated in these patient serums. Then we verified these proteins in four AD
animal models. In brain tissues in different animal models: APP/PS1 transgenic mice, mouse model of Aβ intracerebral ventricular injection, SAMP8 mice and the older rats (>20 months), we found that some proteins expressed in the same way as they expressed in AD serums. These differential proteins included VDAC1, ITI-H4 subunits, Apo-L1, Apo-M and so on. What is interesting is that some of these differential proteins could be recovered when the animals were treated with anti-AD drugs.

**Conclusion:** About 30 differentially expressed proteins were detected in proteomics and 6 of them were validated in both AD patient serums and the brain tissues of AD animal models. They might be recovered by anti-AD agents. Therefore, these proteins might be used for AD diagnosis and also for new drug evaluation.

**Keywords:** Alzheimer’s disease, biomarker, proteomics, drug target

**Disclosures:** X. Wang: None. T. Xu: None. X. Zhang: None. W. Yu: None. Y. Sun: None. X. Rong: None.

**Poster**

292. Alzheimer's Disease: Omics Approaches

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 292.06/E16

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Support:** Kleberg Foundation

**Title:** Neural stem cell-derived exosomes promote synaptic resilience to amyloid beta and tau oligomers through modulation of neuroinflammatory tone via specific miRNAs

**Authors:** *O. ZOLOCHEVSKA¹, E. BISHOP², W. ZHANG², B. TUMURBAATAR², M.-A. MICCI³, G. TAGLIALATELA⁴;
¹Neurol., ²Univ. of Texas Med. Br., Galveston, TX; ³Dept. of Anesthesiol., The Univ. of Texas Med. Br. At Galvesto, Galveston, TX; ⁴Neurol., Univ. of Texas Med. Br. Dept. of Neurol., Galveston, TX

**Abstract:** Alzheimer’s Disease (AD) is the most common form of dementia typically characterized by synaptic loss at early stages, presence and accumulation of aggregated forms of amyloidogenic proteins, and neuronal loss at later stages of the disease. Aging is the greatest risk factor for the disease development, suggesting that the accumulation of different pathological events leads to increased synaptic vulnerability. Therefore, a potential approach for development of a successful therapy against AD is to promote synaptic health and resilience to various pathologic events. We have previously reported that exosomes secreted by hippocampal neural stem cells (NSCs), and not mature neurons (MN), act via specific miRNAs to provide synaptic protection against Aβ and tau oligomers. Delivery of these NSC-specific miRNAs (17, 322, 485)
intracerebroventricularly (ICV) to wild-type mice resulted in preserved long-term potentiation (LTP) during an Aβ and tau oligomer challenge protocol. Moreover, we observed reduced Aβ and tau oligomer binding to synaptosomes isolated from mice treated with these miRNAs or NSC-exosomes. Here we aimed to determine the mechanism of action of NSC-derived exosomes and their bioactive miRNA cargoes in comparison to MN-derived exosomes using transcriptomics and proteomics approaches. Male and female wild-type mice were injected ICV with NSC- or MN-derived exosomes, or a combination of the three selected miRNA cargoes (17, 322, 485). Synaptic fractions were prepared twenty-four hours after the ICV injections and either total RNA or protein were isolated. Transcriptomics and proteomics analysis were performed using Ingenuity Pathway Analysis, PANTHER and Reactome. The transcriptomics analysis identified upregulation of several inflammatory pathways, including TNF, NFκB and IL-8. At the protein level, oxidative phosphorylation, NFAT and PKA pathways were upregulated after treatments with miRNAs and NSC-derived exosomes, while MN-exosomes inhibited those signaling pathways. Remarkably, overall inhibition of cellular signaling was observed only after treatment with MN-derived exosomes. Collectively these data suggest that specific miRNA cargoes enriched in NSC-derived exosomes stimulate the neuroinflammatory tone which plays an important role in synaptic plasticity, thus promoting synaptic resilience to Aβ and tau oligomers.


Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 292.07/E17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 1R01AG062249-01

Title: Behavioral and psychological symptoms of dementia (BPSD) in Alzheimer’s disease: Antemortem clinical assessment and postmortem RNA-sequencing analysis

Authors: *R. KESZYCKI¹, D. FISHER¹, D. BENNETT², R. WILSON², H. DONG¹;
¹Psychiatry, Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; ²Rush Alzheimer’s Dis. Ctr., Chicago, IL

Abstract: By 2050, the global number of patients with dementia is expected to rise to 115.4 million people of which 60-80% will carry a diagnosis of Alzheimer’s disease (AD) specifically. The vast majority of these individuals will experience behavioral and psychological symptoms of dementia (BPSD). Thus, BPSD represents a critical worldwide healthcare issue. It has long been
contended that BPSD should be categorized into domains based commonly co-occurring symptoms that potentially have common underlying etiologies and represent effective treatment targets. The purpose of this study was to choose AD patients who were either highly affected or unaffected-to-mildly-affected on particular BPSD domains. Based on previous research findings, we hypothesized that patients’ symptoms would cluster into: affective symptoms (depression and anxiety), apathy, hyperactivity (irritability, disinhibition, aggression, and aberrant motor behavior), and psychosis (delusions and hallucinations). We performed factor analysis on 660 antemortem behavioral interviews with dementia patients, revealing four factors that were similar to, yet different from, those we had hypothesized. In accordance with our hypothesis, we obtained apathy and affective domains. Regarding psychosis, delusions did not load well onto any of our factors, and aberrant motor behavior loaded strongest with hallucinations. Aggressive symptoms, including impulsive aggression, constituted our last factor. We obtained severity scores for all patients on these four domains who had an AD diagnosis and a behavioral interview within two years before death (n = 100). Within a particular domain, we designated those in the top 70% as “cases” and those in the bottom 30% as “controls.” We selected 55 patients using these cutoffs and gender counter-balancing across all domains’ cases and controls. In the near future, we plan to perform RNA sequencing analysis on several postmortem brain regions from these additional patients. Previously, we conducted preliminary RNA-sequencing analysis on 17 patients’ post-mortem anterior cingulate tissues according to the four domains we had hypothesized. This analysis revealed 57 differentially expressed genes for the affective domain, 44 for the hyperactivity domain, 34 for the psychosis domain, and 20 for the apathy domain. We propose that we will find differentially expressed genes across our adjusted BPSD domains and additional brain regions as well. Future research should consider how epigenetic and interventions upregulating or downregulating these domain-related genes may be helpful in developing treatment strategies.


Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 292.08/E18

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA R01 AG057457
NIA R01 AG059093
NIA 1R01AG057931
Title: Integrative network analysis identifies microglial-specific key drivers for phagosome and abeta-clearance in Alzheimer's disease

Authors: *R. CHANG;
Univ. of Arizona, Tucson, AZ

Abstract: Late-Onset Alzheimer’s Disease (LOAD) results from a complex pathological process influenced by genetic variation, aging and environment factors. Genetic susceptibility factors indicate that myeloid cells such as microglia play a significant role in the onset of LOAD. Here, we developed a computational systems biology approach to construct probabilistic causal and predictive network models of genetic regulatory programs of microglial cells under LOAD diagnosis by integrating two independent brain transcriptome and genome-wide genotype datasets from the ROSMAP and Mayo Clinic studies in AMP-AD consortium. From this network model, we identified and replicated novel microglial-specific master regulators predicted to modulate network states associated with LOAD. We not only experimentally validated three master regulators (FCER1G, HCK and LAPT5) associated with phagocytosis, a process associated with LOAD, that in turn causally links phagocytosis to Abeta burden, and revealed the causal relations among the three, but also we validated the molecular impact these master regulators have on modulating downstream genomic targets by perturbing them in primary human microglia-like cells (MDMi). Thus, we propose three new master regulator genes that emerged from our network analyses as robust candidates for further evaluation in LOAD therapeutic development efforts.

Disclosures: R. Chang: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); INTelico Therapeutics.

Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 292.09/E19

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: A potential role for miRNAs in hippocampal maturation, sex-differences, and disease pathology as revealed by an analysis of the 5XFAD mouse model of Alzheimer's disease

Authors: *R. S. NOWAKOWSKI1, J. L. BUNDY2, C. M. VIED1;
1FSU Col. of Med., Tallahassee, FL; 2Duke Univ., Durham, NC

Abstract: Like many neurological disorders, Alzheimer’s disease has a sex-biased epidemiological profile, affecting approximately twice as many women as men. We investigated
the changes in miRNA levels females and males using the 5XFAD genetic mouse model of Alzheimer’s disease. The 5XFAD mouse genome contains two co-inserted mutated human transgenes (APP and PSEN1) associated with familial Alzheimer’s disease. 5XFAD mice on the C57BL/6J background develop histopathological features of Alzheimer’s disease, such as Aβ42 positive plaques, as early as four months of age. We profiled the miRNAome and the transcriptome of the mouse hippocampus in male and female mice during early stages of disease development (one, two, and four months of age). Our analysis reveals 231 miRNAs that are differentially expressed as a function of age (218 miRNAs), disease status (6 miRNAs) or sex (8 miRNAs). The overlap among the 3 patterns is small (see upper Venn diagram) with only 2 miRNAs differentially expressed by age and disease status and 1 miRNA differentially expressed by both age and sex. To gain insight into the possible role of the differentially expressed miRNAs we downloaded their putative targets from mirdb.org. The total target numbers are large: age (15,521 targets), disease status (1,874 targets) and sex (6,090 targets). The overlap among the 3 patterns, in contrast to the miRNA overlap, is considerable (see lower Venn diagram). The intersection of all 3 sets of targets (i.e., by age, disease status and sex) has 972 targets which from their gene ontology (mousemine.org) is rich in genes involved in CNS and neuron functions and in diseases related to mental illness. Overall, this analysis points to miRNAs as occupying a gene regulatory niche that could potentially mediate many changes in CNS function in normal maturation, sex-related functions and disease pathology.

Disclosures: R.S. Nowakowski: None. J.L. Bundy: None. C.M. Vied: None.
Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 292.10/E20

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R56 AG057469

Title: Mutant presenilin 1 dysregulates exosome cargo derived from human induced pluripotent stem cell (iPSC) neurons, revealed by proteomics analyses

Authors: *A. JONES¹, Q. LIU¹, S. PODVIN¹, C. LIETZ¹, C. MOSIER¹, T. IKEZU², R. RISSMAN¹, S. YUAN¹, V. HOOK¹;
¹UCSD, La Jolla, CA; ²Pharmacol. and Neurol., Boston Univ. Sch. of Med., Boston, MA

Abstract: Exosomes, extracellular vesicles, have been found to participate in Alzheimer's disease (AD) propagation of neuropathology. Prior studies show that plasma neurally-derived exosomes (NDEs) from AD patients, when injected into mouse brain, result in AD-like pathogenesis demonstrated by accumulation of tau aggregates (Winston et al., 2016). These findings suggest that dysregulation of the proteome cargo of NDEs may occur in AD, and raise the question of whether gene mutations of familial AD (FAD) can alter the proteome cargo of exosomes during its biogenesis in human neuronal cells. For this reason, we conducted proteomics analyses of exosomes generated by patient-derived iPSC neurons containing the presenilin 1 (PS1) A246E mutation, compared to those from control iPSC neurons. Exosomes isolated from the media of these neurons were subjected to high throughput proteomics mass spectrometry and bioinformatics analyses, resulting in the identification of over 1,000 proteins. Proteins were categorized as (a) present only in mutant PS1 exosomes (111 proteins), (b) present only in control exosomes (276 proteins), and (c) present in both mutant PS1 and control exosomes (757 proteins). For the large group of proteins shared in mutant PS1 and control exosomes, quantitation revealed distinct patterns of down-regulated and up-regulated proteins in the mutant PS1 exosomes compared to controls, with significance values of p < 0.05. Gene ontology and STRING analysis provided insight into the biological functions of the exosome proteomes, which implicated involvement of vesicle transport and protein localization, exocytosis, and disruption of cellular signaling networks. With respect to tau, western blots showed that the mutant PS1 exosome displayed elevated levels of total tau; these exosomes also contain phospho-tau (Ser396), known to be present in NDEs from plasma of AD patients (Winston et al., 2016). These findings demonstrate that the PS1 A246E FAD mutation dysregulates exosome cargo, via exosome biogenesis, in human patient-derived iPSC neurons.

Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 292.11/E21

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01 AG061787

Title: Exosome protein cargos and dynamics in Alzheimer's disease

Authors: *D. GRAYKOWSKI¹, Y.-Z. WANG¹, J. N. SAVAS²;
¹Neurol., ²Northwestern Univ., Chicago, IL

Abstract: Exosomes are nanosized (50-100 nm in diameter) extracellular vesicles that are a vehicle for cellular communication and waste removal in mammals. In the central nervous system, exosomes are secreted by neurons, astrocytes, microglia, and oligodendrocytes. We are interested in testing the hypothesis that exosomes secreted by neurons play essential roles in synaptic plasticity events while concurrently contributing to the spreading of pathology in disease states including Alzheimer's disease (AD). AD is characterized by the amyloid-beta (Aβ) plaques and neurofibrillary tangles (NFT) pathological hallmarks. Interestingly, exosomes may contribute to the seeding or the prion-like aspects of Aβ or tau in AD models. Thus, exosomes potentially exacerbate AD pathology. However, precisely how exosomes and their protein cargo are perturbed in the context of AD is not well understood. We hypothesize that in the context of different levels of AD pathology in both mouse models and actual AD in humans, the presence of Aβ plaques and NFT's will alter exosome cargo and dynamics. In preliminary experiments, we isolated exosomes from the brains of three lines of 6 months old APP knock-in mouse models: NL, NL-F, and NL-G-F, and analyzed the purified exosomes with tandem mass spectrometry-(MS) based proteomics. We identified several known exosome proteins including CD81, Alix, APP, and Aβ. We look to expand upon these preliminary findings by first verifying the MS results by western blotting and negative staining electron microscopy. Next, we will perform multiplexed-TMT MS experiments on the different lines of APP knock-in mice at 3, 6, 9, and 12-month time points in appreciation of the timeline of increased Aβ levels, synapse loss, and plaque formation. Additionally, we look to investigate exosomes and their respected proteomes in the context of mutant tau via a widely investigated tauopathy model mouse, P301S. The workflow will follow a similar design; optimization of exosome purification from the brain, verification (using immunohistochemistry), and quantitative analysis via multiplexed TMT MS. Lastly, to get a more complete illustration of exosomes in the context of actual AD pathology, exosomes isolated from human AD brain will be analyzed. This part of the study will follow the same biochemical workflow: exosome purification, verification with western blot for the exosome markers Tsg101 and CD63, and multiplexed TMT MS analysis. Taken all together, we
expect this research will begin to clarify the relationship between excitatory synaptic transmission and exosome-based communication in the context of AD-like pathology.

**Disclosures:** D. Graykowski: None. Y. Wang: None. J.N. Savas: None.

**Poster**

**292. Alzheimer's Disease: Omics Approaches**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 292.12/E22

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Support:** NIH T32 5T32AG057468-02

**Title:** Studying hierarchical connectome modularity structure in healthy aging versus Alzheimer's disease patients

**Authors:** *Z. D. MORRISSEY*¹, L. ZHAN², O. A. AJILORE¹, O. LAZAROV³, A. D. LEOW¹; ¹Psychiatry, Univ. of Illinois at Chicago, Chicago, IL; ²Electrical and Computer Engin., Univ. of Pittsburgh, Pittsburgh, PA; ³Dept Anat & Cell Biol, Univ. Illinois, Chicago, Chicago, IL

**Abstract:** In connectomics, modules are a fundamental network component that offers insight into network organization. Neurodegenerative disorders notably disrupt connectivity between brain regions, leading to a breakdown in neuronal communication and modular network architecture. In Alzheimer’s disease (AD), evidence suggests that impairment in key brain networks including the default mode network (DMN) are implicated in AD cognitive decline. To that end, we applied graph theory and the path-length associated community estimation (PLACE) algorithm to study the hierarchical community structure properties of AD patients using DTI data from the Alzheimer’s Disease Neuroimaging Initiative (51 healthy aging subjects (mean age = 69.7), 112 MCI patients (mean age = 71.7), and 39 AD patients (mean age = 75.6)). Using a top-down approach, we found that modularity similarity between healthy-AD notably decreased with further bifurcations; at just 8 communities (Fig. 1A), 18% of nodes were differentially assigned compared to healthy aging controls in areas involved in the DMN, working memory, self-awareness, and language/visual processing (Fig. 1B). In addition, network analyses indicate statistically significant differences between healthy aging and AD subjects in characteristic path length (p < 0.005) as well as network diameter (p < 0.005), indicating that AD structural networks exhibit less efficient network architecture. Node-specific analyses for betweenness centrality, a measure of network integration, likewise indicate the largest group-wise mean differences (healthy-AD) in the posterior cingulate cortex, thalamus, pallidum, hippocampus, and precuneus, suggesting that DMN regions are heavily compromised at the network level (B-H FDR q < 0.2). Together, these results suggest that hierarchical community
structure is compromised in AD networks and offer further insight into how AD pathology affects the network level of the brain.

Disclosures:  
Z.D. Morrissey: None.  
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Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 292.13/E23

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Hong Kong Epigenomics Project

Title: Elucidating retrotransposon dysregulation in Alzheimer’s disease mouse model

Authors: *J. AW, D. LEUNG;
The Hong Kong Univ. of Sci. and Technol., Hong Kong, Hong Kong

Abstract: Transposable elements are mobile stretches of DNA that are ubiquitous in the eukaryotic genome, the largest component of which are retrotransposons. These elements mobilize via RNA intermediates in a “copy and paste” mechanism. Due to their deleterious effects on the genome, most retrotransposons are silenced through mutations, truncations, inversions, deletions and epigenetic mechanisms. However, there is evidence of such sequences being adapted for use in specific cell types. The neuronal gene Arc, a master regulator of synaptic plasticity, is derived from retrotransposon Gag proteins. Moreover, retrotransposons possess cis-regulatory functions. For example, the transcription of the mouse neuronal apoptosis inhibitory protein (Naip) gene initiates from an ORR1E LTR-derived promoter. Recently, retrotransposon dysregulation has been shown to occur in Alzheimer’s disease, but the mechanisms that lead to the dysregulation are yet unclear. In this study, we aim to elucidate the role of these elements in the mouse brains. Using state-of-the-art techniques, we profile transcription and epigenetic marks from four brain regions in the Alzheimer’s disease mouse model R1.40 and wildtype (WT) mice. Our data show that retrotransposons are upregulated in the R1.40 mice, especially in the hippocampus. Interestingly, we also find retrotransposon expression in the WT, hinting at a potential inherent role for retrotransposons in the brain.

Disclosures: J. Aw: None. D. Leung: None.
Support: NRF-2017R1A2B4008456

Title: Functional screening of miR126 targets in *in vitro* and *in vivo* AD models

Authors: H. NOH1, *S. SONG1, K. C. SONNTAG2, H. SEO1;
1Hanyang Univ., Seoul, Korea, Republic of; 2McLean Hospital, Harvard Med. Sch., Belmont, MA

Abstract: Alzheimer’s disease (AD) is chronic neurodegenerative disease caused by loss of neurons and synapses in several brain regions including entorhinal cortex and hippocampus. Alteration of the expression of micro RNA (miRNA), a small non-coding RNA, has been studied for pathological process of several neurodegenerative diseases, as it functions in RNA silencing and post-transcriptional regulation of gene expression. One of the miRNAs, miR126 has been reported as a novel pathological marker of various neurodegenerative diseases such as Parkinson’s disease (PD) and AD, but its pathophysiological targets are poorly understood. In this study, we predicted several genes as potential targets of miR126, using network topologic analysis. To determine transcriptomic changes, we screened the targets of miR126 using next generation sequencing (NGS) analysis in cholinergic neurons after Lenti-miR126 overexpression under AD-like pathological environment induced by amyloid beta 42 (Aβ-42). The selected genes from this screening analysis were functionally evaluated in *in vitro* AD cell model. The function of various target genes of miR126 in this study can reveal the pathophysiological mechanisms and potential therapeutic approaches in AD.


Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 292.15/E25

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant U54AG054345

Title: Estimation of late-onset Alzheimer’s disease progression from post-mortem RNA-seq samples

1Microsoft, Seattle, WA; 2The Jackson Lab., Bar Harbor, ME; 3Sage Bionetworks, Seattle, WA; 4Univ. of Washington, Seattle, WA
Abstract: Background: We infer disease progression for Alzheimer’s disease (AD) using RNA-Seq data from post-mortem brain samples from the Accelerating Medicine Partnership - Alzheimer’s disease (AMP-AD) consortia with a manifold learning method. We test that the estimate of disease progression is concordant with neuropathological and clinical measures of disease and we identify the genetic basis of disease progression based on our estimates of relative disease progression.

Methods: We use a manifold learning approach to learn a tree structured subspace from the RNA-Seq data from the AMP-AD studies. This low-dimensional subspace defines an ordering of samples along the tree - treated as degree of disease progression - based on the similarity in the RNA-Seq profiles. We test for association between the estimated measure of progression and neuropathological, clinical, and cellular measures of disease severity. To identify progression specific pathways, we perform a differential expression analysis on inferred disease progression tree branches and we perform a genome-wide association study to identify loci associated with our quantitative measure of disease severity.

Results: We show that this estimate of disease progression is concordant with neuropathological (p<10^{-4}), clinical (p<10^{-5}), and cellular measures of disease (p<10^{-16}). Our GWAS analysis identifies suggestive associations in ADAMTS14, IL7, and MAN2B1 (p<10^{-5}) and associations in APOE, BIN1, and PTPRD (p<10^{-5}) which have been previously associated with AD or AD endophenotypes. We also identify a potentially ‘resilient’ subset of patients whom may be compensating for prodromal AD associated pathophysiology via activation of protein trafficking, splicing, negative regulation of apoptosis, and prevention of amyloid cleavage.

Conclusions: We develop a novel approach to identify pathways associated with Alzheimer’s disease progression and stage patients given the molecular signature of their disease.


Poster

292. Alzheimer’s Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:/Poster #: 292.16/E26

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: The JPB Foundation
NIH

Title: Chronic gamma entrainment induces a persistent neuroprotective state in mouse model of neurodegeneration

Authors: C. ADAIKKAN, A. MARCO, *K. ABDELAAL, L.-H. TSAI;
Picower Inst. for Learning and Memory, MIT, Cambridge, MA
Abstract: Neurodegenerative diseases, such as Alzheimer’s disease, are characterized by devastating degeneration of brain and cognitive functions. We have recently demonstrated that chronic non-invasive Gamma ENtrainment Using Sensory Stimuli (GENUS) preserves neuronal and synaptic density, limits ventricle expansion, and improves spatial learning and memory in Tau P301S and CK-p25 mouse models of neurodegeneration. Moreover, neuron and microglia specific RNA-sequencing suggest a gene expression shift toward a globally neuroprotective state with reduced neuroinflammation, enhanced synaptic integrity, upregulation of neuroprotective factors, and reduced DNA damage following GENUS. While our findings represented an exciting potential of GENUS for neuroprotection during and up to 24-hour post GENUS regimen, the long-term extent of the observed neuroprotective phenotypes following chronic GENUS has not yet been determined. In the present study, we subjected CK-p25 mice to 1 hour daily GENUS (40 Hz flicker) for 42 days, sacrificed at 7 to 15 days post-GENUS, and then analyzed for extent of detectable neuroprotection. Preliminary analyses revealed that the neuroprotective effects persist for several days after the termination of chronic GENUS. To further examine the molecular mechanism of persistent neuroprotective state induced by GENUS, we assessed epigenetic and gene expression alterations using neuron specific ATAC- and RNA-sequencing, that revealed an increase in chromatin accessibility and upregulation of genes related to synaptic function and vesicle trafficking. By combining pharmacology and GENUS, we are currently establishing the causal role of GENUS induced gene expression alterations to neuroprotection and cognitive processes.

Disclosures: C. Adaikkan: None. A. Marco: None. K. Abdelaal: None. L. Tsai: None.

Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 292.17/E27

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Cure Alzheimer's Fund
CMU Brainhub Fellowship

Title: Amyloid associated epigenomic signatures at promoters of late and early onset Alzheimer’s risk genes in human hippocampal oligodendrocytes

Authors: *E. RAMAMURTHY\textsuperscript{1}, G. WELCH\textsuperscript{2}, J. CHENG\textsuperscript{2}, Y. YUAN\textsuperscript{1}, L. GUNSAUS\textsuperscript{1}, L.-H. TSAI\textsuperscript{2}, A. R. PFENNING\textsuperscript{1};
\textsuperscript{1}Computat. Biol. Dept., Carnegie Mellon Univ., Pittsburgh, PA; \textsuperscript{2}The Picower Inst. for Learning and Memory, MIT, Cambridge, MA
Abstract: Mounting evidence is pointing towards a role for enhancer and promoter regulatory elements in influencing Alzheimer’s disease predisposition and progression through their activity in different cell types. Therefore, to better understand cell type-specific epigenomic mechanisms involved in AD, we isolated major brain cell classes (neurons, microglia and other glia) using fluorescent activated nuclei sorting (FANS) from two brain regions: the dorsolateral prefrontal cortex (dPFC) and the hippocampus of postmortem human brain samples. On each sorted fraction, we performed ChIP-seq of H3K27ac, a histone mark associated with active enhancers and promoters. Then, using peak calling methods and by aggregating information across multiple subjects, we constructed an atlas of H3K27ac peaks enriched in the three cell classes. We compared this atlas with a well powered genome wide association study (GWAS) and report that H3K27ac peaks enriched in microglia have a strong preference for colocalization with AD associated GWAS variants (permutation test adjusted p=9e-06) relative to H3K27ac peaks enriched in neurons and oligodendrocytes. These results support previous observations that microglial and/or myeloid cell gene regulation influences predisposition towards AD.

In parallel, we similarly profiled the cell type-specific epigenomes of postmortem brains of subjects with pathology indicative of late onset Alzheimer’s disease (LOAD). Then, for samples of each sex, brain region and cell type, we compared differences in levels of H3K27ac between subjects with and without amyloid pathology. Our analysis revealed a strong epigenomic signature associated with amyloid load in the human hippocampus that is enriched for promoters of several genes associated with both late and early onset Alzheimer’s disease risk including APP, PSEN1, PSEN2, BACE1, BIN1, PICALM, ADAM10, ADAMTS4, SORL1, FERMT2 and TARDBP (TDP-43). Further, ontological annotation enrichment analysis for genes associated with these peaks revealed processes associated with myelination, amyloid processing and genes differentially expressed upon treatment of monocytes with anti-TREM1 and LPS. We further show that this signature displays a stronger trend in female subjects compared to male subjects. Our study is the first to report genome-wide maps of H3K27ac in different brain cell types. In addition, our analysis reveals an epigenomic module in oligodendrocytes that is likely disrupted in both early and late onset Alzheimer’s disease. This opens up the opportunity to target these specific genomic locations in oligodendrocytes for potential therapeutics.


Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 292.18/E28

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA R01 AG037868
Title: Gray and white matter neurovascular ceruplasmin expression in Alzheimer's disease

Authors: *K. HARGIS-STAGGS*1, E. S. JOHNSON2, P. NELSON3, E. M. BLALOCK4;
1Pharmacol. and Nutritional Sci., 2Dept. of Pharmacol. and Nutritional Sci., 3Univ. of Kentucky, Lexington, KY; 4Dept Pharmacol, Univ. Kentucky Coll Med., Lexington, KY

Abstract: Alzheimer’s disease (AD) is a debilitating and chronic disease targeting the ever-increasing aging population, yet effective therapeutics remain elusive. The majority of AD cases (~90-95%) are sporadic AD (sAD) and a variety of modifiable and unmodifiable risk factors contribute to the development of the disease. For example, aging and being female (~2/3 AD cases) are two key unmodifiable risk factors for sAD, though, it is unclear how this translates into an increased risk. Here, our lab identified a panel of 85 genes that changed with age, worsened with AD, and exacerbated in female sAD subjects. Among these genes, ceruloplasmin (CP) showed robust expression changes in age that were worsened in AD, and exacerbated in females. CP is strongly expressed in neurovascular units (NVUs), especially in white matter. Therefore, we hypothesized that white matter NVUs from the Brodmann area 9 (BA9) region of the frontal cortex will show greater CP expression in sAD females than males. To test this, we used transcriptional profiling of laser-capture microdissected gray and white matter NVUs from FFPE sections in male and female aged control and AD subjects.


Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 292.19/E29

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Institute on Aging-National Institute of Health (R01AG059848)
BrightFocus (A2017346S)
Alzheimer’s Association (AARG-17-528298)
Ben Barres Early Career Acceleration Award (CZI Neurodegeneration Challenge Network)

Title: Single-cell profiling of human neurons with neurofibrillary tangles in Alzheimer’s disease

Authors: *M. OTERO-GARCIA*1, Y. XUE2, Y. DENG4, T. SHAKOURI2, R. KAWAGUCHI3, I. COBOS1;
1Dept. of Pathology, Stanford Univ., Palo Alto, CA; 2Dept. of Pathology, 3Dept. of Psychiatry
and Semel Inst. for Neurosci. and Human Behavior, UCLA, Los Angeles, CA; \(^4\)Dept. of Neurol., The First Affiliated Hosp. of Xi'an Jiaotong Univ., Xi'an, China

**Abstract:** Aggregation of hyperphosphorylated tau in neurofibrillary tangles (NFTs) is closely linked to neuronal death, early memory loss, and the progression of cognitive deficit in Alzheimer’s diseases (AD). Although pathological tau appears to contribute to disease progression via axonal transport defects, synapse dysfunction, and neuroinflammation, our understanding of the cellular and molecular alterations associated with tau aggregation in the brain of AD patients is limited. Here, we developed procedures for high-throughput isolation of individual somas with NFTs from frozen human brain and profiled the transcriptomes of cells with or without NFTs from the prefrontal cortex of AD patients and age-matched controls. By comparing the transcriptomes of single neurons with NFTs to those of neighboring NFT-free neurons, we provide unbiased and precise identification of the cell types exhibiting aggregates. Our data support the previously recognized selective vulnerability of excitatory neurons and further identify the aggregate-prone or resistant subtypes. Differential gene expression analysis between cells with or without NFTs within each cell subtype and across the different cell subtypes identified commonly altered and cell type-specific genes and pathways associated with tau aggregation. Our data provide a framework for improved disease modeling and an unprecedented resource for identifying new biomarkers and targetable molecules and pathways in human AD.

**Disclosures:** M. Otero-Garcia: None. Y. Xue: None. Y. Deng: None. T. Shakouri: None. R. Kawaguchi: None. I. Cobos: None.

**Poster**

292. Alzheimer's Disease: Omics Approaches

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 292.20/E30

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Support:** Alzheimer’s Society of Canada (16 15) the Scottish Rite Charitable Foundation of Canada (15110) the Brain and Behavior Research Foundation (23482) the Department of Defense (PD170089) a Gibby & Friends vs. Parky Award

**Title:** Epigenetic dysregulation of enhancers in neurons is associated with Alzheimer’s disease pathology and cognitive symptoms

**Authors:** *P. LI\(^1\), L. MARSHALL\(^1\), G. OH\(^2\), J. L. JAKUBOWSKI\(^1\), D. GROOT\(^2\), Y. HE\(^3\), T. WANG\(^3\), A. PETRONIS\(^2,4\), V. LABRIE\(^1,2,5\);
Abstract: Epigenetic control of enhancers alters neuron functions and may be involved in Alzheimer’s disease (AD). Here, we identify enhancers in neurons contributing to AD by comprehensive fine-mapping of DNA methylation at enhancers, genome-wide. We examine 1.2 million CpG and CpH sites in enhancers in prefrontal cortex neurons of individuals with no/mild, moderate, and severe AD pathology (n = 101). We identify 1,224 differentially methylated enhancer regions; most of which are hypomethylated at CpH sites in AD neurons. CpH methylation losses occur in normal aging neurons, but are accelerated in AD. Integration of epigenetic and transcriptomic data demonstrates a pro-apoptotic reactivation of the cell cycle in post-mitotic AD neurons. Furthermore, AD neurons have a large cluster of significantly hypomethylated enhancers in the DSCAML1 gene that targets BACE1. Hypomethylation of these enhancers in AD is associated with an upregulation of BACE1 transcripts and an increase in amyloid plaques, neurofibrillary tangles, and cognitive decline.

Abstract: Single cell RNA-seq is rapidly redefining our understanding of cell types in the brain. Limited in the number of genes detected per cell, single cell RNA-seq is too sparse for creating transcriptional regulatory networks (TRNs), which require quantitative expression for all transcription factors and target genes. Bulk tissue RNA-seq provides appropriate coverage of genes for TRNs, but is not cell-type specific. Using single cell RNA-seq from the Allen Brain Atlas, we have redistributed read counts in the AMP-AD bulk RNA-seq for cell-types of the brain, building cell-type specific TRNs. These TRNs represent a directional and mechanistic list of putative transcription factors for nearly all expressed genes in the brain.

We reprocessed all ENCODE brain DHS using Wellington and HINT to identify transcription factor binding sites. We assembled motifs from multiple databases, totaling 1,530 motifs mapping to 1,515 transcription factors. We developed and utilized Transcriptional Regulatory Network Analysis (TReNA), as an R Bioconductor package. Regulatory regions were defined by GeneHancer. TReNA utilizes multiple machine learning techniques to prioritize transcription factors for each target gene. Single cell RNA-seq data from the Allen Brain Atlas was used to define cell types, for redistributing the reads from bulk RNA-seq.

Our TRNs contain a prioritized list of putative transcription factor regulators for all genes in a cell-type specific manner. This information, combined with the footprints (and their genomic locations) allows us to integrate genetic information from GWAS or eQTL analysis to generate testable hypotheses around the functional annotation of variants. These cell-type specific TRNs can be used to identify key transcription factors enriched in gene lists identified in independent perturbation experiments of comparable cell types. We have identified multiple microglia-enriched transcription factors that regulate many differentially and co-expressed genes in AD. These resulting models can be applied to other datasets that generate lists of differentially or co-expressed genes, including single cell RNA-seq data, as well as provide testable hypotheses for non-coding variants of interest. We are actively engaged in testing several hypotheses through experimental means and have made these TRNs publicly available.


Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 292.22/E32

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG054156
Korea Institute of Science and Technology Grant (2E29230, 2E29221, and 2E28030)
Title: Epigenome signatures landscaped by histone H3K9me3 are associated with the synaptic dysfunction in Alzheimer's disease

Authors: M. LEE¹, J. LEE², S. HYEON³, T. D. STEIN⁴, A. C. MCKEE⁵, N. W. KOWALL⁶, J.-I. KIM⁷, D. HWANG⁸, *H. RYU⁹;

Abstract: Despite many advances, the pathogenesis of Alzheimer's disease (AD) and the commonest cause of dementia in the elderly remains incompletely understood. Recently, epigenetic modifications have been shown to play a potential role in neurodegeneration, but the specific involvement of epigenetic signatures landscaped by heterochromatin has not been studied in AD. Herein, we discovered that H3K9me3-mediated heterochromatin condensation is elevated in the cortex of sporadic AD postmortem brains. In order to identify which epigenomes are modulated by heterochromatin, we performed H3K9me3-chromatin immunoprecipitation (ChIP)-sequencing and mRNA-sequencing on postmortem brains from normal subjects and AD patients. The integrated analyses of genome-wide ChIP- and mRNA-sequencing data identified epigenomes that were highly occupied by H3K9me3 and inversely correlated with their mRNA expression levels in AD. Biological network analysis further revealed H3K9me3-landscaped epigenomes to be mainly involved in synaptic transmission, neuronal differentiation, and cell motility. Together, our data shows that the abnormal heterochromatin remodeling by H3K9me3 leads to down regulation of synaptic function-related genes, suggesting that the epigenetic alteration by H3K9me3 is associated with the synaptic pathology of AD.


Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 292.23/E33

Topic: C.02. Alzheimer’s Disease and Other Dementias

Title: The synthetic amyloid beta oligomers induce pathological features associated with Alzheimer’s disease in nonhuman primates
Abstract: As an insidious and slowly progressive neurodegenerative disorder, Alzheimer’s disease (AD) uniquely develops in humans but no other species. Thus, it has been difficult to replicate the progression and typical pathological features of AD in various animal models. The non-human primates share the closest similarities with human and are naturally attractive in modeling AD. However, it still remains largely unclear whether the pathological progress of AD can be reproduced in non-human primates. In this study, synthetic Aβ oligomers (AβOs) were intracerebrally delivered into the cerebral parenchyma of adult cynomolgus monkeys (Maccaca fascicularis). As short as 7 months later, massive Aβ plaques globally developed in the cynomolgus brain as in AD patients. More strikingly, intraneuronal neurofibrillary tangles formed in multiple brain regions and were reminiscent of those in AD patients. AβO-induced monkeys also displayed activated astrocytes and microglia surrounding Aβ plaques and the neuroinflammation was triggered in cynomolgus brain. The degenerative neural cells that closely associated with Aβ plaques were detected in temporal cortex of AβO-induced cynomolgus monkeys. Finally, we confirmed that delivering AβOs into parenchyma but not lateral ventricle induced multiple AD-like features in cynomolgus monkeys. Together, cynomolgus monkeys rapidly developed the early pathological features of AD upon synthetic AβOs infusion, suggesting that the AβO-induced cynomolgus monkey might be a promising research model for uncovering the early pathogenetic events of AD.

Disclosures: F. Yue: None. S. Feng: None. T. Zhang: None. C. Yue: None. N. Jing: None.

Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 292.24/E34

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH grant AG0577277
AARF 16-433173

Title: Role of Unc5c, an Alzheimer’s risk gene in late-onset AD in a novel mouse model

Authors: *D. KARUNAKARAN¹, N. KHALATYAN¹, A. KHATRI¹, B. WANG¹, K. R. SADLEIR¹, J. POPOVIC¹, R. J. WATTS²,³, J. ATWAL³, J. N. SAVAS¹, R. J. VASSAR¹;
¹Neurol., Northwestern Univ., Chicago, IL; ²Denali Therapeut. Inc., South San Francisco, CA; ³Neurosci., Genentech, South San Francisco, CA
Abstract: Alzheimer’s disease (AD) is characterized by amyloid plaques, neurofibrillary tangles, and synaptic and neuronal loss. Recently, a rare autosomal dominant coding mutation, T835M, was discovered in the Un-coordinated 5c (UNC5C) netrin receptor gene that segregated with late-onset AD (LOAD). T835M alters a conserved amino acid in the hinge region of the UNC5C death domain, suggesting the mutation may increase apoptosis. Indeed, in primary hippocampal neurons, overexpression of UNC5C T835M increased cell death in response to neurotoxic stimuli including beta-amyloid (Aβ). These results suggest a mechanism by which UNC5C T835M may confer increased risk of LOAD, however the effects of this mutation in an AD animal model have not yet been explored. We hypothesize that the T835M mutation predisposes to LOAD by exacerbating neuronal death, as observed in the 5XFAD brain, via increased sensitivity to Aβ-induced neurotoxicity and UNC5C death domain activation. Toward this end, we generated a mouse knock-in (KI) model of Unc5c T835M and crossed it with the 5XFAD mouse model of amyloid pathology and neuron loss. Our preliminary results show that homozygous KI mice are very similar to WT littermate controls in terms of the histology, protein and RNA expression or in cell death. However, proteomics analysis of KI and wildtype mice brains showed upregulation of apoptotic proteins and down-regulation of neuronal proteins. We are further investigating mechanisms of cell death and distal phenotypes in 5XFAD; Unc5c T835M KI mice by biochemical, cellular, and unbiased proteomics approaches. Although neuron loss is a cardinal feature of AD, the molecular mechanism of cell death in AD is still unclear. We expect our results to provide valuable insight into the role of UNC5C T835M mutation in Aβ-associated cell death, and thereby identify novel therapeutic targets to prevent neuron loss in AD.


Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 292.25/E35

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RO1 AG043375
PO1 AG107617
PO1 AG014449
R01 AG055328
R56 AG052524
Title: Quantitative analysis of endosomal pathology and basal forebrain cholinergic neuron (BFCN) morphology in young and aged Ts65Dn mice following maternal choline supplementation (MCS)

Authors: *M. K. GAUTIER1,3, M. J. ALLDRED1,4, C. M. KELLEY7, A. SALTZMAN1, S. H. LEE2, S. D. GINSBERG1,4,5,6;


Abstract: Down syndrome (DS) is a genetic disorder caused by triplication of chromosome 21. By middle-age DS individuals develop Alzheimer’s disease (AD) neuropathology including amyloid-β plaques and neurofibrillary tangles, abnormal early endosomes, and degeneration of cholinergic basal forebrain neurons. The trisomic Ts65Dn mouse model of DS/AD recapitulates several key aspects of DS and AD pathology, including cognitive dysfunction, loss of basal forebrain cholinergic neurons (BFCNs), and dysregulation of the endosomal-lysosomal system. We hypothesize that BFCN degeneration stems from deficient neurotrophic support, a biproduct of aberrant septohippocampal endosomal transport. An inexpensive treatment modality, maternal choline supplementation (MCS) attenuates overexpression of genes underlying endosomal defects, indicating MCS may improve endosomal transport. We previously demonstrated that reproducible quantification of endosomal pathology is achievable via z-stack reconstruction using the 3D modeling program Imaris (Bitplane). Results indicate that 11 month old (MO) Ts65Dn mice had significantly increased Rab5-immunoreactive early endosomes per BFCN compared to disomic (2N) littermates when these offspring were fed a normal choline diet. MCS administered during the perinatal period decreased the average number of early endosomes per BFCN in both Ts65Dn and 2N mice, irrespective of genotype and endosomal pathology. To explore the extent to which early choline delivery can affect endosomal phenotype and BFCN survival, we compared Ts65Dn mice at two time-points: 3-4 MO (pre-BFCN degeneration) and 10-12 MO (post-BFCN degeneration). BFCNs were dual labeled with antibodies directed against Rab5 and choline acetyltransferase (ChAT): an early endosome marker and a cholinergic marker, respectively. Quantitative analysis of endosomal compartments was performed using Imaris 9.1 software. Unbiased neuronal counts and density measurements, as well as morphometric analysis of BFCN size, were achieved utilizing specialized macros in ImageJ. Preliminary results reveal no significant differences between Ts65Dn and 2N littermates at 3-4 MO. Comparison of early endosome quantification between young and aged cohorts indicates that the average number of Rab5-positive endosomes per BFCN increases with age regardless of genotype. MCS treatment in utero lessens the magnitude of this increase in both Ts65Dn and 2N offspring at 11 MO, suggesting that early-life MCS treatment may confer long-lasting neuroprotective benefits on vulnerable neuronal populations. One mechanism of action may be through the rescue of endosomal pathology.

Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 292.26/E36

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RO1 AG043375
PO1 AG107617
PO1 AG014449
R01 AG055328
R56 AG052524

Title: RNA-sequencing (RNA-seq) of medial septal nucleus basal forebrain cholinergic neurons (BFCNs) within the Ts65Dn mouse model of Down syndrome (DS) and Alzheimer’s disease (AD) identifies dysregulated transcriptional pathways


Abstract: Basal forebrain cholinergic neuron (BFCN) loss is a hallmark of individuals with Down syndrome (DS) and Alzheimer’s disease (AD). DS subjects also experience hippocampal CA1 pyramidal neuron degeneration and synaptic loss. Further, they develop AD pathology including neurofibrillary tangles and amyloid plaques by the third decade of life. The septohippocampal pathway, including BFCNs which project to CA1 pyramidal neurons exhibit selective vulnerability in both DS and AD patients during disease progression. Current therapeutics have been unsuccessful in slowing disease progression, likely due to the complex pathological interactions and dysregulated pathways that are still poorly understood. The Ts65Dn mouse model recapitulates both the cognitive and morphological deficits of DS and AD, including BFCN degeneration. We utilize this trisomic mouse model to further understand the mechanistic pathways that underlie BFCN degeneration. We performed high-throughput, single population RNA sequencing (RNA-seq) to assess expression level changes in BFCNs from the medial septal nucleus (MSN) in the Ts65Dn mouse and in normal disomic (2N) littermates. Expression profiles from MSN BFCNs were generated by laser capture microdissection (LCM) to isolate ~500 choline acetyltransferase-immunoreactive neurons in adult Ts65Dn and 2N littermates. This procedure enabled quantitative analysis of mRNAs and noncoding RNAs to help understand mechanism(s) underlying neurodegeneration, and link these expression level changes to established pathological hallmarks and cognitive decline for therapeutic development.
in human DS and AD. RNA-seq library preparation was performed on isolated RNA species to determine the viability RNA-seq from individual neuronal cell types for downstream transcriptional analysis. Results indicated unique transcriptomic profiles for MSN BFCNs from Ts65Dn and 2N littermates at ~6 months of age. We further analyzed the resulting differences utilizing Ingenuity Pathway Analysis to link the changes seen in gene expression profiles to canonical pathways, along with dysregulated disease networks and aberrant physiological functions. Preliminary results indicate pathological changes in specific genes within a multitude of pathways, including RNA expression and molecular transport. Validation strategies include qPCR from LCM-Nissl stained neurons and protein assays from regional tissue dissections. The resultant expression profiles are posited to provide key information leading to mechanistic understanding of selective vulnerability within the septohippocampal circuit in models of DS and AD for therapeutic intervention.


Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 292.27/E37

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01 AG043375
       PO1 AG107617
       PO1 AG014449
       R01 AG055328
       R56 AG052524

Title: RNA-sequencing (RNA-seq) of distinct deep and superficial hippocampal CA1 pyramidal neuron populations in a mouse model of Down syndrome (DS) and Alzheimer’s disease (AD)


Abstract: People with Down syndrome (DS) have intellectual disability (ID) and develop hallmark Alzheimer’s disease (AD) pathology during midlife. There are several circuits underlying memory and executive function in the DS and AD brain that are particularly
vulnerable, most notably synaptic and neurodegeneration of the interconnected basal forebrain cholinergic system and the hippocampus. A fundamental lack of knowledge exists as to the etiology and mechanisms of disease progression within the septohippocampal circuit in DS, AD, and relevant models. Studies by Masurkar et al. (2017) demonstrate murine CA1 pyramidal neurons have two distinct sublayers along the radial axis, deep and superficial neurons, that also differ along the transverse axis with regard to innervation and function. While this mediolateral innervation pattern is postulated to have an effect on spatial and non-spatial learning and memory, there is a current lack of knowledge linking gene and encoded protein expression to the learning and memory behaviors thought to be associated with these subregions. Herein, we combined laser capture microdissection of these individual CA1 pyramidal neuron populations (deep and superficial; approximately 500 Nissl-stained neurons per assay) with RNA-sequencing (RNA-seq) to determine differences in gene expression levels that may underlie spatial and non-spatial memory associated with proper CA1 structure and function. Gene expression profiles were accrued from a trisomic mouse model of DS and AD, Ts[Rb(12.17<sup>16</sup>)2Cje (Ts2), which presents phenotypic and pathological features similar to the Ts65Dn mouse model as well as the human condition including synaptic loss and degeneration of basal forebrain cholinergic neurons. Importantly, trisomic mice have deficits in spatial and non-spatial learning and memory linked to the septohippocampal pathway and CA1 pyramidal neurons. Preliminary RNA-seq results indicate that deep and superficial CA1 neurons represent distinct CA1 pyramidal neuron populations with differential expression profiles. We are determining whether they also have significantly different noncoding RNA (ncRNA) profiles. Preliminary profiling results support the hypothesis that both deep and superficial CA1 pyramidal neurons have dysregulated gene expression in Ts2 mice, and we have undertaken pathway analysis to see which individual genes as well as mechanistic pathways are specifically impacted. Understanding expression profiles of distinct cell populations involved in learning and memory may translate into novel treatment options for DS and could possibly lead to novel treatments for cognitive decline in AD.


Poster

292. Alzheimer's Disease: Omics Approaches

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:/Poster #: 292.28/E38

Topic: C.02. Alzheimer's Disease and Other Dementias

Support:  "Retina France" grant
          Fondation de France grant

Title: First identification of ITM2B partners in human retina
Authors: *J. WOHLSCHELEGEL*, M. ARGENTINI, T. LÉGER, C. CONDROYER, C. ZEITZ, I. Audo;

1The Vision Inst., PARIS, France; 2Mass Spectrometry Lab., Inst. Jacques Monod, PARIS, France

Abstract: ITM2B is a type II ubiquitous protein which role remains unclear. ITM2B mutations have been associated with different disorders: mutations leading to longer mutant proteins have been reported in two distinct Alzheimer-like autosomal dominant disorders with early-onset progressive dementia, cerebellar ataxia, spasticity associated and variable degree of deafness, early onset-cataract and vascular retinal abnormalities. Both disorders share neurological features including severe cerebral amyloid angiopathy, non-neuritic plaques, and fibrillary tangles as in Alzheimer Disease. Our group reported a missense mutation in ITM2B underlying an unusual retinal dystrophy with no dementia. This finding would suggest a specific role of ITM2B in the retina. A new alternative transcript leading to exon 1 skipping and resulting in a shorter protein was recently reported. The identification and characterization of retinal transcripts for ITM2B as well as the corresponding proteins and protein partners would bring new insights to the cellular functions of ITM2B in the retina. Using RT-PCR, we demonstrated the presence of at least two transcripts for ITM2B in human retina leading to a 266- and 210-amino acid (aa) proteins. We then performed ITM2B immunoprecipitation followed by quantitative proteomic analysis on normal human retina using two distinct antibodies: one (sc-374362, Santa Cruz), reacting against the 266-aa isoform whereas the other (PA531441, Thermofisher) recognizing both the 266- and 210-aa isoforms of ITM2B. Two pools of ITM2B protein partners were identified for each antibody and compared using ClueGo plugging for functional annotation. Besides common protein partners, specific protein partners for the 210-aa isoform were identified suggesting a role in mitochondrial homeostasis. To our knowledge, this is the first report of ITM2B interactome in human retina. Our findings support a unique and specific role for the newly identified ITM2B protein isoform. Further disease modeling using retinal organoids derived from patients will allow to investigate how the corresponding mutant isoforms impact on protein interactions and will bring new insights in the molecular mechanisms associated with ITM2B-related retinal disease.


Poster

293. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 293.01/E39

Topic: C.02. Alzheimer's Disease and Other Dementias
Support: BRiC INAIL 2016-DIMEIL17

Title: Noise-induced hearing loss accelerates cognitive decline in a mouse model of Alzheimer’s disease

Authors: S. COCCO1, F. PACIELLO2, M. RINAUDO1, G. CONFORTO1, D. TROIANI1, A. R. FETONI2,3, M. V. PODDA1,3, G. PALUDETTI2,3, *C. GRASSI1,3;
1Inst. of Human Physiol., 2Inst. of Otolaryngology, Univ. Cattolica del Sacro Cuore, Roma, Italy; 3Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Italy

Abstract: Chronic exposure to noise induces hearing loss due to cochlear damage within the organ of Corti. Noise-induced peripheral damage also up-spreads to auditory cortex, causing both functional (i.e., reduced basal synaptic transmission) and structural (i.e., reduced spine density in layer 2/3 pyramidal neurons) alterations (Paciello et al., 2018). Epidemiological evidence suggests that hearing loss might affect cognitive functions. Here we investigated the effects of noise exposure on memory performances in a mouse model of Alzheimer’s disease (AD) to evaluate if hearing loss accelerates cognitive decline in AD. Transgenic mice developing a time-dependent AD-like phenotype (i.e., male 3×Tg-AD) were exposed to noise (100 dB, 60 min/day for 10 days) at 2 months of age and they were studied at 3, 6 and 9 months. Auditory brainstem response (ABR) recordings were used to evaluate auditory function. To investigate basal synaptic transmission, electrophysiological recordings were performed at layer 2/3 horizontal connections of auditory cortex brain slices. Novel object recognition (NOR) and open field (OF) tests were performed to evaluate recognition memory and locomotor activity, respectively. Noise exposure induced a significant auditory threshold elevation in 3×Tg-AD mice at each time point studied thus worsening age-related hearing loss. In particular, noise exposure caused a threshold shift of about 35, 15 and 15 dB at middle-high frequencies (12-32 kHz) in 3×Tg-AD mice of 3, 6 and 9 months compared to age-matched controls (P=0.0002, P=0.0004 and P=0.006, respectively). At electrophysiological level, basal synaptic transmission at layer 2/3 horizontal connections was impaired in 3×Tg-AD mice exposed to noise compared to controls (P<0.001 at 3 months, P<0.001 at 6 months, P=0.01 at 9 months). At the behavioral level, cognitive decline was clearly manifested in male 3×Tg-AD mice at 9 months of age, as revealed by NOR tests assessing short-term (NOR STM: P=0.04, 9-month-old vs. 6-month-old 3×Tg-AD mice) and long-term memory (NOR LTM: P=0.03, 9-month-old vs. 6-month-old 3×Tg-AD mice). Of note, noise-induced hearing loss anticipated cognitive deficits inducing memory alterations at 6 months of age (NOR STM: P<0.001; NOR LTM: P=0.01). No changes of locomotor activity were observed in noise-exposed 3×Tg-AD mice compared to controls at any studied ages (P>0.05). Our findings provide novel evidence that hearing loss may accelerate cognitive decline in Alzheimer’s disease and pave the way to further studies investigating the cross-talk between auditory cortex and brain areas directly involved in cognitive functions.

Poster

293. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 293.02/E40

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS074969
          NIH Grant AG064902

Title: Amyloid precursor protein and Aβ in the ear and auditory system - Assessment of possible mechanistic relationships between hearing loss and cognitive decline in Alzheimer’s disease

Authors: *C. M. YUDE1, D. F. WOZNIAK1, K. K. OHLEMILLER2, M. E. WARCHOL2, M. A. RUTHERFORD2, J. R. CIRRITO3;
         1Psychiatry, 2Otolaryngology, 3Neurol., Washington Univ., Saint Louis, MO

Abstract: Many studies demonstrate a relationship between hearing loss and Alzheimer’s disease (AD), in which the degree of hearing loss predicts the rate of cognitive decline (Lin et al., 2013, Hardy et al., 2016). Gates and colleagues (2002) reported that central auditory processing dysfunction may precede the onset of clinical dementia and can be detected many years before the diagnosis of AD. Whether these changes in hearing function occur because of a common underlying pathology or speed up the progression of AD due to the loss of sensory input remains unknown. Basic research into the mechanisms driving the link between hearing loss and AD are needed to provide a better understanding of this relationship. We hypothesize that increased soluble Aβ in the central auditory system drives auditory processing deficits that are detectable before the onset of cognitive impairments. Using the APP/PS1 mouse model of AD and their wildtype littermates (WT), we stained for APP/Aβ in the cochlea, focusing on the afferent synapses between sensory hair cells and auditory nerve fibers. We measured acoustic startle response and prepulse inhibition (PPI) of the acoustic startle response in a group of APP/PS1 and WT mice prior to the development of cognitive deficits, to identify any changes in behaviors involving auditory processing. Soluble and insoluble Aβ levels were measured in the brain and correlated with measures of startle response and PPI. We found an exaggerated startle response in APP/PS1 mice compared to WT littermates, and a significant correlation between soluble Aβ levels in the brain and deficits in PPI. Soluble Aβ could impact central auditory function/processing which could lead to deficits in cognitive function. Understanding factors driving the link between hearing loss and AD may shed light on AD pathology and progressive functional deficits. In addition, hearing diagnostics could be an early behavioral biomarker of emerging AD and may be useful low-cost, clinical screening tools for identifying patients that may benefit from preventative therapies before the appearance of overt cognitive symptoms.

Poster

293. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program#/Poster #: 293.03/E41

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CONACyT CB 250870
         PAPIIT-UNAM IN208616

Title: Intrahippocampal administration of amyloid beta 42 diminishes catecholaminergic axons and disrupts spatial contextual memory

Authors: *L. LANDA NAVARRO, C. VELÁZQUEZ DELGADO, F. BERMÚDEZ RATTONI;
         Neurociencias cognitivas, UNAM IFC, Ciudad de México, Mexico

Abstract: Alzheimer’s disease (AD) is the most prevalent type of dementia in the elderly. Its major characteristics are amyloid-beta (Aβ) aggregates, neurofibrillar tangles of hyperphosphorylated tau, synaptic failure and neuritic dystrophy that lead to cognitive impairment and memory loss.

It has been reported that increased Aβ deposition or its exogenous administration reduces catecholaminergic neurotransmission, impairs recognition memory and converts long-term potentiation in long-term depression in the cerebral cortex and dorsal hippocampus. Recent evidence from our laboratory has shown that Aβ pathologic alterations, in a transgenic mouse model for AD, coincide with catecholaminergic neurotransmission disruption and recognition memory impairment. Moreover, recently we have shown data that links catecholaminergic input into the hippocampus with the encoding of novel contextual information. In this paper, we aim to evaluate the effects of exogenous Aβ administration on long-term spatial and contextual memory evocation and the induced changes in the catecholaminergic system.

Methods: In a Morris Water Maze (MWM) task, we measured the number of crosses with one-way ANOVA Fisher LSD. Also, in a location memory task (OLM) we measured the preference of a novel object location with a two tailed Student's t test. To evaluate the length of tyrosine hydroxylase (TH +) and microtubule-associated protein 2 (MAP2), stereology analyses were evaluated using two-way Fisher LSD ANOVA.

We found that the intrahipocampal administration of Aβ after memory acquisition and before evocation impairs spatial memory (F4,39 = 6.256, P = 0.0005), codification of novel contextual information (t9= 1.446, p= 1.822) and decreases TH + axons after 24 h, but not after five days (F2.21 = 10.58, P = 0.0007). While no differences were found when analyzing the MAP2 +
axons (F2,8 = 1,351, P = 0.3123).
Catecholaminergic neurotransmission and spatial memory evocation are disrupted by intrahippocampal Aβ administration. These findings suggest the importance of the catecholaminergic system in AD that is relevant for the development of new therapeutic targets.

Disclosures: L. Landa Navarro: None. C. Velázquez Delgado: None. F. Bermúdez Rattoni: None.

Poster

293. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 293.04/E42

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Paul B. Beeson Emerging Leaders Career Development Award in Aging 1
K76AG054772
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Alzheimer’s Drug Discovery Foundation
Bernard L. Schwartz Award for Physician Scientists to A.C.P

Title: Divergent roles of astrocytic versus neuronal glutamate transporter EAAT2 deficiency on cognition and overlap with aging and Alzheimer’s molecular signatures

Authors: A. SHARMA¹, S. KAZIM¹, C. LARSON², A. RAMAKRISHNAN¹, J. GRAY², B. MCEWEN², P. ROSENBERG³, L. SHEN¹, *A. C. PEREIRA¹;
¹Icahn Sch. of Med., New York, NY; ²The Rockefeller Univ., New York, NY; ³Boston Children’s Hosp., Boston, MA

Abstract: EAAT2 is the major glutamate transporter in the brain expressed predominantly in astrocytes and at low levels in neurons and axonal terminals. EAAT2 expression is reduced in aging and sporadic AD patients’ brains. EAAT2’s role in cognitive aging and its associated mechanisms remain largely unknown. Conditional deletion of astrocytic EAAT2 with GFAP-CreERT2 and neuronal EAAT2 with Syn-Cre mouse lines in aging mice results in spatial reference learning and long-term memory deficits. Astrocytic, but not neuronal EAAT2 deletion, leads to early deficits in short-term memory and learning. Transcriptomic analysis of hippocampus from astrocytic EAAT2 deficiency demonstrates dysfunction of immune pathways, which negatively correlate with hippocampus dependent cognitive behavior in mice. Astrocytic, not neuronal EAAT2 deficiency in the hippocampus also has overlapping transcriptomic signature with human aging and Alzheimer’s disease.

Poster

293. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: /Poster #: 293.05/E43

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01-AG056060

Title: A novel epigenetic mechanism for synaptic and cognitive deficits in Alzheimer’s disease

Authors: *J. B. WILLIAMS, Q. CAO, W. WANG, Z. YAN; Physiol. and Biophysics, Univ. At Buffalo Jacobs Sch. of Med. and Biomed. Sci., Buffalo, NY

Abstract: Cognitive deficits associated with Alzheimer’s disease (AD) have been linked to synaptic dysregulation in the prefrontal cortex (PFC), a brain region involved in ‘executive’ functions, including working memory and decision making, however, the exact mechanisms remain to be elucidated. We hypothesize that the alteration of transcription of genes regulating synaptic functions in AD could be due to abnormal epigenetic changes in PFC. Consistent with it, we have found the significantly elevated histone 3 lysine 4 trimethylation (H3K4me3) and its catalyzing enzyme Smyd3 in PFC from AD human postmortem tissues and P301S Tau transgenic mouse model of AD. Behavioral assays indicated that systemic administration of the Smyd3 inhibitor, BCI-121, rescued spatial memory and novel object recognition memory deficits in P301S Tau mice. The Smyd3 inhibitor also restored the level of synaptic AMPA and NMDA receptors in PFC of P301S Tau mice. Using chromatin immunoprecipitation sequencing (ChIPseq), we discovered that in PFC of P301S Tau mice, there was the significantly increased enrichment of H3K4me3 at the promoter region of Fbxo2, an E3 ubiquitin ligase controlling the degradation of NMDA receptor subunits, which was associated with the higher level of Fbxo2 mRNA revealed by qPCR assays. These data suggest that the elevated H3K4me3 methyltransferase Smyd3 in AD induces the upregulation of the E3 ligase Fbxo2 in PFC, leading to the increased degradation of NMDARs and cognitive deficits. By targeting the epigenetic factor Smyd3, it provides a novel strategy to restore synaptic function and cognitive behaviors in AD.

Disclosures: J.B. Williams: None. Q. Cao: None. W. Wang: None. Z. Yan: None.
Progressive deterioration of cellular and network dynamics in hippocampus of the novel Alzheimer's mouse model App^{NL-G-F}

Authors: *L. ARROYO GARCÍA, Sr, Y. ANDRADE-TALAVERA, H. BALLEZA-TAPIA, A. G. ISLA, P. NILSSON, A. FISAHN; Neurogeriatrics, Karolinska Institutet, Stockholm, Sweden

Abstract: Alzheimer’s Disease (AD) is one of the most devastating neurodegenerative diseases, characterized by neuronal loss and decrease in cognitive capabilities. The exact cause of AD is still unknown, but the disease involves the misfolding and aggregation of amyloid-β peptide (Aβ). Previously, different mouse models attempted to mimic AD pathology by overexpressing the Aβ precursor protein (APP). This approach generates unwanted side effects in the animals due to the high unphysiological APP levels that interfere with data analysis. Recently, a new APP knock-in mouse model (App^{NL-G-F}) has been generated that shows an aggressively increase of Aβ42 and Aβ42/Aβ40 ratio without APP overexpression and avoids the unwanted side effects of APP overexpression models. These mice develop Aβ plaques from two months of age and cognitive impairment at six months of age.

The aim of this project is to use the App^{NL-G-F} mouse model to investigate the cellular mechanisms responsible for aberrant network oscillations in the gamma-frequency band (30-80Hz) and the neuronal dysfunction that has been observed in AD patients. We used 2-, 4- and 6-months old App^{NL-G-F} and wild type (WT) mice to evaluate their capacity to generate gamma oscillations, to characterize the electrophysiological state (neuronal E_{m}, EPSCs, IPSCs) of fast spiking interneurons (FSP) and pyramidal cells (PC) in the hippocampal CA3 region and to evaluate the action potential synchronization of these neuronal classes during gamma oscillations. Our results show gamma oscillation-impairment in the App^{NL-G-F} mice at 2-months old, and a progressive further deterioration at 4- and 6-months of age. Also, we found alterations in FSP and PC action potential synchronization across the different ages. Together, these results confirm previous acute Aβ studies and provide further evidence for the network and cellular mechanism responsible for the neuronal dysfunction and gamma oscillation disruption in AD. In upcoming work, we will use the App^{NL-G-F} model for evaluation of novel treatment approaches.

Poster

293. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 293.07/F1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01 AG055328

Title: Changes in dentate gyrus granule cell spontaneous excitatory postsynaptic potentials and evoked responses to perforant path stimulation evaluated at 2 months of age in the Tg2576 mouse model of Alzheimer's disease neuropathology

Authors: *D. ALCANTARA-GONZALEZ, E. CHARTAMPILA, H. SCHARFMAN; Ctr. for Dementia Res., Nathan S. Kline Inst. For Psychiatric Res., Orangeburg, NY

Abstract: Increasing evidence suggests that Alzheimer's disease begins earlier than the age when there is classic neuropathology, i.e., amyloid-β plaques and neurofibrillary tangles. In animal models, we previously showed abnormalities as early as 5 weeks of age (Kam et al., 2017). The abnormalities occurred in two mouse models where the precursor to amyloid-β is mutated and overexpressed (Tg2576, APP23). The EEG showed interictal spikes in sleep that increased with age. The spikes were recorded using electrodes over the cortex and hippocampus, and the data suggested emergence in hippocampus, especially in a mouse model of Down's syndrome (Ts65n). To determine changes that might be responsible for interictal spikes, recordings were made in hippocampal slices. Using extracellular recording, the dentate gyrus showed differences between Tg2576 mice and controls (WT). Granule cell (GC) layer recordings showed a larger evoked population spike without a change in the evoked field EPSP in response to electrical stimulation of the major glutamatergic input, the perforant path. GCs were then patched from mice that were 2-2.5 months of age. Spontaneous EPSPs (sEPSPs) showed an increased frequency in Tg2576 mice relative to WT (1.29 ± 0.10 events/s, n=16 vs 2.2 ± 0.18, n=21, respectively; p=0.0003). There was a small but significant decrease in sEPSP amplitude (0.49 ± 0.03 vs 0.43 ± 0.03 mV, respectively; p=0.046). To clarify if the changes in excitatory events could be related to altered intrinsic excitability, intrinsic properties were studied. GCs from Tg2576 mice showed a more hyperpolarized resting membrane potential (RMP; -76.30 ± 1.97 mV, n=22 vs -71.32 ± 1.70, n=19; p=0.034), reduced input resistance (226.1 ± 17.6 vs 271.6 ± 16.4 MΩ; p=0.032), and the time constant was reduced (23.9 ± 1.5 vs 29.9 ± 1.7/sec, p=0.006). The action potential at threshold (evoked by direct current injection from RMP) exhibited an increased time to peak (1.31 ± 0.08 vs 1.08 ± 0.10 ms; p=0.034) and a reduction in the maximum rate of rise (224.3 ± 16.9 vs 283.9 ± 23.3 µV/ms; p=0.021). These data suggest that the changes in sEPSPs are accompanied by altered intrinsic properties. Many intrinsic properties showed decreased excitability. Therefore, the increased frequency of sEPSPs in Tg2576 mice is unlikely
to be a result of changes in intrinsic properties. However, the small decrease in sEPSP amplitudes could be related to the changed intrinsic properties. Together the results suggest several alterations in dentate gyrus GCs in a very early stage of life of the Tg2576 mouse model, supporting the view that many changes occur long before plaque deposition.

**Disclosures:**  
D. Alcantara-Gonzalez: None. E. Chartampila: None. H. Scharfman: None.

**Poster**

**293. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models II**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 293.08/F2

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Support:**  
NIH Grant RF1NS110054  
NIH Grant RO1NS096730  
NIH Grant K99AG059893

**Title:** MRI histopathology correlations of amyloid related imaging abnormalities (ARIA) in post mortem human brain samples

**Authors:**  
*A. SCHERLEK*¹, D. BOCHE², J. A. R. NICOLL², B. J. BACSKAI¹, A. J. VAN DER KOUWE¹, S. M. GREENBERG¹, M. P. FROSCH¹, S. J. VAN VELUW¹;  
¹Massachusetts Gen. Hosp., Boston, MA; ²Univ. of Southampton, Southampton, United Kingdom

**Abstract:** Amyloid-Related Imaging Abnormalities (ARIA) are MRI-defined adverse events frequently observed in the context of anti-Aβ immunotherapy trials in patients with Alzheimer’s disease (AD). ARIA-H include cerebral microbleeds (CMBs) and cortical superficial siderosis (cSS) and ARIA-E vasogenic edema. The underlying histopathology and mechanisms of ARIA remain unknown, although coexisting Cerebral Amyloid Angiopathy (CAA) seems to be a risk factor. We used *ex vivo MRI in post mortem* human brain samples from patients that underwent anti-Aβ immunotherapy during life to determine presence and underlying histopathology of ARIA. Cases were selected from the long-term follow-up study of patients who enrolled in the first clinical trial of active Aβ immunization for AD (AN1792; Elan Pharmaceuticals Inc) and consented to brain donation. 13 cases with available formalin-fixed brain slabs were selected: 11 immunized and 2 placebo. One coronal slab from the occipital lobe per case was imaged on a 7T MRI scanner. The protocol included a T2-weighted (voxel size 300 µm³) and a T2*-weighted gradient echo sequence (voxel size 200 µm³). Each scan was screened for possible ARIA-E and ARIA-H. Representative samples were cut in serial sections and stained with Hematoxylin & Eosin (H&E), Perl’s Prussian blue (for iron), and Von Kossa (for calcifications). The cases were on average 80 ± 9 years old at time of death and mean survival time after first immunization was
110 ± 52 months [range 20-184]. There was evidence of plaque removal in 10/11 (91%) of the immunized cases, as previously reported. On MRI, evidence of possible ARIA-E was observed in 5/11 (45%) immunized cases, appearing as diffuse T2-hyperintensities in the cortex or white matter. No evidence of possible ARIA-E was observed in placebo cases. Histopathology of areas with ARIA-E showed tissue loss and vacuolization on H&E-stained sections, without iron accumulation. Evidence of possible ARIA-H was observed on MRI in 5/11 (45%) immunized cases, appearing as either focal or disseminated cSS. No CMBs were observed on MRI in any of the cases. Histopathology of areas with cSS showed iron-positive vessels and neurons that also stained positive for calcium. Some degree of iron and calcifications was also observed in placebo cases. These preliminary observations suggest presence of ARIA in post mortem brain samples of patients that underwent anti-Aβ immunotheraphy during life. The pathological findings confirm edema and old bleeding events as the substrate of ARIA-E and ARIA-H. Ongoing work is aimed at increasing the number of control cases to determine the specificity of these findings as well as assessing CAA severity in areas with ARIA.


Poster

293. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 293.09/F3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA R21 AG053067
        2 T32 NS 61788-11

Title: Noradrenergic denervation-supersensitivity in the TgF344-AD rat driven by β-adrenergic receptors

Authors: *A. GOODMAN, L. L. MCMAHON;
        Cell Developmental and Integrative Biol., Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: The locus coeruleus (LC) is the sole source of norepinephrine (NE) for most of the brain and maintains everything from cognitive abilities to mood and even normal sleep-wake cycles. Despite the importance of the LC in maintaining a wide-range of cognition/behavior, it is exceedingly understudied in the context of neurodegenerative diseases. In Alzheimer’s disease (AD) the deposition of hyperphosphorylated tau (pTau) first occurs in the LC, and its degradation is correlated with the transition into amnestic mild cognitive impairment. Selective ablation of the LC in animal models of AD cause exacerbation of pathology and
synaptic/cognitive symptoms. Furthermore, degeneration of the noradrenergic (NA) system causes an upregulation of most adrenergic receptors (ARs) leading to a supersensitivity to agonist receptor activation. The novel TgF344-AD rat (containing the APPSwe and PSEN1DeltaE9 mutations driven by a mouse prion promoter) is one of the most comprehensive models of human AD and the first rodent model to display endogenous pTau accumulation in the LC. Previous studies in our lab in the TgF344-AD rat found deficits in basal synaptic transmission at medial perforant path (MPP) to dentate granule cell (DGC) synapses and pathologically increased long-term potentiation (LTP) as early as 6-months of age. In new studies we found that NA fiber density in the dentate gyrus is reduced by an average of 37% (p=0.03) as early as 6-months (and similar amounts in other hippocampal subfields), but in extracellular dendritic field potential recordings at MPP-DGC synapses in acute slices, synaptic potentiation induced by NE [40µM] or the β-AR agonist isoproterenol (ISO) [1µM] is unchanged between Tg and non-Tg littermates. However, by 9-months, [1µM] ISO induces heightened potentiation that is longer lasting (p<0.01) in the Tg rats, suggesting increased sensitivity of β-ARs that is masked with higher concentrations of ISO [10µM]. Furthermore, Tg rat slices have a faster time to reach peak reaction to the application of NE which is abolished with the addition of the β-AR antagonist propranolol [10µM]. Collectively these results are consistent with a heightened function of β-ARs following NA denervation of the hippocampus.

Disclosures: A. Goodman: None. L.L. McMahon: None.

Poster

293. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 293.10/F4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1R01AG047661-01

Title: Investigating the roles of Papez circuit in health and Alzheimer’s disease

Authors: *W.-C. HUANG1, L. LIU1, H. MATHYS1, Z. PENG1, F. GAO1, L.-H. TSAI1,2; 1The Picower Inst. for Learning and Memory, Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA; 2Broad Inst. of MIT and Harvard, Cambridge, MA

Abstract: Loss of memory and cognition are core symptoms of Alzheimer’s disease (AD), but the neural circuits underlying these deficits remain poorly understood. Interrogating disrupted neural circuits in AD might reveal neurobiological substrates leading to cognitive decline and identify potential targets for therapeutic intervention. Accumulating evidence indicates that impaired memory in early AD reflects much wider neurodegeneration in an extended memory network (i.e. Papez circuit). As a subcortical hub of the Papez circuit, the mammillary body...
(MB) receives inputs from the hippocampus, and outputs to the anterior thalamus, which projects back to the neocortex and the hippocampus. Several autopsy studies have reported amyloid deposition, neurofibrillary tangles, reduction of dendritic arbors, and neuronal loss in the MB of AD individuals. Moreover, lesions in the MB result in anterograde amnesia and memory deficits in humans and animal models. However, the mechanisms by which the activity of MB circuits regulates memory and whether impairment of these circuits contributes to the progression of memory decline in AD remain elusive. Here, we use molecular, cellular, and electrophysiological approaches to investigate MB circuits at multiple levels in wildtype and AD model mice. Our findings identify a previously undefined memory retrieval circuit composed of anatomically, genetically, and functionally distinct neuronal subtypes, and highlight the role of this circuit as a critical inflection point in the progression of memory decline in Alzheimer’s disease.

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Poster

293. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 293.11/F5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant AG008702
Sponsored research from Meira GTX
Support from an anonymous foundation to S.A.S

Title: Retromer repletion with AAV9-VPS35 restores endosomal function in the mouse hippocampus


Abstract: Retromer has emerged as a master conductor of endosomal trafficking, and VPS35 and other retromer-related proteins are found to be deficient in late-onset Alzheimer’s disease (AD). Depleting VPS35 in neurons impairs retromer function, affecting for example the trafficking of the amyloid-precursor protein (APP) and the glutamate receptor GluA1. Whether VPS35 repletion, after chronic in vivo depletion, can rescue these impairments remains unknown. Here we set out to address this question by using a viral vector approach for VPS35
repletion. First, we completed a series of studies using neuronal cultures in order to optimize AAV9-VPS35 delivery, and to understand how exogenous VPS35 expression affects its endogenous levels as well as its binding to other retromer proteins. Next, we completed a series of studies in wildtype mice to determine the optimum protocol for in vivo delivery of AAV9-VPS35 to the hippocampus. In wildtype mice/neurons a pattern consistent with autoregulation was observed, whereby the higher the levels of exogenous VPS35 expressed, the lower the levels of endogenous VPS35 were detected. Co-immunoprecipitation studies were performed to establish that exogenous VPS35 binds endogenous retromer core proteins. We relied on information from WT mice studies to deliver optimal dose of AAV9-VPS35 to the hippocampus of mice genetically engineered to have chronic, neuronal-selective, VPS35 depletion. VPS35 repletion in the hippocampus was found to normalize APP cleavage and to restore glutamate receptor levels. Unexpectedly, chronic VPS35 depletion in neurons caused glial activation, similar to the pattern observed in AD, which was also partially normalized by VPS35 repletion. Taken together, these studies strengthen the mechanistic link between retromer and AD, and have therapeutic implications.

Disclosures: Y.H. Qureshi: None. D.E. Berman: None. R.L. Klein: None. V.M. Patel: None. S. Simoes: None. S. Kannan: None. R. Cox: None. S.D. Waksal: Other; Consultant to Meira Gene Therapy and was it’s founder., Working with and am the founder of Equilibre Neuroscience, Visiting Fellow at the Mind Brain Institute And the Appel, Alzheimer’s Research Institute, Cornell Weil medical. B. Stevens: Other; Scientific Advisory Board of annexon Biosciences and Abelian (also founder of later). G.A. Petsko: Other; I am on the Scientific Advisory Boards of MeiraGTx, Proclara Biosciences, QR Pharma and Amicus Therapeutics. As such, I am compensated both monetarily and in the form of stock options. S.A. Small: Other; Scientific Advisory Board of Meira GTx.

Poster

293. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 293.12/F6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: This work was supported by Award Number R01NS092497 (to GT) from National Institute of Health (NIH).

Title: Cleavage of neural cell adhesion molecules by BACE1 is spatio-temporally regulated in vivo

Authors: *W. KIM1, S. LOMOIO1, C. BANEVICIUS2, H. WATANABE2, G. TESCO1;
1Neurosci., Tufts Univ. Sch. of Med., Boston, MA; 2Tufts Univ., Boston, MA
**Abstract:** β-site amyloid precursor protein cleaving enzyme 1 (BACE1) cleaves amyloid precursor protein (APP) to initiate the generation of amyloid beta peptides (Aβ). Studies strongly suggest that Aβ is associated with synaptic dysfunction and neuronal loss in Alzheimer’s disease (AD). Thus, BACE1 is considered a prime therapeutic drug target for lowering Aβ levels in the AD brain. However, very recently, two clinical trials using two different BACE1 inhibitors in patients with prodromal or preclinical AD were halted due to the worsening of cognitive functions, most likely owing to mechanism-based side effects.

In addition to APP, several neuronal proteins, including the neural cell adhesion molecule L1-like protein (CHL1) have been identified as BACE1 substrates and validated in vivo. We report here, for the first time, that neural cell adhesion molecule 1 and 2 (NCAM1 and NCAM2, respectively) are BACE1 substrates in vivo. Neural cell adhesion molecules have been shown to regulate formation, maturation, and maintenance of synapses. Since synaptic loss is one of the earliest signs of AD, the relationship between NCAMs and AD has been studied intensively. Interestingly, reduced synaptic NCAM1 and NCAM2 levels were reported in AD brains.

BACE1-mediated processing of NCAM1, NCAM2 and CHL1 was analyzed in the olfactory bulb and hippocampus of wild type mice and BACE1−/− mice at three different ages (postnatal day 10 (P10), 4 and 12 months). NCAM2 was cleaved in the olfactory bulb of wild type but not BACE1−/− mice at all ages analyzed. Instead, NCAM1 was processed by BACE1 in olfactory bulb at 4 and 12 months of age but not at P10 in wild type mice. Moreover, CHL1 was processed by BACE1 in olfactory bulb at P10 and 4 months of age but not at 12 months of age in olfactory bulb. In the hippocampus, NCAM1 and NCAM2 BACE1-mediated processing was detected only in synaptosomes but not in total homogenates. In contrast, CHL1 processing was clearly detected in both hippocampal homogenates and synaptosomes.

We also found that NCAM2 but not NCAM1 is sequentially cleaved by BACE1 and γ-secretase in vitro. Next, we identified and validated the BACE1 cleavage site of NCAM1 and NCAM2 by mass spectrometry and site-directed mutagenesis.

Taken together, our data demonstrates that the regulation of BACE1-mediated processing of neural cell adhesion molecules (CHL1, NCAM1, and NCAM2) depends on the regions of brain, age, and subcellular localization in vivo, suggesting that BACE1 may play important roles in physiological functions by regulating the proteolysis of substrates spatio-temporally.

**Disclosures:** W. Kim: None. S. Lomoio: None. C. Banevicius: None. H. Watanabe: None. G. Tesco: None.

**Poster**

293. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models II

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 293.13/F7

**Topic:** C.02. Alzheimer's Disease and Other Dementias
Support: NIH AG055497

Title: Converging cellular signaling pathways driving early synaptic pathology in AD

Authors: S. MUSTALY¹, S. SCHRANK¹, J. MCDAID¹, R. A. MARR¹, *G. E. STUTZMANN²;
¹Neurosci., RFUMS/Chicago Med. Sch., North Chicago, IL; ²Ctr. for Neurodegenerative Dis. and Therapeut., Rosalind Franklin Univ./Chicago Med. Sch., North Chicago, IL

Abstract: To date, the cellular mechanisms that directly lead to memory deficits in AD have remained elusive, likely due to insufficient understanding of the basic pathogenic mechanisms that arise early in the disease process. Memory encoding is initiated at the synapse; thus it makes sense functionally that synaptic defects are most highly correlated with memory loss in AD. While aberrant protein aggregations are an attractive target for therapeutic development, their association with cognitive functions and to synaptic properties are not clear. To better identify key cellular mechanisms that drive later memory encoding deficits, we and others are looking to upstream signaling abnormalities that directly couple to synaptic structure and function and occur prior to amyloid and tau pathology. Here, using a combination of patch clamp and field potential electrophysiology, live-cell fluorescent imaging of intracellular signaling and organelle functioning, hippocampal neural network imaging, electron microscopy, and immunoassays, we asked what the functional relationships are among key organelles responsible for protein handling, calcium regulation, and cellular metabolism that can regulate synaptic activity. Our goal was to identify the extent to which dysfunction in one organelle may impact functions in others, thus linking ER calcium and misfolded protein handling, lysosomal-mediated degradation and autophagy clearance, and mitochondrial bioenergetics with synaptic function and vesicle release properties. Using mouse models of AD, human neurons derived from AD patients using direct reprogramming methods, and cellular model systems, we demonstrate that the core later stage features of AD, such as soluble and insoluble amyloid species, phosphorylated tau, and synaptic loss can be linked to altered organelle functionality, particularly those that require acidic compartments and strong ionic gradients. The earlier cellular defects include altered lysosomal pH properties, defective autophagosome clearance, mitochondrial impairments, and neurotransmitter vesicle loss. Further upstream, we identify that intracellular calcium dyshomeostasis originating from ER stores is underlying the respective impairments through a variety of organelle-specific mechanisms, and this is observed in mouse and human neurons expressing AD genotypes. Thus, through stabilizing intracellular calcium release from ER stores, lysosomal-mediated clearance of autophagosomes and the aberrant protein products within are cleared, mitochondrial function is restored, and synaptic structure and memory-encoding properties are normalized.

Poster

293. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 293.14/F8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support:
- NIH Grant AG057767
- NIH Grant AG061937
- IDPH-ADRF Grant 63282003D
- Center for Alzheimer’s disease and Related Disorders at SIU School of Medicine
- SIU School of Medicine Foundation
- Kenneth Stark Endowment
- Fraternal Order of Eagles

Title: Glutamatergic neurotransmission and cognition in health, disease, aging, and intervention: Evidence from mouse models

Authors: *E. R. HASCUP¹, C. A. FINDLEY¹, N. ESPERANT-HILAIRE¹, J. BRITZ², L. SIME¹, S. MCFADDE¹, E. LOKAITIS¹, Y. FANG¹, S. TISCHKAU², H. A. BOGER⁴, A. BARTKE³, K. N. HASCUP¹;

Abstract: It is well established that hippocampal glutamatergic neurotransmission is required for learning and memory. Here, we present data on glutamatergic neurotransmission and cognition from mouse models of normal aging (C57BL/6), successful aging (growth hormone receptor knock out; GHR-KO), metabolic disease (high-fat diet), and Alzheimer’s disease (AD; AβPP/PS1 and APP<sup>NL-F/NL-F</sup>). Mice underwent cognitive evaluation using the Morris water maze (MWM) spatial learning and memory task followed by <em>in vivo</em> electrochemistry to assess glutamatergic neurotransmission independently in the dentate gyrus, CA1, and CA3 subregions of the hippocampus. Regardless of mouse model, we observed that tighter hippocampal glutamatergic signaling led to better performance on MWM memory recall, while elevated basal glutamate was often associated with impaired cognition. Furthermore, when glutamatergic signaling was restored through pharmaceutical intervention with Riluzole, an approved drug for the treatment of amyotrophic lateral sclerosis, cognitive performance was similar to that measured in control mice. Additionally, postmortem brain tissue analysis (mRNA and protein levels, immunohistochemistry, and measures of cellular senescence) revealed potential mechanisms underlying these observed changes in neurotransmission. These mechanisms may...
serve as early biomarkers and therapeutic targets for restoring glutamate signaling and improving patient outcome by delaying or stopping cognitive decline.


Poster

293. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 293.15/F9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG057767
         NIH Grant R01AG061937
         Center for Alzheimer's Disease and Related Disorders
         Southern Illinois University Foundation
         Kenneth Stark Endowment

Title: Hyperglutamatergic signaling throughout disease progression in Alzheimer's mouse models

Authors: *K. N. HASCUP, E. R. HASCUP;
         Neurosci. Institute, Neurology, Ctr. for Alzheimer's Dis. and Related Disorders, Southern Illinois Univ. Sch. of Med., Springfield, IL

Abstract: Alzheimer’s disease (AD) is characterized by severe impairment in new learning and memory, progressive dementia, cerebral atrophy, and eventual death. Increased soluble β-amyloid (Aβ)42 is the earliest detectable biomarker and contributes to the neurotoxicity associated with AD progression. We have demonstrated that soluble Aβ42 elicits hippocampal glutamate release through a presynaptic α7nAChR mechanism. As soluble Aβ42 accumulates during AD progression, this causes a concomitant hyperglutamatergic environment that is hypothesized to underlie the cognitive and functional decline. We measured basal and stimulus-evoked glutamate release in the dentate (DG), CA3, and CA1 of male AβPP/PS1 and APPNL−F/NL−F mouse models of cerebral amyloidosis. At 2-3 months of age, prior to cognitive decline, elevated stimulus-evoked glutamate release was observed in the CA1 of AβPP/PS1 (9.1 ± 1.1 µM, n=12; p<0.001) and APPNL−F/NL−F (5.4 ± 0.4 µM, n=4; p<0.01) mice compared to age-matched C57BL/6 (2.9 ± 0.3 µM, n=10) control mice. By 12-15 months of age, hippocampal hyperglutamatergic signaling became more pronounced in AβPP/PS1 mice when cognitive deficits are observed. This phenotype was reproducible across multiple cohorts of 12-15 month old AβPP/PS1 mice used as controls for various intervention strategies including dietary, 0.9%
saline injection, and 1% sucrose water treatments. Averaging glutamate dynamics across these cohorts, AβPP/PS1 mice had significantly elevated basal glutamate compared to age- and treatment-matched C57BL/6 mice in the DG (1.3 ± 0.1 µM, n=41 vs 0.8 ± 0.1 µM, n=45; p<0.01), CA3 (1.4 ± 0.1 µM, n=41 vs 0.8 ± 0.1 µM, n=46; p<0.0001), and CA1 (1.7 ± 0.1 µM, n=41 vs 0.9 ± 0.1 µM, n=46; p<0.0001). Similarly, average stimulus-evoked glutamate release was elevated in the same cohorts of AβPP/PS1 mice compared to age- and treatment-matched C57BL/6 mice in the DG (9.3 ± 1.0 µM, n=40 vs 4.1 ± 0.4 µM, n=46; p<0.0001), CA3 (7.9 ± 0.8 µM, n=40 vs 5.5 ± 0.5 µM, n=46; p<0.01), and CA1 (8.0 ± 0.6 µM, n=41 vs 3.3 ± 0.3 µM, n=45; p<0.0001). This hyperglutamatergic signaling is a result of elevated vesicular glutamate transporter 1 density in 12-15 month old AβPP/PS1 compared to age-matched C57BL/6 mice in the DG (0.9 ± 0.1, n=6 vs 0.7 ± 0.1, n=13; p=0.2), CA3 (0.7 ± 0.1, n=7 vs 0.5 ± 0.1, n=11; p<0.01), and CA1 (0.9 ± 0.1, n=7 vs 0.5 ± 0.1, n=13; p<0.001). These data support a role of Aβ42 mediated hyperglutamatergic signaling throughout disease progression in mouse models of cerebral amyloidosis that is consistently observed across multiple cohorts of intervention control groups. This elevated glutamatergic signaling may serve as an early biomarker or therapeutic strategy for AD.

Disclosures: K.N. Hascup: None. E.R. Hascup: None.

Poster

293. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 293.16/F10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS041783

Title: Loss of Presenilin function in inhibitory neurons causes impairments of memory, synaptic plasticity and age-dependent neurodegeneration

Authors: *J. KANG, S. LEE, J. SHEN;
Neurol., Brigham & Women's Hospital, Harvard Med. Sch., Boston, MA

Abstract: Mutations in the Presenilin (PSEN1 and PSEN2) genes are the major genetic cause of Alzheimer’s disease (AD). Inactivation of Presenilin (PS) in excitatory neurons of the postnatal mouse forebrain results in memory and synaptic impairment as well as age-dependent neurodegeneration. Although the function of interneurons and the oscillatory network activities they regulate are altered in AD, the role of PS in inhibitory neurons is unknown. Here we generated inhibitory neuron-specific PS conditional double knockout (IN-PS cDKO) mice, in which PS is selectively inactivated by Cre recombinase expressed under the control of the Gad2 promoter. Western analysis confirmed reduction of PS1 in the cerebral cortex and the striatum, in
which ~95% of neurons are GABAergic medium spiny neurons. Interestingly, IN-PS cDKO mice exhibit earlier mortality relative to littermate controls. Moreover, IN-PS cDKO mice at 2 months of age show spatial learning and memory deficits in the Morris water maze, as indicated by significant increases of latency during training and reduced quadrant occupancy in post-training probe trials. Furthermore, paired-pulse facilitation, frequency facilitation, and long-term potentiation are enhanced in the Schaffer collateral pathway of IN-PS cDKO mice at 2 months of age. Importantly, we found age-dependent neurodegeneration in IN-PS cDKO mice, similar to excitatory neuron-specific PS cDKO mice. The number of inhibitory neurons is reduced in the cerebral cortex and the striatum of IN-PS cDKO mice at the age of 9 months but is unchanged at 3 months, relative to littermate controls. Consistent with these findings, the number of apoptotic cells labeled by active caspase-3 immunoreactivity and the TUNEL assay is increased in the cerebral cortex and the striatum of IN-PS cDKO mice at the age of 9 months. Neurodegeneration in IN-PS cDKO mice is also accompanied with gliosis as shown by elevated GFAP and Iba1 immunoreactivity. These findings demonstrate essential roles of PS in synaptic plasticity, learning and memory, and neuronal survival in adult interneurons.

**Disclosures:**  
**J. Kang:** None.  
**S. Lee:** None.  
**J. Shen:** None.

**Poster**

**293. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models II**

**Location:** Hall A  
**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 293.17/F11

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Support:**  
- NIH Grant P20 GM109098  
- NIH Grant P01 AG027956  
- NIH Grant U54 GM104942  
- NIH Grant T32 AG052375  
- NIH Grant K01 NS081014

**Title:** Modeling sporadic Alzheimer’s disease in mice through miR-34a targeted polygenic suppression

**Authors:**  
*S. N. SARKAR*1, E. B. ENGLER-CHIURAZZI1, J. Z. CAVENDISH2, J. M. POVROZNIK1, A. RUSSELL2, D. D. QUINTANA2, P. MATHERS2, J. W. SIMPKINS2;  
1Physiol. and Pharmacol., 2West Virginia Univ., Morgantown, WV

**Abstract:** Autosomal dominant Alzheimer disease (AD) is caused by rare mutations in one of three specific genes namely APP, PS1, and PS2. This contrasts with idiopathic, sporadic AD (sAD), which has a more polygenic risk profile and represents more than 95% of cases. Polygenic risk factors and reduced expression of many genes in sAD impedes the path to
progress in generating a useful mouse model and success for identifying novel target(s) for
disease-modifying therapies. Previously we sought to identify expression of selected microRNAs
in AD brains that maximizes the number of target genes possibly involved in cognitive function.
We have found that miR-34a in human AD patient’s brains and primary neurons repressed
proteins related to synaptic plasticity and energy metabolism. Furthermore, our bioinformatic
analysis of many of the miR-34a targeted genes were predicted to be similarly down regulated as
found in the differentially expressed genes in AD and control human patients from Gene
expression Omnibus (GEO) database. These genes were analyzed for functional and pathway
enrichment analysis of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes
(KEGG) to elucidate the pathological polygenic mechanisms of sAD in mice. To this end, we
have generated conditionally miR-34a overexpressing mice (TetR-TetO-miR-34a-Transgenic
mice) and investigated functional consequences of polygenic suppression of miR-34a target
genes by identifying shared behavioral and molecular signatures characteristics of sAD in
human. Doxycycline-treated mice of either sex exhibited profound behavioral impairment
compared to untreated groups with only 1-2 months of over-expression of miR-34a. Cognitive
impairment of individual mice in T- and Y-maze tasks correlated with elevated miR-34a
expression in many parts of the brain including the hippocampus and prefrontal cortex, regions
which are known to be involved in this task and implicated dysfunction in sAD.
Immunocytochemistry of brain sections from mice show high amyloid β and phosphorylated tau-
specific staining in the hippocampus and cortex. Analysis of protein samples from these mice
revealed that miR-34a targets specific genes involved in structural and functional synaptic
plasticity, amyloid precursor protein (APP) metabolism, phosphorylation-dephosphorylation of
tau, faster spike transmission in inhibitory neurons, and functional hyperemia. Thus, our results
suggest that the polygenetic dysfunction caused by miR-34a may be involved in initiation and or
progression of sAD and disclose miR-34a as a potential therapeutic target.

Disclosures: S.N. Sarkar: None. E.B. Engler-Chiurazzi: None. J.Z. Cavendish: None. J.M.
Simpkins: None.

Poster

293. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 293.18/F12

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH AG053937
            NIH AG057408
Title: ATase1 is the preferential target to rescue the proteotoxicity associated with Alzheimer’s disease

Authors: *L. PUGLIELLI, M. J. RIGBY, V. BANDUSEELA, G. KAUR, R. SULLIVAN, A. LAKKARAJU;
Univ. of Wisconsin Madison, Madison, WI

Abstract: Nε-lysine acetylation in the lumen of the endoplasmic reticulum (ER) has emerged as a novel mechanism for the regulation of protein homeostasis by regulating the selection of correctly folded glycoproteins and the autophagy-mediated disposal of unfolded/misfolded protein aggregates. Acetyltransferase 1 (ATase1/NAT8B) and acetyltransferase 2 (ATase2/NAT8) are ER-resident enzymes responsible for Nε-lysine acetylation; pharmacologic inhibition of both ATase1 and ATase2 results in activation of reticulophagy (ER-specific autophagy), enhanced clearance of protein aggregates within the secretory pathway, and rescue of Alzheimer’s disease (AD)-like neurotoxicity in the mouse. The physiologic and pathologic differences between ATase1 and ATase2 remain uncharacterized, which prevents more targeted therapeutic approaches. To elucidate these differences, we used a combination of structure-biochemistry approaches to dissect the enzymatic properties of the two transferases. We also generated ATase1−/− and ATase2−/− mice. The results show that ATase1 (but not ATase2) can be allosterically activated, thus enabling this transferase to couple the induction of reticulophagy to the levels of acetyl-CoA within the ER in real-time. Consistently, ATase1−/− (but not ATase2−/−) mice displayed increased activation of reticulophagy, thus suggesting improved proteostatic functions. Importantly, neither ATase1−/− nor ATase2−/− mice developed a significant phenotype. To determine the specific translational potential of ATase1 and ATase2 inhibition in AD, we crossed ATase1−/− and ATase2−/− mice with APPswe/PSEN1dE9 mice. The results show that genetic disruption of ATase1 (but not ATase2) improved the lifespan of APPswe/PSEN1dE9 mice. Biochemical and pathologic examination of APP/PS1;ATase1−/− and APP/PS1;ATase2−/− mice is currently underway. In conclusion, our results demonstrate that ATase1 is the preferred target to improve the proteostatic functions of the secretory pathway and rescue associated proteotoxic disease states.


Poster

293. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 293.19/F13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG060203
**Title:** A transient physiological role for amyloid beta oligomers during CNS development

**Authors:** *W. L. KLEIN*¹², N. RAO¹, M. BICCA¹, E. HO¹, H. XIA¹, E. CLINE¹, S. BARTLEY¹, K. L. VIOLA¹, F. G. DE MELLO³⁴; ¹Dept. of Neurobio., Northwestern Univ., Evanston, IL; ²Dept. of Neurol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; ³Inst. de Biofisica Carlos Chagas Filho, Rio de Janeiro, Brazil; ⁴Inst. de Bioquimca Medica, Univ. Federal, Rio de Janeiro, Brazil

**Abstract:** Toxic amyloid beta oligomers (AβOs) accumulate in Alzheimer’s brain and cerebrospinal fluid (CSF). The hypothesis that these toxic AβOs instigate neural damage leading to dementia was first proposed in 1998 based on discoveries that fibril-free synthetic preparations of AβOs rapidly inhibit long-term potentiation and ultimately cause selective nerve cell death, preventable by knockout of the *fyn* gene (MP Lambert et al 1998 PNAS). The AβO hypothesis has been widely investigated since that time (reviewed in E Cline et al 2018 JAD). Detectable by conformation-sensitive antibodies, AβOs manifest in human brain and CSF in an Alzheimer’s disease (AD)-dependent manner and in frequently used transgenic models of familial AD. Experimentally, synthetic and AD brain-derived AβOs instigate cognitive dysfunction and major features of AD pathology such as tau hyperphosphorylation, synapse elimination, and selective nerve cell death. In this study, we have investigated whether AβOs might be present in the central nervous system (CNS) during neural development, which also manifests synapse elimination, selective nerve cell death, and transiently abundant hyperphosphorylated tau (pSer396). Our experiments used embryonic chick, chosen because its Aβ42 sequence is the same as humans. Western blots with AβO-selective antibodies and mass spectrometry confirmed the presence of AβOs in embryonic chick retina. The sodium dodecyl sulfate-stable core of the naturally-occurring embryonic AβOs comprises 9mers and 10mers, which are smaller than the type 2 AβOs prominent in AD brain. Abundance and distribution of AβOs were developmentally regulated in a pattern that was approximately coincident with tau phosphorylation at Ser396. A transient role for AβOs in CNS development may be one of the reasons evolution has conserved a peptide that manifests such deleterious gain-of-function properties in the aging brain. Studies of transient AβOs in the developing retina offer new opportunities for investigating a physiological regulation that is disrupted in AD and perhaps in diseases of the eye such as glaucoma, macular degeneration, and diabetic retinopathy. This study is funded by NIH research grant R21 AG060203

**Disclosures:** W.L. Klein: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Acumen. N. Rao: None. M. Bicca: None. E. Ho: None. H. Xia: None. E. Cline: None. S. Bartley: None. K.L. Viola: None. F.G. de Mello: None.
**Poster**

293. Synaptic Dysfunction in Alzheimer's Disease: In Vivo Models II

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 293.20/F14

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Support:** Canadian Institutes of Health Research

**Title:** Potential roles of APP-cleaved products in an animal model of temporal lobe epilepsy

**Authors:** *S. KAR, A. KODAM, D. OURDEV, M. MAULIK, A. SCHMAUS, A. DAHAL, Y. WANG;
Med., Univ. of Alberta, Edmonton, AB, Canada

**Abstract:** Temporal lobe epilepsy (TLE) is the most common form of epilepsy that originates from the hippocampus and then propagates to other limbic areas such as the amygdala and entorhinal cortex. The main pathological feature associated with TLE is hippocampal sclerosis, which is characterized by atrophy, induration, gliosis, synaptic reorganization and loss of neurons in CA1, CA3 and dentate hilar regions. Some animal models, albeit do not exactly match the complex etiologies identified in humans, are found to recapitulate most of the pathological features observed in TLE. There is evidence that systemic administration of kainic acid (KA) can cause seizures in the CA3 region of the hippocampus that can lead to loss of neurons and astrogliosis characteristic of TLE. Our results show that injection of KA along with seizures, gliosis and loss of hippocampal neurons, can increase the level/processing of amyloid precursor protein (APP), resulting in the enhanced production of amyloid beta (Aβ)-related peptides - which play critical roles in Alzheimer’s disease (AD) pathology. The changes in APP levels/processing were evident primarily in activated astrocytes, implying a role for astrocytic Aβ-related peptides in KA-induced toxicity. Accordingly, we showed that treating rat cultured astrocytes and human U-373 astrocytoma with KA can lead to increased levels of APP and its cleaved products by selectively activating kainate receptor. Additionally, we revealed that KA reduces neuronal viability more in neuronal/astrocyte co-cultures than in pure neuronal culture, and this is attenuated by precluding Aβ production. Collectively, these results indicate that increased production/secretion of Aβ-related peptides from activated astrocytes can contribute to seizure/ neurotoxicity in KA-treated rats. Since KA administration can lead to neuropathological changes resembling TLE, it is likely that APP/Aβ peptides derived from astrocytes may have a role in TLE pathogenesis.

Poster

294. Molecular Underpinnings of LRRK2 Function and Dysfunction

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 294.01/F15

Topic: C.03. Parkinson’s Disease

Support: MEXT Japan

Title: Proteomic analysis of lamellar bodies in Lrrk2 knockout mice

Authors: *M. ARAKI, S. TAKATORI, G. ITO, T. TOMITA;
Pharmaceut. Sci., The Univ. of Tokyo, Tokyo, Japan

Abstract: Parkinson disease (PD) is one of the most common neurodegenerative disorders. PD is characterized by the loss of dopaminergic neurons and formation of Lewy bodies in the substantia nigra. It has been shown that mutations in leucine-rich repeat kinase 2 (LRRK2) cause familial PD (FPD). As FPD mutant LRRK2 exhibits an increased kinase activity, LRRK2 kinase inhibitors are expected to be useful for treatment of PD. Therefore, several brain penetrable LRRK2 inhibitors have been developed. Although these inhibitors effectively suppress the LRRK2 kinase activity in the brain, morphological abnormalities in peripheral tissues were observed in rodents as well as non-human primates treated with these compounds. Especially, in the lung, the number and size of lamellar bodies in alveolar epithelial type 2 cells were significantly increased. Lamellar body is a lysosome-related organelle that stores pulmonary surfactants required for maintaining a low surface tension of alveoli. Given the importance of lamellar bodies in the physiological function of lung, it is possible that the disruption of lamellar body homeostasis upon inhibition of LRRK2 impairs the lung function. To reveal the molecular mechanism of the lamellar body hypertrophy, we undertook a proteomic analysis on the lamellar bodies isolated from mouse lungs. We confirmed that the area and number of lamellar bodies in Lrrk2 knockout (KO) mice (Tong et al., PNAS 2010) were dramatically increased to those in wild-type (WT) mice. Next, we isolated the lamellar bodies from these mice using sucrose gradient centrifugation and compared the protein abundance by a label-free quantitative proteomic analysis. Approximately 1500 proteins were identified from these lamellar body fractions. We found more than two-fold increase in the levels of 103 proteins in the Lrrk2 KO lamellar bodies. A gene ontology enrichment analysis of these proteins revealed a significant enrichment for proteins associated with small GTPase signal transduction and cellular vesicle transport including Rab proteins. These results suggested that the intracellular vesicular transport was changed in the lungs of Lrrk2 KO mice, which potentially lead to the lamellar body hypertrophy.

Disclosures: M. Araki: None. S. Takatori: None. G. Ito: None. T. Tomita: None.
Poster

294. Molecular Underpinnings of LRRK2 Function and Dysfunction

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 294.02/F16

Topic: C.03. Parkinson’s Disease

Support: Intramural Research Program of the National Institute on Aging

Title: LRRK2 regulates microglial neurotoxicity via NFATc2 in synucleinopathies

Authors: *C. KIM¹, A. BEILINA¹, N. SMITH¹, Y. LI², M. KIM³, R. KUMARAN¹, A. KAGANOVICH¹, M. ADAMANTIOS¹, A. ADAM⁴, M. IBA¹, S. KWON¹, S.-J. SHIN⁵, R. RISSMAN⁴, S. YOU³, S.-J. LEE⁵, A. SINGLETON¹, M. COOKSON¹, E. MASLIAH¹;
¹Natl. Inst. on Aging, Bethesda, MD; ²Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD; ³Cedars-Sinai Med. Ctr., Los Angeles, CA; ⁴UCSD, La Jolla, CA; ⁵Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Synucleinopathies are neurodegenerative disorders characterized by abnormal a-synuclein deposition and neuroinflammation. Neuronal-released a-synuclein have been shown to induce neurotoxic microglial responses through Toll-like receptor 2 (TLR2), resulting in the production of pro-inflammatory responses. However, the molecular mechanism is poorly understood. Here, we elucidate a critical role for Leucine-rich repeat kinase 2 (LRRK2), in the activation of microglia by a-synuclein. Microglial exposure to a-synuclein was found to significantly enhance LRRK2 kinase activity. While genetical and pharmacological inhibition of LRRK2 affected the expression of only TNFa and IL-6, overall a-synuclein-mediated microglial neurotoxicity was diminished. LRRK2 also phosphorylates and induces nucleus translocation of the immune transcription factor, nuclear factor of activated T-cells, cytoplasmic 2 (NFATc2). We also identified accumulations of NFATc2 in the brains of synucleinopathy patients and mouse models. Therefore, we propose the modulation of LRRK2 and its downstream signaling mediator NFATc2 as a novel therapeutic strategy for synucleinopathies.

**Poster**

**294. Molecular Underpinnings of LRRK2 Function and Dysfunction**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 294.03/F17

**Topic:** C.03. Parkinson’s Disease

**Support:**
- UK Medical Research Council (MR/K005146/1 to K.J.D.V.)
- Parkinson’s UK Senior Fellowship (F-1301 to H.M.)
- Parkinson’s UK small grant (K-1506 to L.F. and H.M.)
- Academy of Medical Sciences/Wellcome Trust Springboard Award (SBF0021142 to L.F.)
- Rosetree’s Trust M580 (H.M. and R.M.)

**Title:** LRRK2 regulates HDAC6 dependent aggresome formation

**Authors:** R. M. LUCAS¹, C. S. BAUER¹, K. CHINNAIYA¹, A. SCHWARTZENTRUBER², R. M. MACDONALD², M. O. COLLINS², J. O. AASLY³, G. BRØNSTAD⁴, L. FERRAIUOLO¹, H. MORTIBOYS¹, *K. J. DE VOS¹;

¹The Univ. Of Sheffield, Sheffield, United Kingdom; ²The Univ. of Sheffield, Sheffield, United Kingdom; ³St Olav’s Hosp., Trondheim, Norway; ⁴Neurozym, Snasa, Norway

**Abstract:** Mutations in LRRK2 are the most common cause of dominantly inherited Parkinson’s disease. LRRK2 is a multi-domain protein with both GTPase and kinase activities that has been shown to affect various cellular processes including membrane trafficking, axonal transport and protein homeostasis. A main cellular pathway to remove aggregated ubiquitinated proteins is aggrephagy: the cytosolic class IIb histone deacetylase HDAC6 recognizes ubiquitinated misfolded proteins and recruits them to the molecular motor cytoplasmic dynein which transports them to the perinuclear region where they are trapped in aggresomes that are subsequently removed by macroautophagy. We show that LRRK2 regulates HDAC6-dependent aggresome formation. LRRK2 directly interacted with the HDAC6 deacetylase domains via its Roc domain and phosphorylated HDAC6 on serine-22. Serine-22 phosphorylation of HDAC6 enhanced its interaction with cytoplasmic dynein and stimulated recruitment of ubiquitinated proteins to aggresomes. Knockdown or knockout of LRRK2 impaired HDAC6-mediated aggresome formation. Parkinson’s disease mutant LRRK2 G2019S showed reduced interaction with HDAC6 and did not support aggresome formation to the same extent as wild type LRRK2. This was recapitulated in LRRK2 G2019S patient-derived iAstrocytes that showed an aggresome formation defect. In conclusion our data reveal HDAC6 as a target of LRRK2 and suggest that deregulation of HDAC6-mediated aggresome formation and aggrephagy could contribute to the pathology of Parkinson’s disease.

Poster

294. Molecular Underpinnings of LRRK2 Function and Dysfunction

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 294.04/F18

Topic: C.03. Parkinson’s Disease

Support: Parkinson’s Foundation PF-FBS-1894
NIH R01 NS091719
Van Andel Research Institute

Title: ATP13A2 functionally interacts with LRRK2 in Parkinson’s disease models

Authors: *M. ERB\textsuperscript{1}, A. PODHAJSKA\textsuperscript{2}, J. KORDICH\textsuperscript{1}, N. LEVINE\textsuperscript{1}, D. J. MOORE\textsuperscript{1}; \textsuperscript{1}Van Andel Res. Inst., Grand Rapids, MI; \textsuperscript{2}Brain Mind Inst., Swiss Federal Inst. of Technol. (EPFL), Lausanne, Switzerland

Abstract: Parkinson’s disease (PD) is a progressive neurodegenerative movement disorder that results from the death of substantia nigra dopaminergic neurons. Although most cases of PD have no known cause, mutations in over 15 genes cause heritable PD with a surprising number involved in intracellular trafficking pathways. For example, mutations in \textit{leucine-rich repeat kinase 2} (\textit{LRRK2}) cause autosomal dominant late-onset PD, and loss-of-function mutations in \textit{ATP13A2} (\textit{PARK9}) cause autosomal recessive early-onset parkinsonism. Recent studies suggest that \textit{ATP13A2} genetic variants may reduce the age-of-disease-onset in \textit{LRRK2}-linked PD subjects. Using synthetic genetic array screens in \textit{Saccharomyces cerevisiae}, we find that deletion of \textit{YPK9}, the yeast ortholog of \textit{ATP13A2}, enhances human \textit{LRRK2}-induced toxicity. To determine whether \textit{LRRK2} and \textit{ATP13A2} converge in a common PD pathway, we first tested if these proteins interact in HEK-293T cells or rat primary cortical neurons. Using co-immunoprecipitation assays, we find that FLAG-tagged \textit{LRRK2} robustly interacts with V5-tagged \textit{ATP13A2} with a minimal impact of PD-linked \textit{LRRK2} or \textit{ATP13A2} mutations on this interaction. Confocal microscopy further reveals the partial co-localization of FLAG-\textit{LRRK2} and GFP-\textit{ATP13A2} on vesicular structures in cortical neurons. Using Phos-tag assays, WT or mutant \textit{LRRK2} expression fails to induce \textit{ATP13A2} phosphorylation in cells. Oppositely, WT or mutant \textit{ATP13A2} expression has no effect on cellular \textit{LRRK2} phosphorylation (S910, S935, S1292) or substrate phosphorylation (Rab10). Next, we assessed the role of \textit{ATP13A2} in a model of mutant \textit{LRRK2}-induced neuronal toxicity in primary cortical cultures. We find that wild-type \textit{ATP13A2} (but not an ATPase-inactive D513N variant) attenuates the impaired neurite outgrowth induced by G2019S \textit{LRRK2}. Interestingly, \textit{LRRK2} has been shown to modulate
lysosome size, number and acidity, and ATP13A2 expression, in cultured primary astrocytes. We are currently developing models to evaluate whether ATP13A2 overexpression in astrocytes can rescue the impaired lysosomal morphology and function induced by mutant LRRK2. Similar studies are being conducted in an established Ad5-mediated gene transfer rat model of PD where G2019S LRRK2 expression induces robust nigral dopaminergic neurodegeneration. We are evaluating whether lentiviral-mediated expression of ATP13A2 is protective in this LRRK2 model, and whether ATP13A2 knockout mice exhibit enhanced G2019S LRRK2-induced neurotoxicity. We hope to define a novel PD pathway linking LRRK2 and ATP13A2, and to establish ATP13A2 as a promising therapeutic target for disease-modification in PD.


Poster

294. Molecular Underpinnings of LRRK2 Function and Dysfunction

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 294.05/F19

Topic: C.03. Parkinson’s Disease

Support: R01 NS097901
          NUCATS

Title: Leveraging LRRK2 biology to develop therapeutic opportunities for Parkinson's disease

Authors: C. CHEN¹, M. CLUTTER², L. PARISIADOU¹;
¹Dept. of Pharmacol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; ²High Throughput Analysis Lab., Northwestern Univ., Chicago, IL

Abstract: Leucine rich repeat kinase 2 (LRRK2) has been linked to both familial and the most common non genetic form of Parkinson’s disease (PD) and it represents one of the most promising therapeutic targets for PD. Specifically, targeting LRRK2 kinase activity has generated a great amount of interest followed by the development of several LRRK2 kinase inhibitors. Although this remains a priority therapeutic approach, a number of safety liability concerns of the developed inhibitors in peripheral tissues highlights the importance of alternative therapeutic approaches that take into account aspects of LRRK2 biology particular in the neuronal population specifically susceptible to PD. High throughput screening of cellular phenotypes could enable the discovery of new targets that modulate mutant LRRK2 function in an unbiased manner. Our previous studies were among the first to demonstrate that one of the most consistent cellular phenotype that reflect mutant LRRK2 activity is a decrease in neurite outgrowth and neuronal complexity. Here, we employed a cell based imaging assay using primary dopaminergic neurons of mutant LRRK2 mice by measuring neuron outgrowth
amenable to large scale screening. A library of translational compounds was used for screening and those compounds that increased the neuronal complexity of mutant LRRK2 with no significant change in wild-type were set to reflect modification of mutant LRRK2 function. Overall in the present study, we successfully established a platform for the identification of compounds that reverse mutant LRRK2 activity using neuronal populations with maximum disease relevance. The identified top hit compounds represent novel LRRK2 targeted agents in a precise disease relevant context and the present study opens up several opportunities to pursue preclinical development of the identified compounds.

**Disclosures:**  C. Chen: None. M. Clutter: None. L. Parisiadou: None.

**Poster**

294. Molecular Underpinnings of LRRK2 Function and Dysfunction

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 294.06/F20

**Topic:** C.03. Parkinson’s Disease

**Title:** A novel protective role for N-acylphosphatidylethanolamine phospholipase D in 6-OHDA-induced neurodegeneration

**Authors:** *F. PALESE*¹², S. PONTIS³, N. REALINI², D. PIOMELLI¹;
¹Anat. and Neurobio., Univ. of California Irvine, Irvine, CA; ²Drug Discovery and Develop. (D3) Dept., Italian Inst. of Technol., Genoa, Italy

**Abstract:** N-acylphosphatidylethanolamine phospholipase D (NAPE-PLD) is a zinc hydrolase that catalyzes the cleavage of membrane NAPEs into fatty-acid ethanolamides (FAEs), a family of lipid mediators with diverse important biological activities. Biophysical experiments suggest that, in addition to their role as FAE precursors, NAPEs may also serve autonomous structural and signaling functions, such as stimulation of membrane fusion, consolidation of lipid raft structure and stabilization of the lipid bilayer. It is largely known that NAPEs are produced in the brain following several injurious stimuli, however, the functional significance of this accrual is still not clear. In the present study, we used a well established model of Parkinson’s disease, administering the dopaminergic neurotoxin 6-hydroxydopamine (6-OHDA) in the striatum of mice. We report that injections of 6-OHDA cause increase in NAPE and FAE levels in situ, which precedes neuronal cell death. Striatal NAPE, but not FAE accumulation is enhanced in mice lacking NAPE-PLD, which also display a substantial reduction in 6-OHDA-induced neurotoxicity, as assessed by measuring survival of substantia nigra dopamine neurons, integrity of striatal dopaminergic fibers, and striatal dopamine metabolite content. Moreover, reduced neuronal damage in NAPE-PLD/- mice is accompanied by a significant attenuation of the motor response evoked by the dopaminergic agonist apomorphine. Consistent with these results, we find that 6-OHDA incubation stimulates NAPE and FAE formation in cultures of human
catecholaminergic SH-SY5Y cells and that siRNA-mediated NAPE-PLD silencing augments NAPE, but not FAE levels in these cells. NAPE-PLD silencing also protects the cells from 6-OHDA-induced reactive oxygen species production, caspase-3 activation, and death. Simultaneously, we looked at naïve NAPE-PLD-/- mice and NAPE-PLD-silenced cells, to determine whether NAPEs may influence the membrane association of LRRK2, a multifunctional protein involved in Parkinson’s disease. NAPE-PLD deletion and the consequent accumulation of non-metabolized NAPEs are accompanied by a remarkable shift of LRRK2 protein from the membrane to the cytosol and a reduction in total LRRK2 content in mice brain and neuronal cell culture. Conversely, exposure of intact SH-SY5Y cells to an exogenous PLD lowers NAPE levels and enhances LRRK2 association with membranes. These findings point to a previously unrecognized role for NAPE-PLD in the regulation of dopamine neuron survival, which might be linked to the ability of this enzyme to control LRRK2 localization by regulating membrane NAPE levels.

Disclosures: F. Palese: None. D. Piomelli: None. S. Pontis: None. N. Realini: None.

Poster

294. Molecular Underpinnings of LRRK2 Function and Dysfunction

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 294.07/F21

Topic: C.03. Parkinson’s Disease

Title: LRRK2 kinase inhibitor mitigates rotenone-induced cellular senescence in dopaminergic neurons

Authors: *D.-H. HO, I. SON, W. SEOL; InAm Neurosci. Res. Ctr., Gunpo, Korea, Republic of

Abstract: Leucine-rich repeat kinase 2 (LRRK2) is one of the pathogenic factors for Parkinson’s disease (PD). Our previous study demonstrated that LRRK2-mediate p53 phosphorylation increased the induction of p21 and neuronal toxicity. The activated p53-p21 pathway is involved in the cellular senescence. In a previous study, rotenone promoted cellular senescence. The cellular senescence is responsible for the defect of proteolytic pathways in cells. Rotenone-induced LRRK2 kinase activity is reported as a mediator of neuronal death by oxidative stress. Thus, we hypothesized that rotenone-mediated LRRK2 kinase activation would induce senescence in dopaminergic neuron via the induction of p21 following the phosphorylation of p53, thereby deteriorating the aggregation of α-synuclein (αSyn). We treated rotenone with or without GSK2578215A (GSK-KI), an LRRK2 kinase inhibitor, into differentiated SH-SY5Y cells for 48hr. Cell lysates were analyzed by Western blot assay and Cathepsin D (CTSD) activity assay. In addition, we co-treated SH-SY5Y cells with αSyn fibril under the same condition as above and cell lysates were subjected to Western blot analysis. Rotenone promoted
the cellular senescent markers, which are represented by the decrease in phosphorylated Rb and the increase in β-galactosidase levels, and changes of these senescent markers were rescued by the administration of LRRK2 kinase inhibitor. CTSD activities were also changed along with rotenone-induced LRRK2 kinase activity or co-treatment of GSK-KI. The accumulation of αSyn fibril was accelerated by rotenone treatment along with the increase in p62 level and LC3BII/I ratio, but the administration of GSK-KI rescued αSyn accumulation. In reprogrammed human dopaminergic neurons, similar results were found in the treatment of rotenone with or without MLI-2 LRRK2 kinase inhibitor. And the ectopic expression of G2019S LRRK2 mutant in differentiated SH-SY5Y and reprogrammed human dopaminergic neurons from G2019 LRRK2 human PD patient fibroblast also showed the increase of cellular senescence markers. These results suggest that LRRK2 kinase activity would promote the cellular senescence; aggravating accumulation of αSyn via dysfunction of lysosomal clearance.

Disclosures: D. Ho: None. I. Son: None. W. Seol: None.

Poster

294. Molecular Underpinnings of LRRK2 Function and Dysfunction

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 294.08/F22

Topic: C.03. Parkinson’s Disease

Title: The Michael J. Fox Foundation’s efforts to develop new tools for understanding LRRK2 biology and the role of LRRK2 in Parkinson’s disease pathology

Authors: *N. K. POLINSKI1, T. MARTINEZ1, A. TRAN NGUYEN2, D. J. MOORE3, S. M. YOUNG, JR4, A. KALOGEROPULOU5, D. R. ALESSI5, S. PADMANABHAN1, M. BAPTISTA1, K. DAVE1;
1The Michael J. Fox Fndn., New York, NY; 2CNS, 3Ctr. for Neurodegenerative Sci., Van Andel Res. Inst., Grand Rapids, MI; 4Dept. of Anat. and Cell Biol., Univ. of Iowa, Iowa City, IA; 5Univ. of Dundee, Dundee, United Kingdom

Abstract: As the greatest known genetic contributor to Parkinson's disease (PD), the leucine-rich repeat kinase 2 (LRRK2) gene and protein are targets of interest for Parkinson’s disease research and therapeutic development. Unfortunately, critical research tools for understanding the function and role of LRRK2 in disease pathogenesis are lacking. To address this gap, The Michael J. Fox Foundation (MJFF) has taken an active role in designing, validating, and distributing various tools and models that can be used to investigate LRRK2-related mechanisms of PD neurodegeneration or strategies to prevent, slow, or halt disease progression. Here we summarize MJFF-led efforts to develop and characterize a variety of LRRK2-related tools. One such tool is a set of viral vectors that can be used to overexpress wild-type and various mutant forms of LRRK2 in vivo to understand the role of this protein in disease biology, pathways
related to PD, and test therapeutic interventions aimed at reducing LRRK2-related pathology. Characterization data for these viral vectors in the rat brain will be presented, including information on the expression profile and resulting neurodegeneration. In addition, we will be presenting data on a new mouse model that overexpresses Rab29 protein—a protein that increases LRRK2 kinase activity and acts as a substrate for LRRK2 kinase activity. We will provide data on the protein and mRNA expression levels, as well as data demonstrating the effects on LRRK2 kinase activity. Finally, we will include information on how to access these important tools and models. Ultimately, MJFF’s investment in providing the research community with robust, well-characterized tools and models will speed research towards a cure for PD by enabling research, de-risking investment in PD research, and increasing reproducibility by providing the tools to researchers across labs.


Poster

294. Molecular Underpinnings of LRRK2 Function and Dysfunction

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 294.09/F23

Topic: C.03. Parkinson’s Disease

Support: NRF-2017R1A2B4008456

Title: Collapsin response mediator protein (CRMP)-2 is pathological target in LRRK2 R1441G Parkinson's disease model

Authors: *J. SONG, J. JEON, T. KIM, H. SEO;
Hanyang Univ., Seoul, Korea, Republic of

Abstract: Parkinson's disease (PD) is one of the most prevalent chronic neurodegenerative disorders. PD is caused by progressive degeneration of dopaminergic neurons in the substantia nigra. Mutation in leucine-rich repeat kinase (LRRK2) is frequently found in familial and sporadic PD and LRRK2 Roc domain R1441G mutation has been previously studied to explain dopaminergic cell death. In this study, we determined differentially phosphorylated proteins in LRRK2 R1441G transgenic mice using phospho-proteomics technology, and found that several cytoskeletal proteins are involved as phosphorylated targets including collapsin response mediator protein (CRMP)-2. CRMPs have been reported as crucial regulator of microtubule dynamics during neurite/axonal growth. Phosphorylation level of CRMP-2 was increased in LRRK2 R1441G transgenic mice compared to normal mice. When GW5074 was administered to inhibit LRRK2 activity, the level of phosphorylation of CRMP-2 was down-regulated in vivo and
*in vitro* PD models. GW5074 also improved neurite outgrowth of primary cultured cells and improved motor deficits in LRRK2 R1441G mice. These results suggest that cytoskeletal proteins are pathologically targeted in LRRK2 R1441G Parkinson’s disease model and the phosphorylation of specific cytoskeletal protein can explain the mechanism of dysfunctional cytoskeletal transport in PD.

**Disclosures:** J. Song: None. J. Jeon: None. T. Kim: None. H. Seo: None.

**Poster**

**294. Molecular Underpinnings of LRRK2 Function and Dysfunction**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 294.10/F24

**Topic:** C.03. Parkinson’s Disease

**Support:** MJFF Grant 4219720

**Title:** Increased sleep spindle density in LRRK2 G2019S mice

**Authors:** *E. J. MONROE*1, L. M. CROWN4, M. J. BARTLETT5, J.-P. WIEGAND2, A. J. EBY2, T. FALK6, S. L. COWEN3;

1Dept. of Biomed. Engin., 3Dept. of Psychology, 2Univ. of Arizona, Tucson, AZ; 4Dept. of Psychology, Univ. Of Arizona, Tucson, AZ; 5Dept. of Pharmacol., 6Dept. Of Neurol., Univ. of Arizona Col. of Med., Tucson, AZ

**Abstract:** The G2019S mutation of the leucine-rich repeat kinase 2 (LRRK2) gene is the most common genetic cause of Parkinson’s disease (PD). Sleep disorders are a common complaint of early PD patients, suggesting a tie between neuronal networks that regulate sleep and those involved in PD. Sleep spindles are thalamo-cortical oscillations involved in memory consolidation during sleep, and they are generated via GABAergic and glutamatergic neurotransmission. Because previous studies have shown increased glutamatergic activity in neurons of LRRK2 G2019S mice, we hypothesized that sleep spindle activity would be enhanced in LRRK2 G2019S knock-in mice. To test this, we recorded electrocorticographic (ECoG) activity from 7-10 mo. old male mice (n=24 wild-type and n=28 LRRK2 G2019S) before and after they performed either a rotarod-based motor-enrichment task or spent one hour in an unenriched polycarbonate box. While we found that expressing the G2019S mutation did not alter physiological features of the sleep spindles themselves (oscillatory frequency, power, or duration), we did observe that LRRK2 animals showed a significantly higher sleep spindle density (spindles per minute of sleep) compared to wild type animals (2-sample t-test, p=0.04). This supports our hypothesis that increased spindle density represents hyperactivity of cortico-thalamic networks. Additionally, in wild-type animals spindle density in post-task sleep was no different between motor enrichment and unenriched days, suggesting that either the task was not
enriching enough to engage consolidation processes or that the un-enriching task was still sufficiently novel to induce memory consolidation. In contrast, the LRRK2 animals showed greater spindle density following the unenriched experience than following the motor task (paired t-test, p<0.001). The increase in spindle density following the unenriched experience was also significantly greater than for wild type animals (2-sample t-test, p=0.014), suggesting that spindle density increases in LRRK2 animals as sleep progresses. Finally, to determine if these effects were a direct result of excessive LRRK2 kinase activity, we administered MLi-2 (Merck Pharmaceuticals, 60mg/kg in-diet), a LRRK2 kinase inhibitor, in the second week of experiments (n=12 LRRK2, n=14 wild type), and we found no effect of the drug on spindle density or spindle features in either LRRK2 or wild type animals. In sum, the observed increase in sleep spindle density may indicate excessive excitatory activity in LRRK2 cortical neurons. Such excitation could stress cortico-striatal-thalamic loops involved in motor function and contribute to PD.


Poster

294. Molecular Underpinnings of LRRK2 Function and Dysfunction

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 294.11/F25

Topic: C.03. Parkinson’s Disease

Support: NIH (R21 AG058241)
Van Andel Research Institute

Title: Neuroprotective effects of lifespan-extending pathways in a G2019S LRRK2 model of Parkinson’s disease

Authors: *M. SENCHUK1, J. VAN RAAMSDONK2, D. MOORE1;
1Van Andel Res. Inst., Grand Rapids, MI; 2Harvard, Cambridge, MA

Abstract: Increased age is the primary risk factor associated with the development of Parkinson’s disease (PD). Genetic mutations associated with familial PD (i.e. SNCA, LRRK2) are generally well-tolerated until advanced age, suggesting an interplay between PD pathogenesis and normal age-associated cellular decline. Conserved pathways regulating aging have been defined in C.elegans, including the insulin/IGF pathway, dietary restriction, proteostasis, mitochondrial dynamics and reactive oxygen species. Previous studies have established that modulation of the insulin/IGF pathway is neuroprotective in C.elegans and Drosophila models of PD, while compounds inducing lifespan extension including metformin, resveratrol, and rapamycin have been found to be beneficial in cell and animal models of disease. In this study,
we sought to determine whether chronological age can be uncoupled from neurodegeneration to further understand how age-related decline leads to neuronal loss. We employed a *C. elegans* model of PD expressing human G2019S LRRK2 specifically in dopaminergic neurons, combined with genetic deletion or tissue-specific gene silencing of key lifespan-regulating genes, including ife-2(EIF4E) (protein translation), eat-2 (caloric restriction), nuo-6 (mitochondrial dynamics/Complex I) osm-/che (osmotic sensing), gpl-1 (germline ablation) and daf-2/age-1 (insulin signaling). The studied aimed to examine the correlation between lifespan extension and rescue of dopaminergic degeneration, and moreover, to identify pathways impacting LRRK2-mediated phenotypes. Our data demonstrate that PD-related phenotypes can be disassociated from aging *per se*, as not all lifespan-extending mutants can rescue G2019S LRRK2-induced neurodegeneration. Targeting specific age-related pathways (age-1, daf-2, nuo-6) provides the most robust rescue of G2019S LRRK2-induced neurodegeneration, suggesting that these pathways may contain druggable targets for PD. The deletion of age-1 and daf-2 genes were most effective at providing neuroprotection against LRRK2, and accordingly we are currently conducting rescue experiments with pan-neuronal or dopaminergic-specific AGE-1 and DAF-2 transgenes to determine the cell autonomous effects of these lifespan signaling pathways. Similarly, the role of transcriptional regulator daf-16 (FOXO) and cellular energy homeostasis effector aak-1/2 (AMPK) downstream of daf-2/age-1 and metformin pathways, respectively, are being assessed. Our study demonstrates that targeting specific age-related pathways can be neuroprotective in a G2019S LRRK2 animal model of PD.

**Disclosures:** M. Senchuk: None. J. Van Raamsdonk: None. D. Moore: None.

**Poster**

294. Molecular Underpinnings of LRRK2 Function and Dysfunction

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 294.12/F26

**Topic:** C.03. Parkinson’s Disease

**Title:** The G2019S LRRK2 mutation affects the oxidative stress and neurodegenerative response in a novel dual-hit *in vitro* co-culture of Parkinson's disease

**Authors:** *T. J. CHARLTON*, B. M. FENNER, Z. DWYER, W. WILLMORE, S. HAYLEY;

1Neurosci., Carleton Univ., Ottawa, ON, Canada; 2Neurosci., Kings Col., Wilkes-Barre, PA

**Abstract:** The majority of cases of Parkinson’s disease (PD) are idiopathic, with much emphasis recently being placed on a potential causative role for environmental chemical and immune agents. In any case, microglial-driven neuroinflammatory processes are clearly somehow involved in disease genesis and/or development. The inflammatory protein, LRRK2, has been implicated as both a familial and a vulnerability factor in PD and interestingly, is found in high
levels in the immune cells and is known to affect the release of oxidative free radicals. We were interested in assessing the role of LRRK2 in isolated neurons and microglial cells. To this end, primary microglia and dopaminergic cells from wild type mice and LRRK2 G20192 knock-in mice were assessed using a double-hit model of PD. Specifically, microglial were separately pre-treated with LPS and neurons pre-treated with glutamate and then co-cultured together. We found that the release of reactive oxygen species as well as mitochondrial specific superoxides were particularly increased in the G2019S knockin mice. Further, RT-qPCR analyses revealed increased expression of proinflammatory cytokines and the WAVE2 protein in wild type but less so in LRRK2 mutants, which is important in the phagocytic response of microglia. These data are consistent with a role for LRRK2 in PD inflammatory pathology and also suggest a novel dual-hit in vitro model that could be used as testing platform for potential therapeutics.


Poster

295. Alpha-Synuclein Models and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 295.01/F27

Topic: C.03. Parkinson’s Disease

Support: FRQS

Parkinson Canada

Title: Towards the development of a marmoset model of Parkinson's disease based on the spreading of human alpha-synuclein pre-formed fibrils

Authors: *C. KWAN1,2, D. BÉDARD1, M. KANG2, S. G. NUARA2, J. C. GOURDON2, A. MATHIEU3, C. L. TARDIF1, G. MASSARWEH1, T. M. DURCAN1, A. HAMADJIDA1, E. A. FON1, P. ROSA-NETO2, S. FREY4, P. HUOT1;

Abstract: The protein alpha-synuclein is widely disseminated within the brain of patients with advanced Parkinson’s disease (PD) and there is increasing evidence suggesting that this abnormal dissemination and accumulation of alpha-synuclein may be neuro-toxic. Therefore, drugs interfering with alpha-synuclein propagation might perhaps slow PD progression. A key step in the development of such drugs is the development of animal models of PD that recapitulate this feature of the disease. Here, we present preliminary results of a pilot experiment conducted in a male common marmoset which was injected with alpha-synuclein pre-formed fibrils (PFFs) in the putamen. The co-ordinates of the putamen were determined after acquisition
of brain magnetic resonance imaging (MRI) and computed tomography (CT) scans. Baseline striatal dopaminergic innervation was determined by performing positron emission tomography (PET) scan to the vesicular monoaminergic transporter 2 (VMAT2) with $[^{11}\text{C}]$-dihydrotetrabenazine (DTBZ). Endotoxin-free recombinant human alpha-synuclein PFFs were synthesised and injected in the left putamen of a marmoset using a neuro-navigational approach with the Brainsight® software. PET scans with $[^{11}\text{C}]$-DTBZ were obtained 1- and 2-months post-surgery. Two months after intra-putaminal alpha-synuclein PFFs injection, there was a noticeable reduction of binding to the VMAT2 within the striatum. Post-mortem experiments to characterise the extent of dopaminergic lesion within the striatum and the substantia nigra, as well as the extent of alpha-synuclein spreading are on-going and the results will be presented at the conference. These preliminary results suggest that the injection of human alpha-synuclein PFFs in the putamen of the common marmoset might lead to dopaminergic denervation. This represents an exciting first step in the creation of a marmoset model of PD based on the propagation of alpha-synuclein.


Poster

295. Alpha-Synuclein Models and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:/Poster #: 295.02/F28

Topic: C.03. Parkinson’s Disease

Support: Department of Veterans Affairs 5I01RX000441-04
        National Institute of Health 5R01DK100281-03

Title: Early sociability and social memory impairment in A53T mouse model of Parkinson’s disease are ameliorated by chemogenetic modulation of orexin neuron activity

Authors: *M. STANOJLOVIC$^1$, J. PALLAIS$^1$, A. VIJAYAKUMAR$^1$, C. M. KOTZ$^2$;
         $^1$Univ. of Minnesota, Minneapolis, MN; $^2$Integrative Biol. and Physiol., Univ. of Minnesota Twin Cities, Minneapolis, MN

Abstract: Parkinson’s disease (PD) is a multi-layered progressive neurodegenerative disease. Signature motor system impairments are accompanied by a variety of other symptoms such as mood, sleep, metabolic and cognitive disorders. Interestingly, social cognition impairments can be observed from the earliest stages of the disease, prior to the onset of the motor symptoms. In this study we investigated age-related reductions in sociability and social memory in the A53T
mouse model of PD. Since inflammation and astrogliosis are an integral part of PD pathology and impair proper neuronal function, we examined astrogliosis and inflammation markers and parvalbumin expression in medial pre-frontal cortex (mPFC), part of the brain responsible for social cognition regulation. Finally, we used DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) for stimulation/inhibition of orexin neuronal activity to modulate sociability and social memory in A53T mice. We observed that social cognition impairment in A53T mice is accompanied by an increase in astrogliosis and inflammation markers and loss of parvalbumin neurons and inhibitory pre-synaptic terminals in mPFC. Moreover, DREADD induced activation of orexin neurons restores social cognition in the A53T mice model of PD.

**Disclosures:**  M. Stanojlovic: None. J. Pallais: None. A. Vijayakumar: None. C.M. Kotz: None.

**Poster**

**295. Alpha-Synuclein Models and Mechanisms I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 295.03/F29

**Topic:** C.03. Parkinson’s Disease

**Support:** NRF-2016M3C7A1904391

**Title:** Osmotin mitigates susceptibility of alpha-synuclein and mptp induced neuropathology and dopaminergic neuronal death via the AMPK activation

**Authors:** *M.-G. JO, M.-H. JO, M. IKRAM, M.-W. KIM, M.-O. KIM;

Div. of Applied Life Sci. (BK 21), Col. of Natural Sci., Gyeongsang Natl. Univ., Jinju, Korea, Republic of

**Abstract:** Parkinson’s disease (PD) is the age-associated neurodegenerative disorder. It is clinically characterized by tremor, bradykinesia, muscle rigidity and postural instability. The accumulation of pathological α-synuclein as a major component of Lewy bodies is considered as a neuropathological characterization in PD. AMPK is a master regulator believed to play a critical role in the control of energy homeostasis and survival of neurons. Osmotin, an adiponectin homolog, induces the phosphorylation of AMPK which is a downstream marker of adiponectin receptor 1 and exhibits therapeutic effects on PD models. Osmotin mitigated neurological impairment in transgenic mice overexpressing neuron specific enolase (NSE)-controlled human alpha-synuclein. It also attenuated dopaminergic neuronal cell death in the SNpc region and enhanced dendritic complexity and spine density in the hippocampal region of the PD models. Osmotin not only reduced α-synuclein expression but also enhances cell survival and synaptic neurotransmission through enhanced dopamine synthesis. Taken together, our study
suggests that osmotin exerts a potential role as a therapeutic agent against α-synuclein induced neuropathy via activation of AMPK and its associated downstream signaling proteins.


Poster

295. Alpha-Synuclein Models and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 295.04/F30

Topic: C.03. Parkinson’s Disease

Support: NIH R01 NS038065
        NIH R01 NS086074
        NIH R01 NS092093
        NIH NS108686
        NIH R01 AG062135

Title: Alpha synucleinopathy associated cABL activation causes p53 dependent autophagy impairment

Authors: *R. KARIM1, E. E. LIAO2, M. K. LEE3;
2Neurosci., 1Univ. of Minnesota, Minneapolis, MN

Abstract: Studies link c-Abl activation with the accumulation of pathogenic α-synuclein (αS) and neurodegeneration in Parkinson’s disease (PD). Currently, c-Abl, a tyrosine kinase activated by cellular stress, is thought to promote αS pathology by either directly phosphorylating αS or by causing autophagy deficits. We show that the pathologic effects of c-Abl in PD might also involve activation of p53, as c-Abl activation in a transgenic mouse model of α-synucleinopathy (TgA53T) and human PD cases is associated with increased p53 activation. Significantly, active p53 in TgA53T neurons accumulates in the cytosol, which may lead to inhibition of autophagy. Thus, we hypothesized that c-Abl-dependent p53 activation contributes to autophagy impairment in α-synucleinopathy. In support of the hypothesis, we show that c-Abl activation is sufficient to inhibit autophagy in p53-dependent manner and inhibition of either c-Abl, using nilotinib, or p53, using pifithrin-α, was sufficient to increase autophagic flux in neuronal cells. Moreover, nilotinib and pifithrin-α upregulated the phosphorylation of AMP-activated kinase (AMPK) which was accompanied by pS555-ULK1 activation and conversely down-regulated mTORC1 signal molecule ribosomal S6 and translation repressor protein 4EBP1. These results show that c-Abl inhibition induces autophagy-lysosomal process via regulation of p53, which in turn inhibit mTORC1 by ULK1 activation. Finally, we show that pharmacological attenuation of c-Abl activity in the TgA53T model reduces activation of p53, stimulates autophagy, decreases accumulation αS pathology, and delays disease onset. Collectively, our data show that c-Abl
activation by α-synucleinopathy causes p53 dependent autophagy deficits and both c-Abl and p53 represent therapeutic target for PD.

**Disclosures:** R. Karim: None. E.E. Liao: None. M.K. Lee: None.

**Poster**

**295. Alpha-Synuclein Models and Mechanisms I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program#/Poster #:** 295.05/F31

**Topic:** C.03. Parkinson’s Disease

**Support:**
- Multipark Grant 015-03684
- Crafoord Foundation Grant
- Hedlund Foundation Grant

**Title:** Oligodendrocyte dysfunction in Parkinson’s disease: A study case

**Authors:** *C. AZEVEDO, G. TEKU, M. CHUMARINA, M. VIHINEN, L. ROYBON;

**Abstract:** Oligodendrocytes, which are produced during the development and postnatally, are responsible for myelinating axons in the central nervous system. During differentiation, oligodendrocytes undergo morphological changes and express specific markers depending on the state of differentiation. Developing oligodendrocytes are characterized by co-expression of transcription factors SOX10, OLIG2 and NKX2.2. Oligodendrocyte progenitor cells (OPCs) are the first cell type of the oligodendrocyte lineage and express PDGFR-alpha. The transition of OPCs to immature/non-myelinating oligodendrocytes is marked by the modification of cell-surface sulfatide, which is recognized as O4 antigen. Mature-myelinating oligodendrocytes express myelin basic protein (MBP), which is involved in the myelination of axons. An important challenge in disease modeling using stem cells is to produce enough oligodendrocytes to be able to reveal disease mechanisms. Also, recent evidence suggests that oligodendrocytes may play an important role in neurodegenerative diseases. Scaling up the production of oligodendrocytes is therefore of paramount importance for modeling neurodegenerative diseases and study possible disease-related mechanisms. Here, we report a new protocol for generating oligodendrocytes from human pluripotent stem cells, yielding up to 60% of O4-positive oligodendrocyte lineage cells measured by fluorescent activated cell sorting (FACS). Interestingly, oligodendrocytes carrying the A53T variation in the SNCA gene encoding for alpha-synuclein exhibited delayed maturation from OPCs to MBP-positive oligodendrocytes when compared to control cells. Gene expression studies of FACS-purified O4-positive oligodendrocytes supported this evidence: we observed upregulation of OPCs-related transcripts and downregulation of myelin/mature oligodendrocyte-related transcripts in SNCA A53T
variants compared to control. The scaled-up production of oligodendrocytes from diseased pluripotent stem cells should allow further identification of altered cellular networks, potentially revealing oligodendrocytes as novel targets for therapeutic intervention in Parkinson’s disease.

**Disclosures:**  C. Azevedo: None. G. Teku: None. M. Chumarina: None. M. Vihinen: None. L. Roybon: None.

**Poster**

**295. Alpha-Synuclein Models and Mechanisms I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 295.06/F32

**Topic:** C.03. Parkinson’s Disease

**Support:** NTU The Ministry of Science and Technology

**Title:** The propagation of alpha-synuclein in the central nervous system and other organ systems in the Parkinson’s disease rat model


**Abstract:** Parkinson's disease (PD) is the second most common movement disorder, owing to degenerative loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNc) of midbrain. Recent studies showed that the main hallmark of PD is the Lewy body pathology, resulting from aggregation of α-synuclein. The assembly of toxic, oligomeric species of α-synuclein can be further spread out to other brain regions upon PD progression. Moreover, in the past decade, constipation has been found as one of the most common symptoms of PD. Treating the gastrointestinal (GI) complaints appeared to alleviate the movement-related problems associated with PD. In 2003, Heiko Braak proposed that PD may originate in the gut rather than the brain, as evidenced by that Lewy bodies appeared in both brain and GI system in post-mortem samples of PD patients. However, it remains unclear what is the relationship between PD progression and the propagation of α-synuclein aggregates in the gut-brain axis. To answer this question, we examined whether the human pathogenic α-synuclein mutant may propagate from the SNc to the gut or other peripheral organs in a rat model mimicking sporadic PD during adulthood. In this PD model, male Sprague-Dawley rats (~250 g, 2 week-old) were deeply anesthetized for stereotaxic surgery. The 2 μL solution containing DNA plasmids (5 μg/μL) was microinjected into SNc to deliver genes expressing the human pathogenic α-synuclein mutant. Five electric pulses were delivered by homemade platinum electrodes via ear bars, with duration
of 50 msec, intervals of 950 msec, and electric field strength of 133 V/cm. After 3-4 months following *in vivo* electroporation, the motor function and dopamine neuronal loss in the PD rats were examined by gait analysis and immunostaining of the DA neuronal marker, respectively. Furthermore, the PD rats were sacrificed to verify α-synuclein aggregates in the SNc by immunostaining the phosphorylated Ser129 (pS129) of α-synuclein (the pS129 immunoreactivity as the hallmark of α-synuclein aggregates). Finally, by using Western analysis, we found that the tissues from the gut and epididymis fat also presented the pS129 immunoreactivity. These results suggest that the human pathogenic α-synuclein mutant can be propagated from the rat brain to other peripheral organs during the early stage of PD progression.

**Disclosures:** L. Cheung: None. Y. Chen: None. C. Wang: None.

**Poster**

295. *Alpha-Synuclein Models and Mechanisms I*

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 295.07/F33

**Topic:** C.03. Parkinson’s Disease

**Support:** NIH/NINDS NS038377

NIH/NINDS NS082205

NIH/NINDS NS098006

**Title:** Transneuronal Propagation of Pathologic α-synuclein From the Gut to the Brain Models Parkinson’s Disease

**Authors:** *S. KIM*¹, S.-H. KWON¹, T.-I. KAM¹, N. PANICKER¹, S. S. KARUPPAGOUNDER¹, S. LEE¹, J. LEE⁴, W. KIM¹, M. KOOK¹, C. A. FOSS², C. SHEN⁵, S. KULKARNI³, P. J. PASRICA³, G. LEE¹, M. G. POMPER², V. L. DAWSON¹, T. M. DAWSON¹, H. KO¹;

¹Inst. for Cell Engin., ²The Russell H. Morgan Dept. of Radiology and Radiological Sci., ³Ctr. for Neurogastroenterology, Dept. of Med., Johns Hopkins Univ., Baltimore, MD; ⁴Dept. of Pharmacol. and Toxicology, Univ. of Alabama at Birmingham Sch. of Med., Birmingham, AL; ⁵Dept. of Nuclear Med., Shanghai Jiao Tong Univ. Affiliated Sixth People’s Hosp., Shanghai, China

**Abstract:** Analysis of human pathology led Braak to postulate that α-synuclein (α-syn) pathology could spread from the gut to brain, via the vagus nerve. Here, we test this postulate by assessing α-synucleinopathy in the brain in a novel gut-to-brain α-syn transmission mouse model, where pathological α-syn preformed fibrils were injected into the duodenal and pyloric muscularis layer. Spread of pathologic α-syn in brain, as assessed by phosphorylation of serine 129 of α-syn, was observed first in the dorsal motor nucleus, then in caudal portions of the
hindbrain including the locus coeruleus, and much later in basolateral amygdala, dorsal raphe nucleus, and the substantia nigra pars compacta. Moreover, loss of dopaminergic neurons, motor and non-motor symptoms were observed in a similar temporal manner. Truncal vagotomy and α-syn deficiency prevented the gut-to-brain spread of α-synucleinopathy and associated neurodegeneration and behavioral deficits. This study supports the Braak hypothesis in the etiology of idiopathic PD.


Poster

295. Alpha-Synuclein Models and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 295.08/F34

Topic: C.03. Parkinson’s Disease

Support: Michael J. Fox Foundation
T32GM008076

Title: Poly(ADP-ribose) bound α-synuclein fibrils display different binding properties of PET tracers

Authors: *Z. LENGYEL1, L. PUENTES2, J. J. FERRIE3, J. PETERSSON3, R. H. MACH1; 1Dept. of Radiology, 2Dept. of Systems Pharmacol. and Translational Therapeut., 3Dept. of Chem., Univ. of Pennsylvania, Philadelphia, PA

Abstract: α-synuclein (α-syn) is an intrinsically disordered protein that is localized at the presynaptic nerve terminals in the central nervous system. Aggregation of this protein results in insoluble Lewy bodies and Lewy neurites, that is the major hallmark feature in Parkinson’s disease (PD). Traditionally, patients are diagnosed with PD upon the clinical presentation of motor disturbances, however early diagnosis is problematic since these features may also appear in patients with Parkinsonian syndromes. Due to the advances of positron emission tomography (PET) imaging of amyloid β (Aβ) plaques and tau-based pathology in Alzheimer’s (AD) patients, there have been interests in developing PET radiotracers to map aggregated α-syn in PD patients to improve early stage clinical diagnosis of this disease. However, since Aβ and tau have both been found in post-mortem tissue of PD and AD patients, developing a selective α-syn ligand has been extremely challenging. A greater understanding of protein interactions and binding sites for small molecules in α-syn fibrils is important to guide the design of PET radiotracers.
Recently, it was shown that poly(adenosine 5’-diphosphate-ribose) polymerase-1 (PARP-1) modulates α-syn fibril formation in mouse and that α-syn fibrils can, in turn, activate PARP-1 and increase PAR levels. We hypothesize that this PAR/α-syn interaction changes not only the biophysical properties of the fibrils, but also the binding properties of PET radiotracers. Using various biochemical techniques, we characterized α-syn fibrils formed in the presence of PAR and showed that PAR accelerates α-syn fibril formation in vitro and increases fibril stability. We also conducted radioligand saturation binding assay using various radiotracers that bind to different sites in α-syn fibrils. Interestingly, the radiotracer that binds closer to the N-terminus of the protein displayed a significantly different binding curve and dissociation constant in α-synuclein fibrils compared to α-synuclein+PAR fibrils. This study suggests that PAR either blocks the binding sites near the N-terminus or alters the structure of α-synuclein fibrils, thus altering the binding of radiotracer. These findings will aid the development of PET imaging agents and also drugs targeting α-synuclein.


Poster

295. Alpha-Synuclein Models and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 295.09/F35

Topic: C.03. Parkinson’s Disease

Title: Modulation of lipid metabolism alters alpha-synuclein based aggregation and toxicity in Parkinson’s disease

Authors: *J. W. NICHOLATOS, L. A. HRDLICKA, L. DANG, J. GROOT, X. HRONOWSKI, W. D. HIRST, A. WEIHOFEN; Biogen, Cambridge, MA

Abstract: α-Synuclein is the major component of the hallmark aggregates (Lewy bodies) found in Parkinson’s disease (PD) patients. The interaction of α-Synuclein with lipid membranes is emerging as a critical feature in its normal function and its propensity for aggregation and toxicity, which is hypothesized to drive PD pathophysiology. Indeed, familial Parkinson’s mutations, such as E46K, have been shown to alter α-Synuclein lipid membrane interactions. We are investigating the relationship between α-Synuclein and lipid metabolism using the 3K α-Synuclein model, which harbors E46K and two additional lysine mutations in KTKEGV motifs. The 3K α-Synuclein model is more prone to aggregation and cytotoxicity than E46K alone, making it useful to study synuclein pathology. Transcriptional profiling of primary neuronal cultures transduced with 3K α-Synuclein showed enrichment in genes related to lipid biosynthetic processes as significantly altered, this included Stearoyl-CoA desaturase (SCD).
Modulation of SCD, and other identified genes pharmacologically and/or by siRNA, altered 3K α-Synuclein based aggregation and cytotoxicity. Additionally, mass spectrometry of neuroblastoma cells expressing 3K α-Synuclein revealed changes in lipid metabolites, supporting a connection between lipid metabolism and α-Synuclein pathology. Our data suggest that modulation of lipid metabolism can alter α-Synuclein based pathology and may be a viable strategy for novel Parkinson’s therapeutics.

with sex modelled as a covariate.
Significant group differences among component weightings were found for two NMF components (p<0.05), revealing two significant patterns of αSyn PFF-induced pathology. Component 4 revealed a striatal-nigral-hypothalamic pattern, while Component 2 revealed a midbrain-pons-medulla pattern (Fig. 1).
The inoculation of αSyn PFF in the striatum give rise to widespread network patterns of PFF-induced brain morphological changes, particularly involving regions that project to, or receive input from the injection site. These results are in accordance with PD studies in which show prominent αSyn pathology in the neurons of these structures, as well as functional impairment of these structures in patients with PD.

Title: Endogenous astrocytic CDNF protects dopamine neurons in AAV-A53T-α-synuclein rat model

Authors: *K. KYOUNG IN¹, J. JANG², A. HONG², Y. CHUNG², B. JIN¹-²;
¹Dept. of Neuroscience, Grad. School, Kyung Hee Univ., Seoul, Korea, Republic of; ²Dept. of Biochem. & Mol. Biology, Kyung Hee Univ., Seoul, Korea, Republic of

Abstract: Abnormal alpha synuclein (α-syn; SNCA) is one of the components within Lewy body characterized by Parkinson’s disease (PD). A53T mutant α-syn induces mitochondrial autophagy and macroautophagy leading to cell death. Cerebral dopamine neurotrophic factor (CDNF), endoplasmic reticular (ER)-resident protein, is a promising therapeutic target treating PD and inhibits ER stress-induced apoptosis pathway. Here we report that CDNF endogenously produced in astrocytes regulates autophagy pathway in A53T-α-syn-lesioned rat PD model, resulting in improvement of amphetamine-induced rotational asymmetry and increased survival of dopamine neurons in the substantia nigra. The present study is the first description determining function of CDNF in A53T-α-syn-lesioned genetic animal model of PD and might give great insight treating familiar PD therapy.


Poster

295. Alpha-Synuclein Models and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 295.12/F38

Topic: C.03. Parkinson’s Disease
Title: Examination of pathological synuclein phosphorylation in relation to the spatial and temporal progression of tyrosine hydroxylase depletion and other disease-related endpoints in an intracranial seeding mouse model of Parkinson’s disease

Authors: *R. C. GENTZEL, J. SUGAM, D. TOOLAN, W. GOCAL, S. PARMENTIER-BATTEUR, S. M. SMITH, J. MARCUS; Merck and Co., Inc., West Point, PA

Abstract: Parkinson disease (PD) pathology is predominated by aberrant forms of alpha-synuclein protein (aSyn), observable in detergent-insoluble biochemical extractions and phosphorylated at serine 129 (pS129). Transmission of pathological aSyn is thought to occur during PD progression. Supporting this hypothesis, animal models that incorporate intracranial administration of recombinant sonicated aSyn fibrils (aSyn PFFs) result in the formation and spread of synuclein pathology to anatomically interconnected brain regions such as the cortex, amygdala, striatum and substantia nigra. In this work, we describe the phenotypes of a transgenic mouse model (homozygous, heterozygous and wildtype littermates; male mice) expressing human A30P synuclein under the control of the Thy-1 promoter. The A30P mutation of synuclein is a known genetic cause for a familial form of PD. We observe human A30P synuclein protein robustly overexpressed throughout the brain in this mouse; interestingly, pS129 synuclein is observed in only a restricted set of brain structures. Using traditional 2D histology as well as 3D histology (LifeCanvas Technologies), we further investigated the interplay of synuclein pathology, Iba1 and dopamine levels in this mouse model of PD. In A30P mice, aSyn PFF injection resulted in a morphological conversion of endogenous pS129 signal into Lewy neurite-like structures, an indication of synuclein pathology. In addition, administration of aSyn PFF into A30P resulted in marked elevation of Iba-1 staining, indicating a role for activated microglia. aSyn PFF administration into both WT and A30P animals led to reductions in dopamine containing fibers, suggesting neurodegeneration. Although the PFF-induced pSer129 signal in A30P mice was greatly enhanced in comparison to the WT mice, TH reductions in both the WT and A30P animals were comparable over multiple timepoints. Furthermore, when examined at 90 days post-PFF injections, the loss of TH reduction increased as the signal of pSer129 decreased. Additional studies demonstrated that doubling the initial dose of PFF, while directly impacting the amount of pSer129 that was induced, had only minor, if any, impacts on the level of TH depletion that was observed. Having a deeper understanding of the temporal and spatial progression of different PD-related endpoints and their relationships to each other will enable better interpretations of how therapeutic agents can alter PD disease progression.

Poster

295. Alpha-Synuclein Models and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 295.13/F39

Topic: C.03. Parkinson’s Disease

Support: NIH NS088533
American Parkinson Disease Association
Parkinson Association of Alabama

Title: 14-3-3theta reduces behavioral deficits and alpha-synuclein inclusions in the alpha-synuclein fibril model

Authors: *T. A. Y. YACOUBIAN*1, R. N. UNDERWOOD2, M. GANNON3, S. CHANDRA2, A. KLOP3, A. PATHAK3;
1Univ. Alabama Birmingham, Birmingham, AL; 2Neurol., 3Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Alpha-synuclein (αsyn) plays a critical role in Parkinson’s disease (PD). Research points to a prion-like mode for αsyn toxicity: αsyn is released as aggregated species that cause further aggregation and toxicity in neighboring cells. We have studied the role of 14-3-3 proteins in regulating αsyn propagation. 14-3-3s are chaperone-like proteins that reduce protein aggregation, regulate protein secretion, and promote cell survival. We have previously demonstrated the protective effects of 14-3-3s on αsyn propagation and toxicity in the in vitro fibril model. Here we have expanded our studies to test the effect of 14-3-3s on αsyn propagation in an αsyn in vivo protofibril (PFF) model. To test whether 14-3-3θ blocks αsyn inclusion formation in the PFF model, we injected transgenic cortical 14-3-3θ overexpressing (OE) mice and wild type littermate controls with PFFs in the striatum at 8 weeks and performed behavioral analysis at 3- and 6-months post injection followed by immunohistochemical evaluation of αsyn pathology and cell death. PFF-injection induced a deficit in wildtype mice in the cortically-dependent social dominance test which was rescued by 14-3-3θ OE at 6 months. At 3 months, αsyn inclusion counts were reduced by ~50% in the cortex of 14-3-3θ mice compared to wildtype mice injected with PFFs. No differences in αsyn inclusion counts were observed in the substantia nigra, where 14-3-3θ is not overexpressed in this transgenic mouse line. At 6 months, αsyn inclusion counts in wildtype mice was dramatically reduced in the cortex, presumably due to loss of neurons. However, αsyn inclusion counts in 14-3-3θ mice at 6 months was comparable to that seen at 3 months. These data suggest that 14-3-3θ slows inclusion formation and delays neurodegeneration. We are currently analyzing neuronal counts in these groups. We have also investigated the impact of 14-3-3 inhibition in the in vivo PFF model. Difopein is a pan 14-3-3 peptide inhibitor that we have previously shown to increase αsyn propagation in
Difopein-expressing PFF-injected mice showed increased social dominance deficits at both 3 and 6 months post injection in comparison to wildtype PFF-injected mice. We are currently examining αsyn inclusion counts and neuronal loss in these mice. We conclude that 14-3-3θ regulates αsyn propagation and may serve as a target for therapeutic intervention in Parkinson’s disease.


Poster
295. Alpha-Synuclein Models and Mechanisms I
Location: Hall A
Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM
Program #/Poster #: 295.14/F40
Topic: C.03. Parkinson’s Disease
Title: Could an anti-inflammatory mediator be the culprit in Parkinson’s disease?
Authors: *J. DELA CRUZ;
InTouch BioSolutions LLC, Moreno Valley, CA
Abstract: The etiology of Parkinson’s disease (PD) is not known. We uncovered a pathway that we propose explains the events that result in aggregation of alpha-synuclein (α-syn) and subsequent neurodegeneration in synucleinopathy. Alpha-MSH is a 13 amino acid peptide that functions in pigmentation and energy homeostasis. It is an anti-inflammatory mediator produced by activated microglial cells and regulates inflammation in the brain. Alpha-MSH is implicated in synuclein disease and is elevated in cerebrospinal fluid of patients with PD and Multiple System Atrophy (MSA). PD patients administered with the peptide exacerbated symptoms. In contrast, pharmacologic reduction of alpha-MSH resulted in improved symptoms. Using a human pigmented cell line (MNT-1) as a model of neuromelanin-containing neurons, we observed induction of synuclein pathology after incubation with alpha-MSH. The peptide triggered aggregation of α-syn and impaired autophagy, which was measured by a decrease in melanin. With autophagy impaired, the cells failed to adjust to declining levels of glucose in culture and continued a rate of metabolism that deprived nutrients to vulnerable cells that died by apoptosis. Synuclein pathology in PD also show evidence of impaired cellular autophagy, diminished neuromelanin and display Lewy bodies (LB) composed of aggregated α-syn. In addition, neurons that contain LBs appear normal, while surrounding cells have degenerated and died by apoptosis. The receptor for alpha-MSH is expressed by nigral dopaminergic neurons. We tested whether exogenous alpha-MSH was sufficient to initiate synuclein disease in vivo. Mice administered intranasal alpha-MSH exhibited progressive decline in gait, a prevalent condition seen in patients with PD. Moreover, we observed LB-like aggregate of alpha-synuclein in the substantia nigra. Injury or infection in the brain induces inflammation and activation of
microglial cells. During resolution, microglial cells produce alpha-MSH to reduce inflammation. Our observations suggest synuclein pathology is initiated when bystander cells are exposed to elevated levels of alpha-MSH. Aggregation of a-syn is induced and autophagy is impaired. Furthermore, cells fail to slow metabolism under low nutrient conditions, depriving neighboring cells of vital nutrients. As vulnerable cells die, another round of inflammatory response is initiated leading to chronic cell loss that manifest in a synucleinopathy. Our current goal is to confirm the central role of alpha-MSH in synuclein disease in non-human primates, to design novel therapeutic strategies against PD, MSA and Dementia with LB, that target alpha-MSH and related pathways.

Disclosures:  J. Dela Cruz: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent pending holder.

Poster

295. Alpha-Synuclein Models and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 295.15/F41

Topic: C.03. Parkinson’s Disease

Support: NIH Grant F31NS106733
APDA Research Grant

Title: Rab27b regulates alpha synuclein release, toxicity and clearance

Authors: *R. N. UNDERWOOD, B. WANG, T. YACOUBIAN;
Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Alpha synuclein (αsyn) is the primary component of proteinaceous aggregates termed Lewy Bodies that pathologically define Parkinson’s Disease (PD). αSyn is hypothesized to spread through the brain in a prion-like fashion by misfolded protein forming a template for aggregation of endogenous αsyn. The release and uptake of αsyn from cell to cell are essential processes for this prion-like spread. αSyn does not have a signal peptide for classical secretion and is thought to be released through non-classical secretion mechanisms regulated by a family of proteins called Rab GTPases. Rab27b is one of several GTPases essential to the endosomal-lysosomal pathway and is necessary for the proper localization of endosomal compartments. We have developed an in vitro doxycycline-inducible αsyn model in M17 neuroblastoma cells (termed ISYN cells). Induction of αsyn expression by doxycycline in ISYN cells causes a corresponding increase in the release of αsyn into the conditioned media (CM). When transferred to separately-cultured primary neurons, thisαsyn-enriched CM is toxic to these neurons. We found that upon αsyn induction Rab27b protein expression increased by ~2 fold in the ISYN
Similarly we observed a ~2 fold increase in Rab27b expression in the postmortem human brain lysates from PD patients compared to healthy controls. To examine the impact of Rab27b dependent pathways on αsyn release and toxicity, we knocked down Rab27b expression by lentiviral transfection of shRNA. shRNA knockdown of Rab27b decreased αsyn release into the CM by ~40%. Surprisingly, despite the reduction in αsyn release, CM from induced ISYN cells in which RAB27b was knocked down cells induced greater toxicity in separately cultured SH-SY5Y cells compared to control; this toxicity is ablated by αsyn immunodepletion. We hypothesized that this paradoxical increase in toxicity but decreased paracrine release may be due to changes in the autophagic pathway with Rab27b KD leading to decreased clearance of misfolded αsyn species. Rab27b KD leads to increased formation of endogenous LC3 positive puncta. In addition, Rab27b KD leads to decreased LC3 turnover with bafilomycin treatment, indicating a defect in autophagic flux. These data indicate a potential role for Rab27b in the release, toxicity and clearance of αsyn and ultimately in PD pathogenesis.


Poster

295. Alpha-Synuclein Models and Mechanisms I

Location:  Hall A

Time:  Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 295.16/F42

Topic:  C.03. Parkinson’s Disease

Title:  Cytosolic trapping of a mitochondrial heat shock protein is an early pathological event in synucleinopathies

Authors:  *T. F. OUTEIRO, E. M. SZEGO;
Exptl. Neurodegeneration, Univ. Med. Ctr. Goettingen, Goettingen, Germany

Abstract:  Alpha-synuclein (aSyn) accumulates in intracellular inclusions that are associated with synaptic and mitochondrial dysfunctions in Parkinson’s disease (PD) and other synucleinopathies, but the precise underlying mechanisms are still elusive. Here, we identified the 10 kDa heat shock protein (HSP10) as a key mediator of aSyn-induced mitochondrial impairment in striatal synaptosomes. We found an age-associated increase in the cytosolic levels of HSP10 in transgenic mice expressing human aSyn, alongside with a concomitant decrease in the mitochondrial levels of the protein. This resulted in a reduction in the levels of superoxide dismutase 2, a client protein of the HSP10/HSP60 mitochondrial folding complex that handles mitochondrial reactive oxidative species, and in synaptosomal spare respiratory capacity, confirming functional impairments. Importantly, overexpression of HSP10 ameliorated aSyn-associated mitochondrial dysfunctions and delayed aSyn pathology both in vitro and in vivo. Altogether, our data indicate that increased levels of aSyn induce mitochondrial deficits, including decreased ATP production on demand, reduce mitochondrial membrane potential and
decrease efficient handling of reactive oxygen species, at least partially by sequestering HSP10 in the cytosol and preventing it from acting in mitochondria. Importantly, these mitochondrial alterations manifested first at presynaptic terminals and, at later stages, induced energy deficits and increased ROS production, which in turn slowed down the synaptic machinery, further contributing to aSyn pathology. Our study provides novel mechanistic insight into synucleinopathies and opens new avenues for addressing underlying cellular pathologies.

Disclosures: T.F. Outeiro: None. E.M. Szego: None.

Poster

295. Alpha-Synuclein Models and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 295.17/F43

Topic: C.03. Parkinson’s Disease

Support: NIH/NIEHS 1R01ES024745
NIH/NINDS F31NS105245

Title: Synonymous single nucleotide polymorphism alters SNCA mRNA and protein homeostasis in vitro

Authors: *K. M. VON HERRMANN¹, K. W. HORN², L. A. SALAS¹, F. L. ANDERSON¹, B. C. CHRISTENSEN¹, M. C. HAVRDA¹;
¹Geisel Sch. of Med. at Dartmouth, Hanover, NH; ²Dartmouth Col., Hanover, NH

Abstract: Synonymous single nucleotide polymorphisms (sSNPs) scattered throughout the genome are capable of dramatically altering mRNA processing and/or protein translation. sSNPs have been reported to eliminate mRNA splice sites, disrupt mRNA secondary structure, alter mRNA stability, or impact codon optimality, resulting in changes at the mRNA and protein level. Considering the importance of mRNA and protein homeostasis in the context of neurodegenerative disease, we investigated the impact of synonymous SNPs on alpha-synuclein protein in vitro. SNCA codes for the 140 amino acid, intrinsically disordered alpha-synuclein protein comprised of three main regions, the amphipathic helical N terminus, the hydrophobic central region, and the acidic disordered C terminus. Under normal conditions alpha-synuclein is involved in synaptic vesicle transport, while under pathological conditions including Parkinson’s disease, the protein aggregates into various oligomers, protofibrils, and fibrils and can be eventually sequestered into Lewy body inclusions and Lewy neurites. Alpha synuclein oligomers and protofibrils are cytotoxic, and emerging data indicate that pathogenic fibrils can be transmitted from cell-to-cell, propagating the pathology. The influence of synonymous SNPs on alpha-synuclein aggregation is not clearly understood. Many sSNPs identified by GWAS are deemed “likely benign” and remain uncharacterized. Experimental interrogation of synonymous
genetic variants in genes involved in proteinopathy like SNCA are important to determine if this class of genetic variation impacts the development of disease. We selected five of the many SNCA sSNPs and utilized site-directed mutagenesis and transfection to produce multiple independent polyclonal HEK293 cell lines carrying sSNP sequences. To characterize the impact of the sSNPs on mRNA and protein in vitro we then conducted real-time PCR and immunoblotting, as well as immunoprecipitation and solubility assays. Expression of SNCA harboring the sSNP rs76642636 resulted in significantly lower SNCA mRNA and monomeric alpha-synuclein protein levels, contrasted with an increase in high molecular weight alpha-synuclein protein species. These findings were replicated in differentiated SH-SY5Y cells and primary cortical neurons. Ongoing studies suggest that despite encoding the same amino acid, synonymous SNPs have the capacity to significantly alter the protein lifecycle with the potential to influence protein homeostasis and function in the context of proteinopathies like Parkinson’s disease.


Poster

295. Alpha-Synuclein Models and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 295.18/F44

Topic: C.03. Parkinson’s Disease

Title: Synucleinopathy in action: From motion to vision

Authors: *E. DE LEONIBUS¹², E. MARROCCO³, F. ESPOSITO³, A. CARBONCINO⁴, V. TARALLO⁴, F. G. ALVINO³, A. INDRIERI³, S. DE FALCO⁴, B. FRANCO³, M. DE RISI³, E. M. SURACE⁶;¹TIGEM, Pozzuoli, Italy; ²Inst. of Cell. Biol. and Neurobio. (IBCN), Monterotondo (Rome), Natl. Res. Council, Italy, Rome, Italy; ³Telethon Inst. of Genet. and Med. (TIGEM), Pozzuoli, Italy; ⁴Inst. of Genet. and Biophysics, CNR, Naples, Italy; ⁵Telethon Inst. of Genet. and Med. (TIGEM), Camposano, Italy; ⁶Dept. of Translational Medicine, "Federico II" Univ., Naples, Italy

Abstract: The neuropathological hallmarks of Parkinson’s disease (PD) are loss of dopaminergic (DA) neurons in the substantia nigra (SN) and formation of intraneuronal protein inclusions termed Lewy bodies, composed mainly of α-synuclein (α-syn) protein. Abnormally accumulating α-syn leads to progressive DA neuronal loss, motionless and dementia. This late stage is preceded by a variety of early signs including emotional, autonomic and memory symptoms. More recently, much attention is also directed toward the eye, as it has been shown that α-syn accumulation in the retina and visual acuity defects are early markers of synucleinopathy (Ortuño-Lizarán et al., 2018).
We have recently reported that adeno-associated viral vector-mediated overexpression of α-syn (AAV-α-syn) in the midbrain of adult mice induces highly selective striatal-dependent motor learning-induced metaplasticity deficit (Giordano et al., 2018). This early stage is followed by progressive loss of nigral DA neurons and spreading of α-syn in other brain regions, associated to bradykinesia, increased anxiety and endophenotypes of psychosis recapitulating early stages of dementia with Lewy Bodies. In this study, using the same experimental approach, we explored the synaptic and behavioral effects of α-syn overexpression in different brain regions, including the retina. We show that α-syn leads to a time-dependent degeneration of dopamine amacrine cells in the retina, impaired visual acuity and altered contrast-light sensitivity evaluated with an electroretinogram (ERG). The visual and ERG impairments are rescued by systemic administration of L-DOPA.

Our study provides the first experimental evidence showing that α-syn exerts harmful effects on DA neurons in the retina leading to impaired vision sensitive to L-DOPA treatment. These findings have high translational relevance as the eye is gaining momentum for studying neurodegenerative disorders due to its easy accessibility (London et al., 2013). Indeed, it represents an ideal model organ for both an early identification of protein aggregates and for testing novel therapeutic approaches.


Poster

295. Alpha-Synuclein Models and Mechanisms I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 295.19/F45

Topic: C.03. Parkinson’s Disease

Support: Sigrid Juselius Foundation

Title: Deep convolutional neural network-based analysis of Lewy body pathology in mice

Authors: *M. T. A. AIRAVAARA1, I. PARKKINEN2, K. ALBERT3, K. PITKÄNEN5, S. BLOM5, K. C. LUK6, M. H. VOUTILAINEN4, J. KORPIKOSKI7; 1Neurosci. Center, Hifife, Univ. of Helsinki, Helsinki, Finland; 2Inst. of Biotechnology, HiLIFE, Univ. of Helsinki, Helsinki, Finland; 3Inst. of Biotechnology, HiLIFE, 4Dept of Biotechnology, Univ. of Helsinki, Helsinki, Finland; 5Aiforia Technologies Oy, Helsinki, Finland; 6Dept of Pathology and Lab. Med., Univ. Pennsylvania, Philadelphia, PA; 7Inst. of Biotechnology, Univ. of Helsinki, Helsinki, Finland
Abstract: Robust and reliable counting and analysis of p129S-alpha-synuclein-positive (alpha-synuclein phosphorylated at S129) inclusions of Lewy bodies (LBs) and Lewy neurites (LNs) is a necessity in Parkinson’s disease research as these inclusions are characteristic features in the brains of both sporadic and familial forms of Parkinson’s disease patients. Current analysis of LBs and LNs is performed using either stereology, which is time-consuming and prone to interobserver variation or using automated image analysis methods which are limited by their ability to recognize LBs and LNs in the brain sections reliably. In contrast, deep convolutional neural networks (CNN) algorithms provide high performance pattern recognition making them an ideal solution for automated analysis of LBs and LNs. Intra-striatal injection of preformed alpha-synuclein fibrils was used to induce alpha-synuclein pathology in the mouse brain. Paraffin sections from brains were stained with a p129S-alpha-synuclein antibody and counterstained with hematoxylin. Digital whole-slide images of brain sections were acquired with a digital slide scanner at 0.22 µm/pixel resolution and uploaded to Aiforia® cloud platform for supervised CNN training. CNN algorithms were trained in Aiforia® to detect intracellular LBs and LNs automatically in digitalized images. The algorithm measured the length, width, area, and location for individual LNs and LBs. The combination of cloud-computing and CNN-based detection of p129S-alpha-synuclein immunoreactivity enables quantitative and reproducible high-throughput analysis of LNs and LBs in the mouse brain. The algorithm can be trained to detect LBs and LNs in any sample regardless of tissue or species, provided they can be stained with p129S and hematoxylin. This enables a variety of Lewy pathologies to be examined yielding length, area, and width distribution curves from large brain regions.

Disclosures: M.T.A. Airavaara: None. I. Parkkinen: None. K. Albert: None. K. Pitkänen: A. Employment/Salary (full or part-time); Full time salary. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ownership interest. S. Blom: A. Employment/Salary (full or part-time); Full time salary. K.C. Luk: None. M.H. Voutilainen: None. J. Korpikoski: None.

Poster

296. Mouse Models of Tauopathies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 296.01/F46

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Support: Merck & Co

Title: A novel tau transgenic mouse model overexpressing human tau and an aggregation-prone tau sequence shows tau hyperphosphorylation and behavioral deficits in the Morris water maze

Authors: *T. W. ROSAHL1, G. B. VARTY3, S. M. SMITH2, R. C. GENTZEL4, N. CARROLL5, L. SILENIEKS5, G. A. HIGGINS6, J. SCHACHTER2;
Abstract: Tauopathy is a group of neurodegenerative diseases characterized by hyperphosphorylation, misfolding and aggregation of Tau protein, as well as behavioral deficits and neuronal loss. Here, we developed a novel tau Tg mouse model which overexpresses 5-fold all six isoforms of the WT human tau (GEM1) and a tetracycline responsive, Camk2a-activated transgene carrying a human Tandem Repeat Tau (TRT) sequence at lower expression levels (GEM2). The TRT sequence serves as a tau aggregation primer accelerating the tau pathology without having to use known tau mutations like eg P301L, etc. Indeed, expression of TRT in HEK293 cells results in formation of soluble, hyperphosphorylated, high molecular weight tau oligomers. Brains were collected from WT controls, GEM1, and GEM2 mice, as well as from the GEM1 and GEM2 composite mouse (GEM3), at various ages, for biochemical and histological evaluation. Despite the high overexpression of WT human tau, GEM1 showed only a modest increase in tau hyperphosphorylation in sagittal sections stained for the tau phospho (Ser202, Thr205) specific antibody AT8, a marker for pathologically phosphorylated tau. In contrast, the GEM2 and GEM3 transgenic mice showed a dramatic increase in tau hyperphosphorylation. Similar results were obtained with staining of hippocampus and cortex for AT180 antibody, which recognizes hyperphosphorylated Thr231, and for PHF1 antibody, which recognizes tau phosphorylated at Ser396/404. Behaviorally, 20-month old GEM3 mice showed moderately increased locomotor activity, but normal performance in the rotarod assay, compared to WT controls. In the Morris Water Maze, GEM3 mice showed equivalent performance to age-matched WT mice in a 5-day cued learning task, but impaired learning a hidden platform task. GEM3 mice also exhibited spatial learning deficits in three probe tests (e.g., Day 10 probe test: Percent of time spent in island quadrant; WT: 51.5 ± 6.4; GEM3: 31.7 ± 3.8; P<0.01) and analysis of swim patterns indicated search strategies reflective of impaired learning. In summary, this novel tau transgenic mouse model recapitulates key aspects of Alzheimer’s Disease-like symptoms including tau hyperphosphorylation and learning and memory impairments.

Disclosures: T.W. Rosahl: A. Employment/Salary (full or part-time); Merck & Co. G.B. Varty: A. Employment/Salary (full or part-time); Merck & Co. S.M. Smith: A. Employment/Salary (full or part-time); Merck & Co. R.C. Gentzel: A. Employment/Salary (full or part-time); Merck & Co. N. Carroll: A. Employment/Salary (full or part-time); InterVivo Solutions. L. Silenieks: A. Employment/Salary (full or part-time); InterVivo Solutions. G.A. Higgins: A. Employment/Salary (full or part-time); InterVivo Solutions. J. Schachter: A. Employment/Salary (full or part-time); Merck & Co.
Detection of alpha-synuclein aggregates in the brain of two different mouse strains of tauopathy model, rTg4510 and PS19

Authors: *Y. Takaichi¹, T. Washinuma¹, H. Inoue¹, Y. Ano², J. K. Chambers¹, K. Uchida¹, A. Takashima³, H. Nakayama¹;
¹Lab. of Vet. Pathology, The Univ. of Tokyo, Tokyo, Japan; ²Res. Labs. for Hlth. Sci. & Food Technologies, Kirin Company, Limited, Kanagawa, Japan; ³Gakushuin Univ., Tokyo, Japan

Abstract: Introduction: The accumulation of specific phosphorylated protein aggregates in the brain is a hallmark of neurodegenerative disorders. Specifically, hyperphosphorylated tau (hp-tau) accumulates in tauopathies and phosphorylated α-synuclein (p-αSyn) accumulates in α-synucleinopathies. Co-deposition of different pathological proteins is common in the brains of patients with neurodegenerative diseases. In this study, we examined the hp-tau-dependent p-αSyn aggregation in the brain of two strains of the tauopathy model mouse.

Methods: Two strains of the tauopathy model mouse, rTg4510 mice that overexpress human P301L mutant tau, and PS19 mice that overexpress human P301S mutant tau were used. As controls, the background strains, FVB/N-C57BL/6J and C57BL/6J mice were examined, respectively. The rTg4510 and PS19 mice were euthanized at 10 and 13 months of age, respectively, and the brains were collected. The deposition of hp-tau and p-αSyn in the brain of these mice were examined by immunohistochemistry.

Results: Human hp-tau and mouse p-αSyn aggregates were detected within the same neuronal cells mainly in the cerebral cortex of rTg4510 mice and the cerebral cortex and brain stem of PS19 mice. In rTg4510 mice, numerous p-αSyn-positive grains, occasionally p-αSyn-positive spherical Lewy body (LB)-like inclusions, and minute and uniform neuronal granules were observed. On the contrary, in PS19 mice, in addition to p-αSyn-positive spherical LB-like inclusions, and minute and uniform neuronal granules in the cerebral cortex, many p-αSyn-positive dense, uniform aggregates and p-αSyn-positive spherical LB-like inclusions were detected in the brain stem. Semi-quantitative analysis revealed a significant regional correlation between hp-tau and p-αSyn accumulations. After proteinase K-treatment, αSyn-positive aggregates were detected in the cerebral cortex of rTg4510 mice, but not in PS19 mice.

Conclusion: The present results indicate that endogenous mouse αSyn is phosphorylated and accumulates in neurons with hp-tau aggregation, and suggest that the overexpression of human P301L and P301S mutant tau may enhance endogenous αSyn phosphorylation and
aggregation. Therefore, kinases and phosphatases which control the phosphorylation process of both tau and αSyn may be involved in the mechanism of the co-deposition. The morphological variations of p-αSyn aggregates and different proteinase K-resistance of αSyn aggregates between the two tauopathy mice may imply the direct interaction of tau and αSyn aggregates. This synergic effect of tau and αSyn accumulations may exacerbate the brain pathology.


Poster

296. Mouse Models of Tauopathies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 296.03/DP04/G2

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Support: Swedish Research Council
Knut and Alice Wallenberg Foundation
Bertil Hållsten Foundation
Torsten and Ragnar Söderberg Foundation
Swedish Brain Fund
Stratneuro Initiative
Västerbotten County Council

Title: Transmissibility of SOD1 prion strains between mice expressing different mutant human SOD1s

Authors: *E. EKHTIARI BIDHENDI1, P. ANDERSEN2, S. MARKLUND1, T. BRÄNNSTRÖM1;

Abstract: Background. Two structurally different human (h) SOD1 aggregate strains, A and B, can arise in CNS of transgenic (Tg) mice expressing hSOD1 variants (Bergh et al. 2015). When inoculated into spinal cords of Tg mice both strains transmit exponentially growing templated hSOD1 aggregation and premature paralysis (Bidhendi et al. 2016, 2018).

It is known from the prion protein field that there are species barriers for prion transmission and that mutations/polymorphisms in a given species can determine susceptibility to prion transmission. Here we investigate if such transmission barriers exist for hSOD1 aggregate/prion strains when prepared from, and inoculated into Tg mouse expressing different hSOD1 variants.

Methods. hSOD1 A and B seeds were prepared from mouse spinal cords by ultracentrifugation
through a density gradient (Bidhendi et al. 2016). Seeds were microinoculated into lumbar spinal cord of adult recipient Tg mice.

**Results.** We have shown that A-prions prepared from hSOD1\(^{G85R}\), hSOD1\(^{G127X}\) Tg mice and B-prions from hSOD1\(^{D90A}\) mice efficiently seed aggregation and motor neuron disease in hSOD1\(^{G85R}\) mice (Bidhendi et al. 2016, 2018). We have also found that strain A seeds from hSOD1\(^{G85R}\) mice efficiently seed aggregation and disease in both hemi and homozygous hSOD1\(^{D90A}\) mice. Likewise strain B seeds from hSOD1\(^{D90A}\) mice transmit disease to hemi and homozygous hSOD1\(^{D90A}\) mice. A second passage strain B seed from hSOD1\(^{G85R}\) mice transmitted disease to hSOD1\(^{G85R}\) mice, but without any enhanced efficiency compared with such seeds from hSOD1\(^{D90A}\) mice. Finally, strain A-like seeds from hSOD1\(^{G127X}\) mice efficiently transmitted disease to both hemi and homozygous hSOD1\(^{G127X}\) mice (unpublished data).

However, strain A seeds from hSOD1\(^{G93A}\) Tg mice did transmit strain A aggregation and disease to hSOD1\(^{G85R}\) mice, but apparently with low efficiency. The lifespans of recipient mice were longer than expected from the dose of hSOD1\(^{G93A}\) aggregates that was inoculated. Finally, strain A seeds prepared from paralytic hSOD1\(^{WT}\) mice (Graffmo et al. 2013) have so far failed to transmit disease to hSOD1\(^{G85R}\) mice.

**Conclusions.** Our experience suggests that hSOD1 A and B prions on different hSOD1 mutation backgrounds in most cases freely transmit disease to Tg mice expressing other mutant hSOD1s. However, strain A seeds on hSOD1\(^{G93A}\) background apparently showed reduced efficiency in hSOD1\(^{G85R}\) mice, and we have so far failed to transmit ALS with strain A seeds from hSOD1\(^{WT}\) Tg mice.

**Disclosures:** E. Ekhtiari Bidhendi: None. P. Andersen: None. S. Marklund: None. T. Brännström: None.
GUPTA¹, P. BICKFORD², D. LEE¹;
¹Dept. of Pharmaceut. Sci., Univ. of South Florida, Col. of Pharm., Tampa, FL; ²Dept. of Mol. Pharmacol. and Physiol., Univ. of South Florida, Morsani Col. of Med., Tampa, FL

Abstract: Tauopathies consist of a group of neurodegenerative diseases characterized by intracellular protein aggregates formed by abnormal accumulation of microtubule-associated protein tau. Tau aggregates are often diagnosed as neurofibrillary tangles in postmortem tauopathy brain tissues and correlates closely to brain atrophy and memory deficits. Clinical phenotypes of tauopathies manifest as cognitive impairment and behavioral disturbance. Tau remains a central target for drug discovery, but currently there are no effective treatments that treat disease. Our previous work revealed a unique interaction between tau biology and arginine metabolism. G protein coupled receptor (GPCR) family C, group 6 member A (GPRC6A) was a putative L-arginine extracellular sensor and recently linked to mechanistic target of rapamycin complex 1 (mTORC1) pathway \textit{in vitro}. Our preliminary work suggests hyper-mTORC1 signaling in both human Alzheimer’s disease patient brains and animal models of tauopathy. We aim to decrease GPRC6A signaling, which is predicted to inhibit mTORC1 signaling, promote tau degradation, and mitigate cognitive impairment in a mouse model of tauopathy. We used recombinant adeno-associated virus (rAAV) encoding shRNA specific to mouse GPRC6A or scrambled shRNA control. The rAAV constructs were bilaterally delivered into anterior/ entorhinal cortex and hippocampus of tau P301S (PS19) mice and non-transgenic littermates at the age of 6 months. After four months’ viral incubation, we measured a series of mouse behavioral tests associated with tau neuropathology. The shRNA rAAV successfully downregulated mouse GPRC6A expression both \textit{in vitro} and \textit{in vivo}. Long-term \textit{in vivo} downregulation of GPRC6A significantly changed tau P301S mouse behaviors, including improved spatial working memory measured in radial arm water maze (RAWM) and enhanced fear associated recall in fear conditioning (FC) measurement. In summary, this is the first evidence to show that central nervous system GPRC6A repression \textit{in vivo} can partially rescue components of the tau phenotype. Further biochemistry and immunohistochemistry analysis of key proteins involved in mTORC1 signaling and different aspects of the tau phenotypes are warranted to validate the effects of GPRC6A repression. Therapeutics that modulate GPRC6A activity may potentially provide new treatments to improve cognition and mitigate tauopathies in human patients.


Poster

296. Mouse Models of Tauopathies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 296.05/G4
**Topic:** C.05. Tauopathies/Tau-dementias/ and Prion Diseases

**Support:** CurePSP Grant  
CurePSP URSO Summer Fellowship  
ASPET SURF Grant

**Title:** Progress towards the first preclinical model of progressive supranuclear palsy

**Authors:** *G. S. KING, K. M. VEROS, S. D. CLARK;*  
Pharmacol. and Toxicology, Univ. At Buffalo- SUNY, Buffalo, NY

**Abstract:** The brainstem structure pedunculopontine tegmentum (PPTg) has become a focus in the study of Parkinsonisms, such as Progressive Supranuclear Palsy (PSP). Pathologically, PSP shows an extensive loss of PPTg cholinergic neurons, as well as aggregates of the microtubule stabilizing protein tau, which are greatest in areas innervated by the PPTg. PSP symptomology includes deficits in acoustic startle reflex (ASR), motor function and coordination, and working memory. Currently, there are no pharmacotherapies available for PSP patients and drug discovery is hindered by the lack of an adequate preclinical model. Previously, selective cholinergic PPTg lesions in rats induced PSP-like symptomology and pathology including motor deficits, a significantly reduced ASR, substantia nigra (SN) dopaminergic loss, and enlarged lateral ventricles. However, lesioned rats showed no evidence of pathological tau. Building upon previous efforts, we hypothesize that the loss of PPTg cholinergic neurons, coupled with deposition of tau in areas seen in PSP, will mimic PSP symptomology and pathology. Male and female ChAT-Cre Long Evans rats were injected in the PPTg with a virally mediated (adeno associated virus) tau construct or GFP construct as a control. Following surgery, rats were tested in a battery of behavioral paradigms: ASR, marble burying, locomotor activity, horizontal ladder, hindlimb clasping, and an operant task. As early as four weeks post-surgery rats exhibited significant PSP-like motor deficits, which did not diminish over the duration of 20 weeks. Cognitively, operant testing revealed an intact working memory, but gave insight to other potential deficits also analogous to PSP.

While histology is still in progress, preliminary results confirm a significant loss of PPTg cholinergic neurons as well as a trend towards loss of dopaminergic SN neurons. Presence of phosphorylated tau is also evident, further indicating PSP-like pathology. To determine the progression of pathology, brains will be analyzed for presence of various forms of pathological tau as well as ventricle width. Thus far, both behavior and histology results suggest the induction of tau in the cholinergic PPTg may be an adequate animal model for PSP, and could be used in drug discovery efforts.

**Disclosures:** G.S. King: None. K.M. Veros: None. S.D. Clark: None.
Title: The anti-tau antibody HJ8.5 reduces tau seeding and aggregation in vitro and in vivo

Abstract: Emerging evidence suggests the progression of tau pathology in neurodegenerative diseases such as Alzheimer’s disease (AD) and progressive supranuclear palsy (PSP) is due, at least in part, to the trans-neuronal propagation of misfolded tau capable of templating tau aggregation in neurons that take up the pathological “seed”. Anti-tau antibodies have been proposed as a potential therapeutic intervention based on the theory that they will bind to seeding competent tau in extracellular space and reduce subsequent downstream neuronal seeding and intracellular tau aggregation. HJ8.5 is an anti-tau antibody that binds the N terminal of tau. Using ELISAs, it was demonstrated that HJ8.5 bound to tau in soluble and insoluble fractions isolated from AD and mouse tau transgenic brains as well as to monomeric and paired helical filament (PHF) recombinant tau. In functional in vitro assays, HJ8.5 decreased tau aggregation induced by the application of seeding competent tau to biosensor cells expressing a tau repeat domain containing the P301S mutation. The antibody reduced tau aggregation induced by total AD brain lysate, by sarkosyl insoluble tau from AD brain lysate and by sonicated recombinant PHF tau. HJ8.5 was also tested in an in vivo seeding model. In the model, AD brain lysate was injected into the hippocampus of young, pre-pathology rTg4510 transgenic mice expressing human tau with the P301L mutation. Three weeks after injection, brains were analyzed for the presence of aggregated tau in the ipsilateral hippocampus using AT100 immunohistochemistry. Dosing mice with HJ8.5 prior to the injection of AD brain lysate led to a dose dependent reduction in AT100 immunoreactivity in the hippocampus. The level of target engagement required to reduce AD brain lysate-induced tau aggregation in the rTg4510 in vivo seeding model was calculated by determining the degree of reduction of unbound tau in the CSF seen at efficacious brain concentrations. Reductions in in vivo tau aggregation induced by HJ8.5 were correlated with the level of target engagement in the CSF. These data support the seeding and propagation theory of...
tau pathology progression and suggest antibodies such as HJ8.5 are capable of reducing pathological tau-induced seeding.

**Disclosures:**  
**L.E. Rueter:** A. Employment/Salary (full or part-time); Employee of AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Own AbbVie stock.  
**A. Striebinger:** A. Employment/Salary (full or part-time); Employee of AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); May own stock.  
**S. Barghorn:** A. Employment/Salary (full or part-time); Employee of AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); May own stock.  
**H. Wu:** A. Employment/Salary (full or part-time); Employee of AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); May own stock.  
**J. Brewer:** A. Employment/Salary (full or part-time); Employee of AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); May own stock.  
**C. Hamilton:** A. Employment/Salary (full or part-time); Employee of AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); May own stock.  
**R. Chang:** A. Employment/Salary (full or part-time); Employee of AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); May own stock.  
**T. Dellovade:** A. Employment/Salary (full or part-time); Employee of AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); May own stock.  
**K. Schlegel:** A. Employment/Salary (full or part-time); Employee of AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); May own stock.  
**M. Mezler:** A. Employment/Salary (full or part-time); Employee of AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); May own stock.  
**F. Le Prieult:** A. Employment/Salary (full or part-time); Employee of AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); May own stock.  
**K. Kaygisiz:** A. Employment/Salary (full or part-time); Employee of AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); May own stock.  
**K. Diry:** A. Employment/Salary (full or part-time); Employee of AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); May own stock.  
**D. Clausznitzer:** A. Employment/Salary (full or part-time); Employee of AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); May own stock.
**Title:** Microbiome manipulation modifies tau-mediated neurodegeneration in *Drosophila melanogaster*

**Authors:** H. KOHL, V. HYDE, *K. M. LOHR; Biol., Washington and Jefferson Col., Washington, PA

**Abstract:** The deposition of the microtubule-associated protein tau is a hallmark pathology of the family of neurodegenerative diseases known as tauopathies, including Alzheimer’s disease, frontotemporal dementia, and chronic traumatic encephalopathy. Despite progress in the study of tau-mediated neurodegeneration, the field has shown that most tauopathy cases do not have a single genetic cause. Instead, genetic contributions may interact with peripheral or environmental factors to contribute to disease. Recently, the gut microbiome has emerged as a potential modifier of brain function in human, rodent, and invertebrate models via changes to neurotransmitter levels and systemic inflammation. We show that Drosophila expressing human tau in neurons have a progressive deficit in fecal deposition compared to control flies. Further, tau-expressing flies grown with a limited microbiome using an axenic preparation show enhanced neurodegenerative outcomes, as shown by locomotor activity deficits and increased brain vacuole formation. 16S rDNA analysis was also used to define the typical microbiome composition in tau and control flies at multiple time points. Taken together, these data suggest that tau-expressing flies have an innate deficit in gut function and that manipulation of the gut microbiome is capable of altering neuronal health in this Drosophila model of human tau expression.

**Disclosures:** H. Kohl: None. V. Hyde: None. K.M. Lohr: None.
Abstract: Objective: Validated animal models of neurodegenerative disease are required to understand disease mechanisms. In order to validate animal models, it is necessary to understand the impact on individual neurons in affected brain regions. Here we assessed the electrophysiological properties of striatal neurons, and behavioral measures in a transgenic model of Pick's disease (the K3 mouse) compared with wildtype mice.

Methods: All experiments were approved by the Animal Ethics Committee of the University of Sydney. The electrophysiological discharge properties (n = 66) and synaptic input properties (n = 5) of mouse striatal neurons were characterized in two experimental groups; wildtype mice (n=38), and K3 transgenic mice (n=28), all on the C57BL/6 background. For behavioral studies, twenty-one mice were used, comprised of both wildtypes (n=10) and K3 mice (n=11).

Electrophysiological recordings were made from coronal slices (200 μm) distributed evenly across the entire striatum in whole-cell current-clamp and voltage-clamp mode at room temperature. Voltage-clamp recordings to interrogate synaptic input profile were carried out under pharmacological blockade using known excitatory and inhibitory receptor blockers. For behavioral procedures an outcome devaluation test was used to quantify the formation of habitual behavior.

Results: The proportion of striatal neuron discharge profiles in wildtype mice was significantly altered when compared with the K3 transgenic group (p= 0.006). In general, this alteration was characterized by a shift towards burst firing discharge profiles in the K3 transgenic mice. Furthermore, the K3 transgenic mice showed significantly higher subthreshold excitatory postsynaptic potentials activity at rest (p= 0.03). The passive membrane properties including input impedance and capacitance of striatal neurons were not significantly different between both mouse groups. The synaptic input profile of striatal neurons is predominantly excitatory in nature. Results of the outcome devaluation test indicated a reduced ability for K3 mice to form habits relative to wildtype mice, and thus behaved in a goal-directed manner, suggestive of impairments in the dorsal striatum.

Conclusion: Neurons in the striatum of K3 transgenic mice display a hyper-excitabale state, which may contribute to reduced habit formation when compared with wildtype mice.

**Poster**

**296. Mouse Models of Tauopathies**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 296.09/G8

**Topic:** C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

**Support:** TauRx Therapeutics, Singapore

**Title:** Neuropsychiatric symptoms in mouse models of AD and bvFTD

**Authors:** *E. S. E. DREESEN, L. ROBINSON, V. MELIS, P.-H. MOREAU, C. HARRINGTON, C. M. WISCHIK, G. RIEDEL;* Univ. of Aberdeen, Aberdeen, United Kingdom

**Abstract:** For Alzheimer’s disease (AD) and other forms of dementia a lot is known about cognitive symptoms and their neurobiological basis. However, more recently studies have focused on the importance of different neuropsychiatric symptoms (NPS) discriminating between the different types of dementia. Possible recurring NPS in AD are psychosis, depression, anxiety and apathy, while patients with the behavioural variant of frontotemporal dementia (bvFTD) exhibit e.g. increased impulsivity, disinhibition, hyperphagia and apathy. These symptoms have a critical impact on the quality of life of both patients and caregivers and a considerable economic impact. To study NPS, their neurobiological basis as well as their presence and influence on disease progression, preclinical trials should be done in mouse models. The research objective of this exploratory study was to assess NPS in two tau transgenic mouse models described in Melis et al. (2015). Briefly, L1 overexpresses a truncated repeat domain of the tau fragment to model AD while L66 overexpresses full-length tau with two pathogenic point mutations (P301S and G335D) to represent bvFTD. Focus lay on behavioural tests for anxiety, depression, inhibition and motor impairment as well as cognition.

**Methods:** Experiments were performed with female mice, aged 3 and 6 months and compared to NMRI controls. Experiments were carried out in accordance with the European Communities Council Directive (63/2010/EU) and a project license with local ethical approval under the UK Animals (Scientific Procedures) Act (1986). Sample sizes were based on power calculations and all experiments were performed blind and counterbalanced. Behavioural tests done: elevated plus maze, open field, light-dark box, forced swim test, sucrose preference test, pre-pulse inhibition (PPI), delay discounting, Y-maze and Barnes maze.

**Results:** A hyperactive, anxiolytic phenotype with impaired PPI, spatial reference and working memory was found in L1 animals. L66 mice presented with neophobia as well as impaired PPI and working memory. No evidence of a depressive phenotype was found in either model.

**Conclusions:** It appears that our tau-based models display NPS together with cognitive impairments. The type of NPS found in L1 and L66 mice is in accordance with predictions for
human patients with AD and bvFTD, respectively, and provides an excellent non-clinical test bed for the development of new drug therapies. It is paramount to include tests for NPS as they can have a major influence on behaviour seen in cognitive tests. Furthermore, these tests are well suited to discriminate the different types of dementia when establishing new models.

**Disclosures:** E.S.E. Dreesen: None. L. Robinson: None. V. Melis: None. P. Moreau: None. C. Harrington: A. Employment/Salary (full or part-time); TauRx Therapeutics Ltd. C.M. Wischik: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); TauRx Therapeutics Ltd.. G. Riedel: None.

**Poster**

296. Mouse Models of Tauopathies

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 296.10/G9

**Topic:** C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

**Support:** Institutional start-up funds to S.M.F.
Institutional start-up funds to C.M.D.C.

**Title:** Behavioral characterization of sensorimotor, neuropsychiatric, and cognitive function in transgenic mice overexpressing human tau


**Abstract:** Tauopathies are a group of diseases characterized by abnormal tau phosphorylation and accumulation in neurons in the brain. Tau pathology is associated with neurodegeneration and a wide spectrum of behavioral symptoms. Alzheimer’s disease, progressive supranuclear palsy (PSP), and chronic traumatic encephalopathy are all tauopathies. Common behavioral symptoms include neuropsychiatric conditions such as depression, anxiety and cognitive dysfunction. Sensorimotor function can also be affected as is evident in PSP. Currently, there are few symptomatic treatments and no disease-modifying therapies for tauopathies. This is due, in part, to a lack of understanding of the timecourse and progression of behavioral alterations in these diseases. Thus, a tauopathy model that develops a broad spectrum of behavioral anomalies would be optimal in preclinical trials testing potential therapeutics. In this study, we sought to characterize sensorimotor, neuropsychiatric, and cognitive function in mice that selectively overexpress all human tau isoforms (hTau). Male and female C57Bl/6 (n=9), Tau knockout (Tau +/-; n=8), and hTau (n=9) mice were tested on a battery of behavioral assays to determine the effect of hTau on sensorimotor, neuropsychiatric, and cognitive behaviors. At 13-15 months of
Male and female hTau mice showed sensorimotor deficits compared to control that differed depending on the test. Males displayed decreases in hindlimb stepping, stride length, and locomotor activity, while female hTau mice reared significantly less than control in the activity tests. Female hTau mice were more affected in the neuropsychiatric tests compared to control and males. Latency to approach in novelty suppressed feeding was increased in hTau females compared to control and they drank less sucrose solution in the sucrose preference test of anhedonia. Cognitive function was also more affected in female hTau than males. Female hTau mice showed reduced investigation and impaired discrimination in an object recognition test. These results show overexpression of human tau leads to impairments in multiple aspects of behavior particularly in female mice. These data will help inform preclinical testing of novel therapeutics and facilitate translation to human tauopathies.


Poster

296. Mouse Models of Tauopathies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 296.11/G10

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Title: Age-dependent and region-specific miRNAs deregulation in the hTau mouse model of tauopathy

Authors: *E. LAURETTI, D. PRATICO;
Lewis Katz Sch. of Med. at Temple Univ., Philadelphia, PA

Abstract: Tauopathies are a class of neurodegenerative disorders characterized by accumulation of abnormally phosphorylated tau in association with progressive loss of memory and cognitive functions. Despite intensive research the mechanisms responsible for tau pathology is still unknown. Small non-coding RNAs, microRNAs (miRNAs), are post-transcriptional regulators involved in the modulation of cellular processes including tau metabolism. Analysis of the expression profile of miRNAs in the brain of tauopathy patients has identified several miRNAs that are dysregulated, however, it is unclear whether those alterations are secondary contributing factors to the pathogenesis of these disorders. To tackle this question, we characterize the miRNAs expression profile in distinct brain regions and in different stages of the pathological phenotype in the hTau mouse model of human tauopathy with the goal of correlating them to the onset and progression of the pathological changes. Thus, we analyzed miRNAs expression levels in the cortex and hippocampus of hTau and wild type mice utilizing the Mouse Neurological Development & Disease miScript miRNA PCR Array and results were further confirmed by qPCR (n=6/8 per group) looking at three different age-time points (3, 6 and 12 months). Next, to
explore whether these changes correlate with the age- and region-specific development of tau neuropathology, we performed behavioral tests to assess learning and memory and also looked at synaptic dysfunction, tau metabolism and neuroinflammation in wild-type and hTau mice at the time points and in the brain regions of interest. Notably, we found six miRNAs (miR132-3p, miR22-3p, miR298-5p, miR489-3p, miR455-5p and miR146a-5p) upregulated in the hippocampus of hTau mice at 12 months of age, and among them miR132-3p, miR22-3p and miR-146a-5p were already upregulated at 6 months before the development of memory and cognitive impairment and tau pathology. No changes instead, were observed in the cortex of the same animals. In conclusion, our data show differential expression of specific miRNAs at both presymptomatic and symptomatic stages of tauopathy in the hippocampus of hTau mice and suggest that these miRNAs might be part of the pathological process and/or contribute to the disease progression by targeting crucial key signaling pathways.

Disclosures: E. Lauretti: None. D. Pratico: None.

Poster

297. ALS and Motor Neuron Disease

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 297.01/G11

Topic: C.06. Neuromuscular Diseases

Support: Muscular Dystrophy Association
Wallin Neuroscience Discovery Fund
Engebretson Drug Design and Discovery Fund

Title: Two axes of CHCHD10S59L dominant toxicity: TDP-43 and PINK1

Authors: *N. KIM, Y.-J. CHOE, M. BAEK;
Pharm. Practice and Pharmaceut. Sci., Univ. of Minnesota, Duluth, MN

Abstract: Mutations in coiled-coil-helix-coiled-helix-domain containing 10 (CHCHD10) have been identified as a genetic cause of amyotrophic lateral sclerosis and frontotemporal dementia. However, the disease-causing mechanism and the nature of mutations have not been fully understood. In this study, we generated a Drosophila model to study the function of CHCHD10 and disease-causing mechanisms. Mutant dCHCHD10S59L expression caused dominant toxicity in Drosophila eyes, motor neurons and muscles as well as HeLa cells while other mutants (R15L, P34S, G58R, and G66V) showed various effects in Drosophila and Hela cells. With the dCHCHD10S59L Drosophila model, we found that CHCHD10S59L is a dominant gain-of-toxicity mutant forming protein aggregates in mitochondria. Expression of CHCHD10S59L affects TDP-43 solubility and mitochondrial translocation. In addition, the PINK1/Parkin pathway is activated by dCHCHD10S59L. Blocking TDP-43 mitochondrial
translocation peptide inhibitors mitigated CHCHD10<sup>S59L</sup>-induced mitochondrial fragmentation. Surprisingly, co-expression of CHCHD10<sup>WT</sup> reduced insoluble TDP-43 and mitochondrial translocation. On the other hand, RNAi-mediated reduction of PINK1 and Parkin expression significantly rescued abnormal phenotypes in <i>Drosophila</i> tissues and HeLa cells. Furthermore, treatment of newly developed peptide inhibitors of PINK1 reversed CHCHD10<sup>S59L</sup>-induced mitochondrial phenotypes in HeLa cells. With our <i>Drosophila</i> model, we found two downstream substrates of PINK1, mitofusin, and mitofillin, are involved in the toxicity-generating process. Recently developed mitofusin2 agonists also restored mitochondrial functions disrupted in HeLa cells and <i>Drosophila</i> models for dCHCHD10<sup>S59L</sup> and C9orf72. Our data suggest that mitochondrial translocation of TDP43 and chronic activation of the PINK1-mediated pathways by CHCHD10<sup>S59L</sup> generates dominant toxicity in our model systems and suggest TDP43 mitochondrial translocation and the PINK1-mediated pathways as potential therapeutic targets for mutant CHCHD10-induced degenerative diseases and other related diseases.

**Disclosures:** N. Kim: None. Y. Choe: None. M. Baek: None.

**Poster**

297. ALS and Motor Neuron Disease

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 297.02/G12

**Topic:** C.06. Neuromuscular Diseases

**Support:** the Blazer Foundation

**Title:** Perturbed atlastin-1 results in axonal and synaptic defects in human cortical neurons

**Authors:** *Y. MOU<sup>1</sup>, Y. DONG<sup>3</sup>, S. MUKTE<sup>1</sup>, S.-C. ZHANG<sup>4</sup>, X.-J. LI<sup>2</sup>;<br>2Dept. of Biomed. Sci., <sup>3</sup>Univ. of Illinois, Rockford, IL; <sup>3</sup>Waisman Ctr., Univ. of Wisconsin - Madison, Madison, WI; <sup>4</sup>Waisman Ctr., Univ. Of Wisconsin - Madison, Madison, WI

**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). Why axonal defects occur specifically in cortical PNs and how synaptic connections between cortical PNs and their targets are affected in HSPs remain largely unknown. SPG3A, the most common early-onset form of HSP, is caused by mutations in the ATL-1 gene that encodes atlastin-1. We have previously modeled axonal degeneration of SPG3A using patient-specific iPSCs. Here, to further dissect the role of atlastin-1, we first knocked in an ATL-1 mutation (ATL161 line, p.A161P) using CRIPSR-cas9 mediated homologous recombination, which generated both heterozygous and homozygous SPG3A isogenic human pluripotent stem cell (hPSC) lines (c. G>C). The mutation of ATL-1 (p.A161P) induced axonal defects, including decreased axonal outgrowth, increased axonal swellings and impaired axonal
synaptophysin transport in cortical PNs. Interestingly, heterozygous mutation results in axonal defects at a comparable level to homozygous mutations. This is coinciding that SPG3A is autosomal-dominant and suggests that a critical levels of atlastin is needed to maintain normal axonal function. 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. The SPG3A and control hPSC lines were differentiated into cortical PNs (ChR2+), which were then co-cultured with their target cells, spinal MNs derived from their corresponding regular hPSC (without ChR2). After the immunostaining, we observed a dramatic decrease in the numbers of the Synapsin+/EYFP+/PSD95+ synaptic clusters in the SPG3A 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 perturbed atlastin-1 induced disease-specific axonal defects and the impaired synaptic connections between cortical PNs and spinal MNs in a SPG3A co-culture model, which will serve as a unique system to study the pathogenic mechanism and explore the treatment for HSPs.

Disclosures:  Y. Mou: None. Y. Dong: None. S. Mukte: None. S. Zhang: None. X. Li: None.

Poster

297. ALS and Motor Neuron Disease

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 297.03/G13

Topic: C.06. Neuromuscular Diseases

Title: Abnormal interaction between p97/VCP and a concealed UBX domain in adjacent missense alleles (A988V and T994I) of the Menkes disease copper transporter ATP7A cause an isolated distal motor neuropathy resembling Charcot-Marie-Tooth disease type 2 (CMT2)

Authors: *L. YI1, K. MATHEWS2, S. G. KALER3;
1NICHD/NIH, Bethesda, MD; 2Univ. of Iowa Stead Family Children’s Hosp., Iowa City, IA; 3NICHD, Bethesda, MD

Abstract: The copper transporter ATP7A is an eight-transmembrane protein critical for normal copper homeostasis. Mutations in ATP7A lead to severe infantile-onset Menkes disease (MNK), milder Occipital Horn Syndrome (OHS) or an isolated adult-onset distal motor neuropathy. The missense mutations A988V, T994I, and P1386S cause distal motor neuropathy without copper metabolism abnormalities, as documented in three unrelated families with clinical phenotypes similar to Charcot-Marie-Tooth disease, type 2 (CMT2). P1386S causes unstable insertion of the
eighth transmembrane segment, preventing proper position of the carboxyl-terminal tail, and disturbs the normal physical interactions of ATP7A with adaptor protein complexes 1 and 2 (AP-1, AP-2). ATP7A-P1386S leads to abnormal axonal localization in transfected NSC-34 motor neurons, and alters calcium signaling following glutamate stimulation. The ATP7A-related distal motor neuropathy caused by the two other ATP7A missense mutations, A988V and T994I, both within the 6th transmembrane segment, involves abnormal interactions with p97/VCP, a hexameric AAA ATPase that has a diversity of biological functions. Our immunoprecipitation studies revealed that both ATP7A-A988V and ATP7A-T994I physically interact with p97/VCP, whereas wild type ATP7A and ATP7A-P1386S do not. We identified a concealed UBX domain in the third lumenal loop between the fifth and the sixth transmembrane domains of ATP7A that interacts with the N-terminal domain of p97/VCP. Since inherited mutations in p97/VCP itself may cause motor neuron degeneration phenotypes including CMT2, hereditary spastic paraplegia, and ALS, the isolated motor neuron disease associated with these unique ATP7A missense mutations may provide insight and opportunity to dissect the mechanism(s) of p97/VCP-mediated motor neuron degeneration of varied molecular basis.

Disclosures:  L. Yi: None. K. Mathews: None. S.G. Kaler: None.

Poster

297. ALS and Motor Neuron Disease

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 297.04/G14

Topic: C.06. Neuromuscular Diseases

Support: NIH R21 NS111248

Title: NEK1 loss-of-function in iPSC-derived motor neurons causes ALS-related phenotypes

Authors: *R. P. MARYERS¹, E. L. DALEY¹, L. TELLEZ¹, J. E. LANDERS², E. KISKINIS¹;¹Neurol., Northwestern Univ., Chicago, IL; ²Neurol., Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease characterized by the degeneration of motor neurons (MNs), leading to eventual paralysis and death; at present, no effective therapy exists. Three independent exome sequencing studies reported a significant enrichment of heterozygous loss of function mutations in the NIMA-related kinase 1 (NEK1) gene in ALS patients that may account for up to 3% of all ALS cases. NEK1 is a cell cycle kinase involved in the DNA damage response, ciliogenesis, and apoptosis. Here, we modeled NEK1-associated ALS using patient-derived motor neurons and siRNA-mediated knockdown in control motor neurons. We observed ALS-related phenotypes, including
a redistribution of TDP-43 from the nucleus to the cytoplasm and higher levels of DNA damage. This study provides support for a key role of NEK1 in ALS-related motor neuron dysfunction.


**Poster**

**297. ALS and Motor Neuron Disease**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 297.05/G15

**Topic:** C.06. Neuromuscular Diseases

**Support:** VA Merit Award
NIH R01 NS067283
NIH R01 NS109537

**Title:** Oligodendrocytes dysfunction by EWSR1 deficiency impairs myelination of motor neurons in amyotrophic lateral sclerosis (ALS)

**Authors:** *J. LEE*1,2,3, P. T. NGUYEN4, E. KIM5, A.-Y. CHUNG5, N. W. KOWALL1,2,3, S. LEE6, H.-C. PARK5, H. RYU1,2,3,4;

**Abstract:** EWSR1 (Ewing Sarcoma breakpoint region 1/EWS RNA binding protein 1), a multifunctional protein, was found to be associated with amyotrophic lateral sclerosis (ALS). However, the EWSR1-dependent pathological mechanism in CNS and ALS has not been fully investigated yet. We found that the immunoreactivity of EWSR1 was significantly decreased in oligodendrocytes in the spinal cord of ALS patients and ALS transgenic [mutant SOD1 (G93A)] mice. Demyelination-associated axonal pathology was observed in the spinal cord of ALS patients and SOD1 (G93A) mice. In addition, the levels of PLP, MBP and OLIG2 were significantly down regulated in the ventral horn of G93A mouse spinal cord. To determine the Ewsr1 deficiency-associated gene expression signatures, we performed whole RNA-sequencing from the spinal cords of wild type (WT) and Ewsr1KO mice. We found that myelination and axon ensheathment-related genes including Plp1, Mbp, and Utg8 were markedly down regulated in Ewsr1KO mice. EWSR1 modulated the transcription of Plp1 through the EWSR1-binding element in the 5’-UTR region. Moreover, knockdown of ewsr1 in G93A zebrafish exacerbated impaired oligodendrocyte maturation and poor motor neuron myelination. Our data indicate that
EWSR1 plays a *bona fide* role in oligodendrocyte activity and its deficiency impairs oligodendrocyte-derived gene expression, leading to the axonal damage of motor neurons in ALS. Together, our study indicates that EWSR1 dysfunction is a molecular marker of ALS pathology and a potential therapeutic target for restoration of oligodendrocyte function.


**Poster**

297. ALS and Motor Neuron Disease

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 297.06/G16

**Topic:** C.06. Neuromuscular Diseases

**Support:** CONACYT 0262111

**Title:** Amyotrophic lateral sclerosis associated with the frontotemporal dementia spectrum. Gene variants of FUS and GRN

**Authors:** *M. J. MORALES-ARMENATA*¹, P. YECAS-GÓMEZ², E. S. VARGAS-CañAS³, M.-C. BOLL⁴, E. ÁLVAREZ⁵, T. ZÚÑIGA-SANTAMARÍA², D. F. MOLOTLA-TORRES², I. FRICKE-GALINDO²;

¹Univ. Veracruzana, Minatitlán, Mexico; ²Neurogenetics, ³Nerve and Muscle Clin., ⁴Neurol., Inst. Nacional De Neurología Y Neurocirugía, Ciudad de México, Mexico; ⁵Univ. Autónoma de la Ciudad de México, Ciudad de México, Mexico

**Abstract:** Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by the progressive loss of motor neurons, producing muscle weakness, paralysis and death. A large number of genes are implicated in familiar and sporadic cases, like so in the spectrum associated with Frontotemporal Dementia (FTD), among which, there are *FUS* and *GRN*. *FUS* (16p11.2) codes for a nuclear protein involved in the biogenesis, regulation and transport of RNA. The *GRN* (17q21.31) has cell regulation, proliferation and differentiation functions. We identify gene variants in *FUS* in Mexican patients with ALS and evaluate variants in *GRN* in patients with ALS-FTD. We studied 157 patients who met The Escorial clinical criteria of ALS. Prior informed consent, a peripheral blood sample was taken for genomic DNA extraction. Exons 5, 6, 14 and 15 of the *FUS* gene were amplified by PCR and analyzed by Sanger type sequencing. In addition, in 11 patients with a history of probable FTD, the *GRN* gene was analyzed. We find in *FUS*, the pathological mutation p. P525L in an individual with brachial juvenile presentation in sporadic case. As well as 5 variants of uncertain significance; p.Y239C; p.G488G C> T; intronic variant c.524_21T> C; two in 3′UTR c. *48G> A and c. *41G> A. In *GRN*, 2 variants were identified: p.D591V; c. *78C> T in 3′UTR in a homozygous and heterozygous manner.
The evaluation of these variants in FUS and GRN confirm the presence of the DFT-ELA spectrum. Interestingly, the p.P525L mutation of FUS affects the C-terminal domain of nuclear localization signaling, rich in glycine, critical for protein aggregation, with an early onset presentation corresponding to the ELA6 phenotype. The variants were presented in sporadic cases. In addition, in 36% of cases DFT-ELA were associated with mutations in the GRN gene, highlighting the variant in 3’UTR c. *78C>T in homozygous state as a risk factor.


Poster

297. ALS and Motor Neuron Disease

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 297.07/G17

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant R01 NS078145
NIH Grant R01 NS090352

Title: ALS-linked FUS induces nuclear pore defects in patient-derived motor neurons

Authors: *Y.-C. LIN, M. S. KUMAR, D. GRUNWALD, D. A. BOSCO;
Umass Med. Sch., Worcester, MA

Abstract: FUS is an DNA/RNA binding protein that shuttles between the nucleus and cytoplasm and is actively involved in nucleocytoplasmic transport (NCT). Mutations in FUS cause amyotrophic lateral sclerosis (ALS), a fatal and incurable motor neuron disorder. Compromised NCT has recently emerged as a pathogenic mechanism underlying ALS and other neurodegenerative diseases. Here, we uncovered nuclear pore defects, including reduced nuclear membrane occupancy and abnormal clustering of pores, in motor neurons derived from human induced pluripotent stem cells (iPSCs) harboring an ALS-linked FUS mutation. Further, the Ran gradient was significantly altered in these neurons. As Ran is a master NCT regulator, these results are consistent with disrupted NCT in ALS-FUS. Importantly, these NCT-related defects were rescued by genetically correcting the FUS mutation in iPSCs. To gain insight into how expression of mutant FUS causes nuclear pore defects, we probed for potential interactions between FUS and a class of low complexity nucleoporin proteins called Nups. The major function of Nups is to maintain a functional and dynamic NCT. We detected an interaction between FUS and Nups via co-immunoprecipitation in different cellular models. Further, interactions between FUS and Nup were detected in situ within human neurons through a proximity ligation assay, which reported a significantly higher signal for mutant FUS and Nups.
within the cytoplasm. This observation may account for the reduced occupancy of pore proteins at the nuclear membrane within the patient line, and is consistent with the pathological accumulation of NCT-related proteins within post-mortem ALS tissues. Intriguingly, both FUS and Nup proteins are known to undergo liquid-liquid phase separation (LLPS). Liquid-like properties allow proteins to rapidly concentrate and disassemble under various conditions. Indeed, we found that recombinant Nup62 and FUS proteins co-localize within liquid droplets in vitro, implicating aberrant LLPS of these proteins in the nuclear pore defects observed in human neurons. Collectively, these findings implicate aberrant Nup interactions in the pathogenic mechanism of ALS-FUS, and support the premise that the NCT pathway is a viable therapeutic target for neurodegenerative disorders.


Poster

297. ALS and Motor Neuron Disease

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 297.08/G18

Topic: C.06. Neuromuscular Diseases

Support: ALS Association Milton Safenowitz Post-doctoral Fellowship
          NIH Grant R21NS09837
          NIH Grant R01NS10575

Title: Accumulation of cytoplasmic Nup62 initiates TDP43 aggregate formation

Authors: *A. GLEIXNER1, J. GALE2, J. R. MANN3, K. E. COPLEY4, J. C. MAUNA6, C. J. DONNELLY5;
         2Sch. of Med., 3Neurobio., 4Neurosci., 5Dept. of Neurobio., 6Univ. of Pittsburgh, Pittsburgh, PA;
         Neurobio., Univ. of Pittsburgh Dept. of Neurobio., Pittsburgh, PA

Abstract: ALS and FTD are fatal, neurodegenerative diseases that both present with cytoplasmic mislocalization and aggregation of TAR DNA-binding protein 43 (TDP43). TDP43 is an RNA and DNA binding protein that is predominantly located in the nucleus of healthy cells. However, in ALS and FTD, TDP43 is redistributed to the cytoplasm where it forms insoluble aggregates and these are pathologically hyperphosphorylated, ubiquitinated, and p62-positive. Several genetic mutations in the TDP43 gene have even been linked to familial ALS and FTD cases. TDP43 mutations have also been shown to cause neurotoxicity. In TDP43 Drosophila models, neurotoxicity was reduced by mutating the phenylalanine-glycine (FG) domain of nucleoporin 50. The concentrated region of FG repeats in FG nucleoporins creates an area of hydrophobicity. A similar phenomenon is observed in the low complexity domain of TDP43. Therefore, we sought to examine whether FG nucleoporins are associated with TDP43 aggregate formation. We
hypothesized that these characteristics may favor interactions between FG nucleoporins and TDP43 that eventually yield insoluble TDP43 aggregates. Our studies revealed that the cytoplasmic accumulation of the FG nucleoporin Nup62 colocalizes with TDP43. These TDP43 aggregates are insoluble and mimic hallmark pathology typically observed in neurodegenerative diseases. Further characterization of the mechanism by which Nup62 seeds TDP43 aggregation may lend insight into the mechanism driving TDP43-aggregate formation in ALS and FTD.


Poster

297. ALS and Motor Neuron Disease

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 297.09/G19

Topic: C.06. Neuromuscular Diseases

Title: Mitochondrial dysfunction and energy metabolism deficits in post-mortem C9ORF72 ALS motor cortex


Abstract: Previous studies have demonstrated that mitochondrial dysfunction is a pathogenic mechanism in ALS. Abnormal mitochondrial structure, alteration in mitochondrial protein transport and trafficking, and deficits in electron transport by complexes I-IV have been described in cellular and animal models of ALS as well as in patients. Importantly, these alterations lead to energy imbalance, oxidative stress, apoptosis, and impaired calcium signaling. Impairments in mitochondrial function have been described in the SOD1, wild-type and mutant TDP43 mice and a recent study demonstrated that oxidative stress and DNA damage were increased in iPSC-derived C9ORF72 motor neurons in an age-dependent manner. Our genetic studies have unveiled a novel genetic variant in the WW domain-containing oxidoreductase (WWOX) gene in 4,366 ALS samples from Project MinE which is completely absent in gnomAD. WWOX is involved in a number of critical cellular functions one of which is to decrease oxidative stress by interacting with mitochondria. Therefore, we hypothesized that mutations in WWOX may lead to oxidative stress in ALS. We have begun to address this hypothesis by measuring alterations in mitochondrial function in post-mortem samples from
ALS patients. Our results indicated that WWOX protein levels are significantly decreased in post-mortem ALS motor cortex, supporting our hypothesis. In addition, there was a significant decrease in mitochondrial proteins involved in the electron transport chain (SDHB, UQRC2, ATP5A, COXII, NDUFB8) and the citric acid cycle (SDHB) in C9ORF72 ALS motor cortex compared to control. Furthermore, there is a significant decrease in peroxiredoxin 5 (PRDX5), an antioxidant enzyme targeted to the mitochondria during inflammation, in ALS patient plasma, suggesting that PRDX5 may be a novel biomarker for ALS. Together, these findings suggest that mutations in WWOX may lead to mitochondrial dysfunction and oxidative stress in ALS, specifically in patients with C9ORF72 mutations.


Poster

297. ALS and Motor Neuron Disease

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 297.10/G20

Topic: C.06. Neuromuscular Diseases

Support: NIH K08NS104273
AAN/ALSA Clinician Scientist Development Award
F-prime/Fidelity Biosciences Research Initiative

Title: C9orf72 arginine-containing dipeptide repeat proteins disrupt nuclear import

Authors: *L. R. HAYES1, K. BOWEN1, L. DUAN1, P. KALAB3, J. D. ROTHSTEIN2; 2Brain Sci. Inst., 1Johns Hopkins Univ., Baltimore, MD; 3Johns Hopkins Whiting Sch. of Engin., Baltimore, MD

Abstract: Disruption of nuclear transport is increasingly recognized as an important pathophysiologic mechanism in neurodegenerative disease, including C9orf72-mediated amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Dipeptide repeat proteins (DPRs) produced by translation of the C9orf72 G4C2 hexanucleotide repeat expansion have been implicated in nuclear transport disruption, but the precise mechanism remains unclear. Here, we adapted the permeabilized cell nuclear import assay for primary cortical neurons, and tested the effect of each of the five C9orf72 DPRs (poly-GP, poly-GA, poly-GR, poly-PR, and poly-PA). We found dose-dependent inhibition of nuclear import by poly-GR and poly-PR. Both triggered visible aggregate formation in the assay, and mass spectrometry analysis of the aggregates confirmed sequestration of numerous RNA processing proteins, low complexity domain-containing proteins, and proteins involved in nucleocytoplasmic transport, including karyopherins, Ran cycle proteins, and some nucleoporins. These protein-protein interactions
were validated by Western blot, FRET, and bead halo studies, which confirmed the high affinity of poly-GR and poly-PR for importin β. Consistent with these data, we observed loss of importin β from the nuclear membrane in postmortem motor cortex and spinal cord from C9orf72-ALS/FTD patients. High concentrations of total cellular RNA reduced DPR-induced aggregation and rescued nuclear import. In summary, poly-PR and poly-GR inhibit nuclear import in an in vitro assay, via a mechanism involving disruption of importins and aberrant nucleoporin interactions. RNA-based approaches may offer a method for blocking these interactions and restoring nuclear import.

Disclosures: L.R. Hayes: None. K. Bowen: None. L. Duan: None. P. Kalab: None. J.D. Rothstein: None.

Poster

297. ALS and Motor Neuron Disease

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 297.11/G21

Topic: C.06. Neuromuscular Diseases

Support: NIH RO1 F041854
        NIH T32-NS007222-36UM
        NIH F32 AG059362-01

Title: Ubiquilin-2 efficiently lowers levels of common disease proteins, including the ALS-linked protein TDP-43

Authors: *J. WELDAY1, *J. E. GERSON2, S. S. PISTORIUS3, J. D. GREGORY2, L. M. SHARKEY4, H. L. PAULSON5;
1Univ. of Michigan- Ann Arbor, Ann Arbor, MI; 3Neurosci., 2Univ. of Michigan, Ann Arbor, MI; 4Neurol. Dept., Univ. Michigan, Ann Arbor, MI; 5Prof, Neurol, Univ. of Michigan Dept. of Neurol., Ann Arbor, MI

Abstract: Ubiquilin-2 (UBQLN2) is a homeostatic regulatory protein that functions primarily in the ubiquitin-proteasome system and autophagy to shuttle ubiquitinated proteins for degradation. When mutated, UBQLN2 directly leads to frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS), which are adult-onset neurodegenerative diseases characterized primarily by the presence of trans-active DNA binding protein-43 (TDP-43) inclusions in the cytoplasm. To investigate the ability of UBQLN2 to regulate FTD/ALS pathology, we first transfected HEK-293 cells to express TDP-43 in combination with UBQLN2 or with UBQLN2 knocked-down. The ability of UBQLN2 to regulate other common aggregation-prone disease proteins, including tau and alpha-synuclein, was also evaluated. Co-expressed UBQLN2 markedly lowered levels of wildtype TDP-43, as well as other common disease proteins. Conversely,
knockdown of UBQLN2 significantly elevated levels of wildtype TDP-43. However, mutations in TDP43 decreased the ability of UBQLN2 to lower TDP43 levels. To confirm that UBQLN2 directly interacts with disease proteins, UBQLN2 was immunoprecipitated from HEK-293 cells expressing both UBQLN2 and either tau, α-synuclein or TDP43. To determine how UBQLN2 regulates endogenous levels of disease proteins in vivo, UBQLN2 transgenic and UBQLN2 KO mice were analyzed for levels of TDP43, tau and α-synuclein via Western blot. While soluble levels of TDP43, tau and α-synuclein were unchanged, UBQLN2 lowered levels of endogenous, insoluble disease proteins when overexpressed. Given that mutated UBQLN2 has been shown to directly cause FTD/ALS, understanding its role in toxic protein regulation may ultimately help elucidate disease progression and onset. Further study will evaluate how disease mutations alter the function of UBQLN2 in regulating disease proteins. Our findings indicate the potential importance of UBQLN2 in regulating ALS/FTD pathology and highlight a new role for regulating levels of other common neurodegenerative disease proteins such as tau and α-synuclein.


Poster

297. ALS and Motor Neuron Disease

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 297.12/G22

Topic: C.06. Neuromuscular Diseases

Support: NIH NS045195

Title: Defects in muscle ion channels contribute to motor dysfunction in spinal and bulbar muscular atrophy

Authors: *Y. Xu1, M. Katsuno2, H. Adachi3, G. Sobue2, M. Breedlove1, C. Jordan1;
1Michigan State Univ., East Lansing, MI; 2Nagoya Univ. Grad. Sch. of Med., Nagoya, Japan; 3Univ. of Occup. and Envrn. Hlth. Sch. of Med., Kitakyushu Fukuoka, Japan

Abstract: Spinal-bulbar muscular atrophy (SBMA) is characterized by progressive muscle weakness linked to a polyglutamine expansion in the androgen receptor (AR). Contractile dysfunction is a core trait of SBMA. We previously reported that diseased fibers in SBMA mouse models are partially depolarized, suggesting alterations in membrane excitability. To explore specific mechanisms, we examined the action potential (AP), a hallmark indicator of membrane excitability, using two-electrode intracellular recording of fibers in 3 well-studied SBMA mouse models-AR97Q, knock-in (KI) and myogenic. We find APs are uniformly slower
in all three models and smaller in two. The partial depolarization contributed to the reduced AP magnitude, but even at -80 mV, the threshold for excitation was significantly increased, linked to a loss of voltage-dependent sodium channels we reported previously. We also find reduced chloride and potassium conductance, indicating perturbations in these channels that contribute to the prolonged repolarization of APs and reduced input conductance of SBMA fibers at rest. The sodium/potassium pump maintains the normal resting membrane potential (RMP) of muscle fibers is also defective in diseased fibers in all 3 models. In sum, disease impairs specific ion channels and pumps in the muscle membrane, underlying effects on the RMP, the size and kinetics of APs, and decreased membrane excitability, all likely contributing to the striking deficits in contractile force in SBMA. Chronic depolarization of muscle RMP not only impairs excitation-contraction coupling, but also would trigger increases in resting intracellular Ca\textsuperscript{2+}, activating Ca\textsuperscript{2+}-dependent degradative pathways. These several mechanisms underlying muscle dysfunction in SBMA are potential targets for therapeutic invention.


Poster

297. ALS and Motor Neuron Disease

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 297.13/G23

Topic: C.06. Neuromuscular Diseases

Support: NIH R01NS065263
The Robert Johnston Foundation,
ALSA 18-IIA-420

Title: Two tales of one function physicochemical epidemiology evidence that loss of angiogenin function promotes amyotrophic lateral sclerosis onset but improves survival after onset

Authors: *K. C. ALURI\textsuperscript{1}, J. SALISBURY\textsuperscript{2}, J. N. AGAR\textsuperscript{1};\textsuperscript{1}\textsuperscript{1}Chem. and Chem. Biol., Northeastern Univ., Boston, MA; \textsuperscript{2}Brandeis Univ., Waltham, MA

Abstract: Over twenty mutations in angiogenin (ANG) are associated with familial (f) amyotrophic lateral sclerosis (ALS) ALS. The prevailing hypotheses—which had not been statistically evaluated—were that these mutations contribute to fALS through either loss of ANG ribonuclease activity or a loss of nuclear translocation ability. To test these and other hypotheses, we correlated various physicochemical properties of ANG-ALS variants with ALS clinical outcomes. Using well-established statistical methods such as non-parametric correlation coefficients, Kaplan-Meier analysis, and Cox proportional hazards analysis we 1) demonstrate that loss of ANG function strongly correlates with more rapid ALS onset; and 2) demonstrate
that loss of ANG function strongly correlates with longer survival of ALS patients following ALS onset (increased duration). Notably, the observation that loss of ANG function correlates with increased duration contradicts current models of ANG-associated ALS. These data should be taken into consideration for ANG-replacement therapy development and clinical trial design for ALS because they indicate that 1) presymptomatic ANG replacement would extend the life of patients by an average of 13 years, but paradoxically 2) post-symptomatic ANG replacement could decrease the lifespan of ALS patients by two years. In addition, these observations can serve as an exemplar for growth factors “switching” from survival-promoting to toxic following the onset of disease.

**Disclosures:** K.C. Aluri: None. J. Salisbury: None. J.N. Agar: None.

**Poster**

**297. ALS and Motor Neuron Disease**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 297.14/G24

**Topic:** C.06. Neuromuscular Diseases

**Support:** This work was supported by the grants from Les Turner ALS Foundation.

**Title:** Exosomes secreted by diseased ALS cerebral cortex include messages to modulate disease progression

**Authors:** *E. XIE¹, M. GAUTAM¹, J. R. BRENT¹, N. KOCAK¹, O. GOZUTOK¹, H. Y. SHIN¹, N. L. ANGELONI², C. S. THAXTON²,³, P. OZDINLER¹;

¹Dept. of Neurol., ²Dept. of Urology, Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; ³Intl. Inst. for Nanotechnology (IIN), Northwestern Univ., Evanston, IL

**Abstract:** Exosomes are mediators of intercellular communication between different cells in the body and are small vesicles typically 30-150nm in size. They carry a cargo of important biomolecules (protein, RNA, miRNA and lipids), which is used to transmit signal to recipient cells. Their ability to cross the blood brain barrier enables flow of information to distant locations within central nervous system (CNS), and between the CNS and the periphery. Building evidence suggests the importance and involvement of cortical component of motor neuron circuitry in the establishment and progression of disease pathology in amyotrophic lateral sclerosis (ALS). This study was conceived to identify differential protein content of the exosomes secreted by mixed cortical neuron cultures from 3 day old hSOD1⁹³A and prpTDP-43 A⁵¹T mice, two well-defined mouse models of ALS. We investigated if diseased cortical neurons utilize exosomes to inform other cells and neurons about their physiological condition and whether this information serves to propagate the disease or information as a “warning signal” to other cells and neurons. Our preliminary results suggest that hSOD1⁹³A and prpTDP-
cortical neurons include a very distinct set of proteins in exosomes, some of which are common and some are unique. Proteins present in these early-age exosomes suggest encoding a “warning signal” to other cells and neurons. This study begins to shed light on how diseased cortical neurons utilize exosomes to communicate their disease state. Our findings may reveal novel treatment strategies to prevent disease progression and to improve motor neuron health in ALS and related motor neuron diseases.


**Poster**

**297. ALS and Motor Neuron Disease**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 297.15/G25

**Topic:** C.06. Neuromuscular Diseases

**Title:** Answer ALS: On the road to identifying biological subgroups in sporadic ALS thru a population based multi-omics

**Authors:** *J. D. ROTHSTEIN*1, C. N. SVENDSEN2, L. M. THOMPSON3, E. FRAENKEL4, J. VAN EYKE5, D. SAREEN6, N. J. MARAGAKIS7, J. BERRY8, T. THOMPSON9, E. BAXI1; 1Neurol., Johns Hopkins Univ., Baltimore, MD; 2Regenerative Med., Cedars-Sinai Med. Ctr., West Hollywood, CA; 3Psychiatry/Neurobiology and Behavior, Univ. California, Irvine, CA; 4MIT, Boston, MA; 5Cedars Sinai, Los Angles, CA; 6Cedars Sinai, Porter Ranch, CA; 7Dept Neurol, Johns Hopkins Univ. Dept. of Neurol. and Neurosurg., Baltimore, MD; 8Mass Gen., Boston, MA; 9UC Irvine, Irvine, CA

**Abstract:** Answer ALS was conceived and organized as a comprehensive multi-omics approach to ALS to ascertain, at a population level, the various clinical-molecular-biochemical subtypes of ALS. This national program has enrolled over 1000 ALS and ALS/FTD patients along with a cohort of >100 matched control patients. Patients were recruited at 8 national ALS centers and followed longitudinally over one year. A smartphone-based system was employed to collect deep clinical data including fine motor activity, speech, breathing and linguistics/cognition. In collaboration with IBM Watson, analytics of speech patterns reveals a strong correlation between clinical progression indices and speech. In parallel, iPS motor neurons were blood-derived from each patient and these cells underwent multi-omic analytics including: whole genome sequencing, RNA transcriptomics, ATAC-Seq, proteomic, and in a subset microbiome. HIPPA compliant cloud data bases were employed to store all data. More than 500 whole genomes have been completed and over 500 iPS cell lines generated. More than 500 iPS cell lines have already been generated from these patients. Integrated clinical and biological signatures are now being
generated using bioinformatics, statistics and computational biology to establish patterns that may lead to a better understanding of the underlying mechanisms of disease. Early multi-omics analysis of a trial subset of 100 sporadic and familial ALS and control iPS motor neuron cell lines were used to determine if biological subgroups could be identified. Early analytics from this multi-omics data set form this large and first cohort of human motor neurons suggest subset of patient will be identified. C9orf72 patients were found have prominent defect in nuclear transport, chromatin remodeling and RNA metabolism as fundamentally altered pathways with candidate pathways modulating drugs identified. Open access has already provided data and the iPS cell lines to dozens of researchers worldwide. Relevant pathways and molecular targets are being verified in post mortem brain tissue. A web portal for open source sharing of all data has been developed for widespread community based data analytics. These studies demonstrate distinct reliably identifiable subgroups among the sporadic and familial patients and the great utility in iPS based approaches to disease pathophysiology and therapy discovery.


Poster

297. ALS and Motor Neuron Disease

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 297.16/G26

Topic: C.06. Neuromuscular Diseases

Support: National Key R&D Program of China (No. 2016YFA0501902)  
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Title: Poly(adp-ribosylation) regulates stress granule dynamics, phase separation, and neurotoxicity of disease-related ma-binding proteins

Authors: *Y. DUAN*¹,², A. DU¹, J. GU¹,², C. LIU¹,², Y. FANG¹,²;  
¹Interdisciplinary Res. Ctr. on Biol. and Chemistry, SIOC, Chinese Acad. of Sci., Shanghai, China; ²Univ. of Chinese Acad. of Sci., Beijing, China

Abstract: Mutations in RNA-binding proteins (RBPs) localized in ribonucleoprotein (RNP) granules, such as hnRNP A1 and TDP-43, promote aberrant protein aggregation, which is a pathological hallmark of various neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Protein posttranslational modifications
(PTMs) are known to regulate RNP granules. In this study, we investigate the function of poly(ADP-ribosylation) (PARylation), an important PTM involved in DNA damage repair and cell death, in RNP granule-related neurodegeneration. We reveal that PARylation levels are a major regulator of the assembly-disassembly dynamics of RNP granules containing disease-related RBPs, hnRNP A1 and TDP-43. We find that hnRNP A1 can both be PARylated and bind to PARylated proteins or poly(ADP-ribose) (PAR). We further uncover that PARylation of hnRNP A1 at K298 controls its nucleocytoplasmic transport, whereas PAR-binding via the PAR-binding motif (PBM) of hnRNP A1 regulates its association with stress granules. Moreover, we reveal that PAR not only dramatically enhances the liquid-liquid phase separation of hnRNP A1, but also promotes the co-phase separation of hnRNP A1 and TDP-43 in vitro and their interaction in vivo. And we also found that both genetic and pharmacological inhibition of PARP mitigates hnRNP A1- and TDP-43-mediated neurotoxicity in cell and Drosophila models of ALS. Together, our findings suggest a novel and crucial role for PARylation in regulating the dynamics of RNP granules, and that dysregulation in PARylation and PAR levels may contribute to ALS disease pathogenesis by promoting protein aggregation. Finally, we are trying to investigate the mechanism that PARylation regulates the physiological properties of hnRNP A1, and ongoing experiments include mass spectrometry to identify proteins that interact with hnRNP A1 via the PARylation or PAR-binding to regulate its nucleocytoplasmic transport and stress response.


Poster

297. ALS and Motor Neuron Disease

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 297.17/G27

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant AG051513
        NIH Grant AG047612

Title: High spatio-temporal resolution locomotor analysis of Sod mutants revealed distinct modes of hyperactivity in the circadian behavior induced by oxidative stress in *Drosophila*

Authors: *A. U. UEDA*¹, C.-F. WU²;
        ¹Dept. of Biol., ²Univ. of Iowa, Iowa City, IA

Abstract: Functional interplay between K+ channels and reactive oxygen species (ROS) signaling have been implicated in the circadian patterns of sleep-wake cycles (Kempf et al.,
Hyper-excitable mutants of several K+ channel genes have been shown to decrease total time of sleep with increased locomotion activity during both day and night (Cirelli et al., 2005; Bushey et al., 2007). The enzyme Superoxide dismutase (Sod) is responsible for clearance of the harmful ROS generated in oxidative respiration processes. We discovered that Drosophila Sod mutants also display increased locomotor activity and altered sleep duration, but in a distinct mode of activity patterns.

We monitored fly locomotion behavior using the TriKinetics multibeam counter, which is capable of continuously recording the locomotor activity of a single fly traversing along a narrow glass tube (65 mm length, 5 mm diameter) in multiple (17) zones covering the entire tube length. The activity counts within each zone are continuously recorded at a 1-minute time bin. The results for wild-type (WT) and K+ channel mutant flies were in general consistent with previous reports based on counts of midline crossing for a fly in the tube of the same dimension. WT flies typically remained inactive for most stretches of time and the majority of activity occurred in bouts around dawn and dusk. In contrast, both K+ channel mutants Shaker (Sh) and Hyperkinetic (Hk), which are defective in transient K+ current IA, showed elevated total activity counts in the 24-hour period with decreased number of time periods of inactivity and markedly increased nocturnal activity.

Mutant flies of sod1 alleles produced greatly enhanced local activity of fragmented translocation movements confined in a few adjacent zones. This is in stark contrast to Sh and Hk, which produced sustained long-distance translocation activity, especially during light-off periods. Strikingly, total activity in WT and Sh mutants were accounted for by a few extremely long-duration runs (about 30 to 40%, >1 hr) which were rarely observed in Sod1 mutants (<10%). Using the definition of inactivity >5 min for sleep, Sod flies clearly showed a deficiency in sleep. Even though they seldom traversed the midline, they displayed sustained short bouts of local activity throughout day and night. Future genetic and circuit analysis may reveal the molecular and cellular bases of the distinction between these two modes of hyperactivity caused by K+ channel defects and ROS stress.

Disclosures: A.U. Ueda: None. C. Wu: None.

Poster

297. ALS and Motor Neuron Disease

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 297.18/G28

Topic: C.06. Neuromuscular Diseases

Support: Farber Family Foundation
Family Strong for ALS Foundation
Title: Aberrant production of netrin-1 by astrocytes contributes to motor neuron death in the SOD1-G93A mouse model of ALS


Abstract: Mutations in the SOD1 gene cause ~20% of inherited ALS. The underlying molecular mechanisms involve a combination of intrinsic motor neuron and glia-induced toxicity. Although the contribution of astrocytes towards motor neuron death in ALS has been well established, the identity of the mediators of this toxicity is not well understood. We previously showed in the SOD1-G93A mouse model of ALS that caspase-3 cleaves the astroglial glutamate transporter GLT-1, releasing a sumoylated fragment (CTE-SUMO1), which accumulates in the nucleus of astrocytes over disease. Astrocytes harboring CTE-SUMO1 initiate non-cell autonomous motor neuron toxicity. Microarray analysis of these CTE-SUMO1 astrocytes revealed higher expression of netrin-1. While in the normal adult CNS mainly oligodendrocytes express and release netrin-1 to regulate axon homeostasis and synaptic maintenance, spinal cord astrocytes have not been shown to express netrin-1. We provide here the evidence of netrin-1 expression in astrocytes of the symptomatic SOD1-G93A mice. Interestingly, netrin-1 is also elevated in motor neurons of the presymptomatic and disease onset stages of the SOD1-G93A mice. Expression of netrin-1 was also detected in astrocytes in post-mortem spinal cord tissue and iPS cells-derived astrocytes of sporadic ALS patients. Intrathecal injection of netrin-1 in SOD1-G93A mice decreased the number of ChAT+ motor neurons. Neutralization of netrin-1 in human iPSC derived astrocyte-conditioned media using anti-netrin-1 antibodies significantly rescued the motor neuron directed toxicity. Acute stimulation of mature primary motor neurons with netrin-1 (25 nM) elicits a robust Ca\textsuperscript{2+} influx response. Microelectrode analysis of motor neurons treated with 25 nM netrin-1 showed changes in the frequency of firing both in the short term and long term. Aberrant production and release of netrin-1 by astrocytes may, therefore, be one of the pathogenic, non-cell autonomous mechanisms of motor neuron toxicity and be a potential novel molecular target for intervention.


Poster

297. ALS and Motor Neuron Disease

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 297.19/G29

Topic: C.06. Neuromuscular Diseases

Support: Independent Research Fund Denmark
Title: Progressive changes to nodes of ranvier of motor axons in the SOD1\textsuperscript{G93A} mouse model of amyotrophic lateral sclerosis

Authors: M. KADLECOVA\textsuperscript{1}, K. P. DIMINTIYANOVA\textsuperscript{2}, D. B. JENSEN\textsuperscript{2}, *C. F. MEEHAN\textsuperscript{2}; \textsuperscript{2}Dept. of Neurosci., \textsuperscript{1}Univ. of Copenhagen, Copenhagen, Denmark

Abstract: Background: Our electrophysiology experiments (see linked abstract: Dimintiyanova et al.) show a decrease in K\textsuperscript{+} currents during periods of active degeneration, suggesting either an alteration in the resting membrane hyperpolarization or a change in the K\textsuperscript{+} conductance. The later could due to either a change in the number, density or distribution of proteins at and around the node of Ranvier.

Aim: We performed immunohistochemistry for proteins at both proximal and distal nodes of Ranvier to investigate possible changes at disease onset in the same mouse model.

Methods: Immunohistochemistry was performed on the lumbar ventral roots and the mostly motor quadriceps nerve in 10 SOD1\textsuperscript{G93A} (high copy number) mice (5 male, 5 female) and 10 aged/sex matched wild type littermate controls at 18 weeks of age. Behavioural tests using the rotorod and grip strength test identified this as the time point for symptom onset. We identified nodal Na\textsuperscript{+} channels using a Pan-NaV channel antibody and Kv1.2 potassium channels at the juxtaparanode (JPX). The paranode was identified using antibodies against the scaffolding protein Caspr\textsubscript{1}.

Results: A significant decrease in nodal width was observed in both ventral roots (P<0.0001, 36%) and more distally in the quadriceps nerve (P=0.0005, 16%), consistent across sexes. This resulted in an overall reduction in the surface area by 27% in the ventral roots and 14% in the quadriceps nerve. JPX width was also significantly reduced both in the ventral roots (P<0.0001, 28%) and quadriceps nerve (P=0.0463, 9%) indicating that the reduction in nodal width is not due to a focal constriction of the node. The distance between the node and the JPX (measured by Caspr\textsubscript{1} labelling) was significantly increased in the SOD1\textsuperscript{G93A} mice both at the ventral roots (P<0.0001, 12%) and the quadriceps nerve (P<0.0001, 18%). The changes in the axonal geometry appear to be similar at proximal and distal regions of the axon. However, at the distal axon we more frequently observed signs of disruption of nodal organization, such as mislocalization of paranodal proteins to the node and JPX.

Conclusion: Our data shows changes in nodes of Ranvier structure in SOD1\textsuperscript{G93A} mice both proximally and distally but with distal nodal disruption consistent with a dying back process.

**Poster**

**297. ALS and Motor Neuron Disease**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 297.20/G30

**Topic:** C.06. Neuromuscular Diseases

**Support:** Independent Research Fund Denmark  
Læge Sofus Carl Emil Friis og Hustru Olga Doris Friis’ Project Grant

**Title:** Impaired axonal potassium channel function in the SOD1\(^{G93A}\) mouse model of amyotrophic lateral sclerosis

**Authors:** *K. P. DIMINTIYANOVA\(^1\), D. B. JENSEN\(^2\), C. F. MEEHAN\(^3\), M. MOLDOVAN\(^1\);  
\(^2\)Dept. of Neurosci., \(^1\)Univ. of Copenhagen, Copenhagen, Denmark; \(^3\)Univ. of Copenhagen,  
København N, Denmark

**Abstract:** *Background:* Nerve excitability testing using threshold tracking has shown abnormalities in ALS patients, including a reduction in K\(^+\) currents in motor axons. However, a previous study on the most commonly used ALS mouse model (SOD1\(^{G93A}\), high copy number) up to 19 weeks of age could not confirm these observations, suggesting that the changes observed in patients are not well represented in this model (Boerio et al., Muscle and Nerve 2010). In contrast, we have observed K\(^+\) channel abnormalities preceding axonal degeneration in another ALS model: the SOD1\(^{G127X}\) mouse (Moldovan et al., Exp Neurol. 2012; Maglemose et al. Exp Neurol. 2017).

**Aim:** The aim of this study was to investigate the progression of nerve excitability changes in male SOD1\(^{G93A}\) mice up to 22 weeks of age, with an emphasis on K\(^+\) currents.

**Methods:** We investigated progressive changes in motor nerve conduction and excitability under Ketamine-Xylazine anaesthesia. Aged-matched wild type littermates served as controls. The right tibial nerve was stimulated at the ankle and the evoked compound muscle action potential (CMAP) was recorded from the plantar muscles. Multiple measures of nerve excitability were tested by threshold-tracking using the clinical TRONDNF protocol. The threshold was defined based on the stimulus-response relationship (MScan) as the current required to evoke a 40% CMAP amplitude. For motor unit number estimation (MUNE) we used the recently developed MScanFit MUNE method.

**Results:** SOD1\(^{G93A}\) mice showed a progressive decrease in CMAP amplitude associated with a 6-fold reduction in the number of motor units which was partly compensated for by an increase in the largest motor unit fraction (a marker of collateral reinnervation). The largest CMAP changes occurred from 18 to 22 weeks and were preceded by progressive abnormalities in excitability measures consistent with a reduction in K\(^+\) currents: larger than normal threshold changes during both depolarizing and hyperpolarizing threshold electrotonus and larger than normal deviations.
during the subnormal and supernormal period of the recovery cycle. The other excitability measures provided no evidence for a hyperpolarizing change in the resting membrane potential which could have accounted for the reduction in $K^+$ currents.  

**Conclusion:** Our data suggest that in SOD1$^{G93A}$ mice there was a progressive reduction in the $K^+$ conductance along the distal motor axon preceding the drop in CMAP occurring in the late disease stages, similar to changes previously reported in human sporadic ALS by the same measures. Thus, it is likely that the $K^+$ channel impairment underlies periods of active motor axon degeneration in the patients and ALS models alike.

**Disclosures:** K.P. Dimintiyanova: None. D.B. Jensen: None. C.F. Meehan: None. M. Moldovan: None.

**Poster**

297. ALS and Motor Neuron Disease

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 297.21/G31

**Topic:** C.06. Neuromuscular Diseases

**Support:** NIH Grant GM88804  
AG047612  
AG051513

**Title:** Aberrant synaptic physiology and morphology in *drosophila* larvae of superoxide dismutase 1 (Sod1) mutant alleles

**Authors:** *X. XING, A. UEDA, T. O’HARROW, C.-F. WU;*  
Biol., Univ. of Iowa, Iowa City, IA

**Abstract:** It has been established that mutations in the gene Sod1, which encodes the cytoplasmic Cu/Zn superoxide dismutase (Cu/Zn Sod), are associated with familial Amyotrophic Lateral Sclerosis (ALS), a degenerative disease in motor neurons. The Sod1 enzyme is responsible for scavenging cytosolic reactive oxygen species (ROS). *Drosophila* Sod1 share a highly conserved sequence with human Sod1, and the mutant flies show increased sensitivity to ROS generating agents (e.g. paraquat), greatly decreased lifespan, and weakened motor capability. This provides an opportunity to study the function of Sod1 in *Drosophila* motor neurons, in which the larval neuromuscular junction (NMJ) is widely used as a platform for studying genetic mutations and pharmacological manipulations that affect the development and physiology of motor synapses. The flat and transparent anatomy of the larval NMJ preparation facilitates both electrophysiological recording and optical imaging. We combined these two approaches to reveal the consequences of Sod mutations, including Sod$^{p108}$, the well-characterized functionally null, and Sod$^{G85R}$ and Sod$^{H71Y}$, the orthologs of two mutations found in
human ALS patients. Focal electrophysiological recording showed that these Sod alleles had lower transmission failure rates at low external Ca\textsuperscript{2+} levels and altered frequencies of spontaneous miniature excitatory junction potentials (mEJPs) along the synaptic terminals compared with wild-type (WT) larvae. Treatment with K\textsuperscript{+} channel blockers such as 4-aminopyridine (4-AP) or quinidine produced more extreme supernumerary firing in Sod\textsuperscript{1} mutants. Similarly, supernumerary transmitter release could be observed in double mutants of Sod\textsuperscript{p108} crossed to K\textsuperscript{+} channel mutations such as ether-a-go-go or Sh. suggesting that Sod impaired K\textsuperscript{+} channels. The morphology of synaptic boutons was visualized with fluorescently-tagged anti-horseradish peroxidase (antiHRP). Interestingly, Sod\textsuperscript{G85R} and Sod\textsuperscript{H71Y} both displayed oversized boutons compared to WT, without obviously altering the number of synaptic boutons. The results illustrate the importance of Sod1 in the development and function of motor neurons, which may reflect aspects of the early symptoms of ALS.

**Disclosures:** X. Xing: None. A. Ueda: None. T. O'Harrow: None. C. Wu: None.

**Poster**

297. ALS and Motor Neuron Disease

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program#/Poster #:** 297.22/G32

**Topic:** C.06. Neuromuscular Diseases

**Support:** NIH R01
NS091299
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HHMI Gilliam Fellowship for Advanced Studies
Target ALS
Barrow Neurological Foundation

**Title:** Mechanisms underlying the neuroprotective effect of phosphofructokinase overexpression in ALS

**Authors:** *S. LOGANATHAN\textsuperscript{1}, E. MANZO\textsuperscript{1}, J. BARROWS\textsuperscript{1}, D. SHREINER\textsuperscript{1}, D. BARRAMEDA\textsuperscript{1}, I. LORENZINI\textsuperscript{2}, A. STARR\textsuperscript{2}, T. KOVALIK\textsuperscript{2}, R. BOWSER\textsuperscript{2}, R. SATTLER\textsuperscript{2}, D. ZARNESCU\textsuperscript{1,3,4},

\textsuperscript{1}Mol. and Cell. Biol., Univ. of Arizona, Tucson, AZ; \textsuperscript{2}Neurol., Barrow Neurolog. Inst., Phoenix, AZ; \textsuperscript{3}Neurosci., \textsuperscript{4}Neurol., The Univ. of Arizona, Tucson, AZ

**Abstract:** Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disorder for which there is no cure. ALS affects upper and lower motor neurons leading to loss of motor coordination, irreversible paralysis, respiratory failure and eventually death within 2-5 years of diagnosis. 90% of ALS cases are sporadic (sALS) with no clear genetic connection. TDP-43 is
involved in pathology of at least 97% of ALS cases. Our lab has developed a *Drosophila* model of ALS based on overexpression of human TDP-43 that recapitulates multiple disease aspects including cytoplasmic aggregates, neuromuscular junction (NMJ) abnormalities, reduced lifespan, and locomotor defects. TDP-43 alters the synaptic vesicle (SV) cycle at the NMJ in flies. Observations in humans and animal models show evidence of metabolic dysregulation and oxidative stress mediated damages in ALS. Our lab has shown that glycolysis upregulation, specifically an increase in Phosphofructokinase-1 (PFK1; PFK in *Drosophila*) provides a compensatory, neuroprotective effect in *Drosophila* models of ALS based on TDP-43 proteinopathy. Recent findings from other groups show that under stress PFK accumulates in *C. elegans* at synapses where it is required for SV cycling. In addition, pyruvate, the end product of glycolysis, which was also found to be increased in multiple ALS models has been shown to have strong Reactive Oxygen Species (ROS) scavenging capacity, protecting mitochondrial membrane potential and regulating cell metabolism. Based on these findings, we hypothesize two mechanisms by which increased PFK could mitigate neuronal dysfunction in ALS. We are currently testing whether overexpression of PFK mitigates motor neuron dysfunction by 1) mitigating SV cycling deficits at the larval NMJ or by 2) antioxidant activity of pyruvate. I will present our results on SV cycling and oxidative stress under the conditions of PFK overexpression in several ALS models. These findings may uncover therapeutic strategies for ALS.

**Disclosures:**  

**Poster**

297. ALS and Motor Neuron Disease

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 297.23/G33

**Topic:** C.06. Neuromuscular Diseases

**Title:** Postnatal selective elimination of slow-type motor neurons induces late-onset neuromuscular deficits in mice: Red muscle atrophy, posture abnormality and kinetic tremor

**Authors:** K. KAMISHIMA\(^1\), T. KOYAMA\(^1\), L. OHGAKI\(^1\), T. YAMANAKA\(^2\), S. ITOHARA\(^3\), N. OGIHARA\(^4\), S. SAWANO\(^5\), W. MIZUNOYA\(^6\), *H. MISAWA\(^1\);  
\(^1\)Pharmacol., Fac. of Pharmacy, Keio Univ., Tokyo, Japan; \(^2\)Lab. of Structural Neuropathology, Doshisha Univ. Grad. Sch. of Brain Scien, Kyoto, Japan; \(^3\)RIKEN Ctr. for Brain Sci. - Wako, Wako, Japan; \(^4\)Biol. Sci., The Univ. of Tokyo, Tokyo, Japan; \(^5\)Food and Life Sci., Sch. of Life and Envrn. Science, Azabu Univ., Sagamihara, Japan; \(^6\)Animal Sci. and Biotech., Sch. of Vet. Medicine, Azabu Univ., Sagamihara, Japan
Abstract: Motor neuron diseases are devastating conditions that selectively affect motor neurons (MNs) and innervated muscle fibers. Recent studies have identified various responsible genes and genetic mutations associated with the disease. However, detailed pathological mechanisms still remain elusive because of the intricate interplay between MNs and muscle fibers following MN denervation and compensatory re-innervation. VACHT-Cre is a Cre driver mouse line that expresses Cre recombinase selectively in FR-type (fast-twitch fatigue resistant) and S-type (slow-twitch fatigue resistant) MNs (Misawa et al., genesis, 54, 568-572, 2016). The FR-type and S-type MNs are known as slow-type MNs innervating slow (red) muscle fibers (type IIa and type I muscle fibers) responsible for long-lasting and low-intensity contractions. In order to selectively eliminate the slow-type MNs, VACHT-Cre mice were crossbred with NSE-DTA mice in which the cytotoxic diphtheria toxin A (DTA) subunit was expressed after Cre-mediated DNA recombination. The VACHT-Cre;NSE-DTA mice (designated as “delta SlowMN mice”) were born normal but progressively manifested various characteristics that included body weight loss, kinetic tremor, muscular atrophy and an increase in innervation ratio. These features were evident from around 20 weeks of age. Although the delta SlowMN mice showed a short life span (average life was about 40 weeks), they survived long enough to analyze middle- and late-phase consequences caused by type-selective MNs loss. Kinetic tremor was remarkable in the head and neck and occurred possibly due to peripheral mechanisms of unknown origin. Muscular atrophy was apparent in red muscles (such as the soleus and diaphragm) which are innervated by slow-type MNs. Alterations in composition of muscle fiber types were also evident in red/white mixed muscles. The increase of innervation rate was detected with EMG, probably reflecting compensatory re-innervation by remaining fast-type MNs. These features were obviously different from those of ALS-associated mutant SOD1 mice in which fast-type MNs are initially affected. The delta SlowMN mice could be a novel tool to study pathophysiological consequences of type-selective motor neuron degeneration and resulting muscle responses.


Poster

298. Mechanisms of Neurotoxicity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 298.01/G34

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH Grant R01 ES023864
 NIH Grant P01 AG055367
Title: Traffic related air pollution nanosized particulate matter shows batch differences in biological activity in vitro and in vivo

Authors: *H. ZHANG¹, A. HAGHANI¹, A. MOUSAVI², M. H. SOWLAT², M. Cacciottolo¹, C. AGOSTINO¹, C. SIOUTAS², T. E. MORGAN¹, C. E. FINCH¹, H. FORMAN¹;
¹Leonard Davis Sch. of Gerontology, ²Viterbi Sch. of Engin., USC, Los Angeles, CA

Abstract: Air pollution is associated with numerous health disorders including increased risk of Alzheimer disease (Cacciottolo et al 2017, PMID 28140404). Assessment of air pollution toxicity is complicated by inherent variations in composition of air pollutants among sources, locations, seasons, and weather, which may result in variable experimental responses. The current study documents divergent biological responses of nano-sized particulate matters (nPM) collected at different calendar times at the same site near a Los Angeles freeway. A new assay for genomic inflammatory responses was developed using human THP1 monocytes transgenic for a NF-κB reporter. A panel of nPM batches showed up to 10-fold variation in NF-κB activation. These batch variations showed corresponding differences in in-vivo responses of young adult C57BL/6J mice exposed to nPM for 8 weeks (exposure similar to Woodward et al 2017, PMID 28212893). The nPM batch with 2-fold higher NFκB activity in vitro (11.7±0.23 vs 5.6±0.77) caused a 2-fold increases in hippocampal Iba1 and soluble Aβ42 peptide in cerebral cortex. While the nPM with less NF-κB activity did not alter Iba1 or Aβ42 peptide levels, it still altered mRNA levels in cerebral cortex. To identify factors contributing to batch differences in biological activities, we compared their endotoxin content, lipid peroxidation, cellular toxicity, total organic compounds (TOC), and inorganic components. Pro-inflammatory activity of nPM batches correlated positively with endotoxin, lipid peroxidation, and TOC, but was negatively correlated with some metals. This in-vitro transcription-based assay for air pollution toxicity may increase the reproducibility of studies by identifying batch differences in air pollution particles. Further studies of fractioned nPM may identify synergies among the components of air pollution that impact neurotoxicity (Forman and Finch 2018, PMID 29407794). This study is funded by NIH grants R01 ES023864 (HJF) and P01 AG055367 (CEF).


Poster

298. Mechanisms of Neurotoxicity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 298.02/G35

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection
Support: ES023780
ES022845

Title: Sex specific blood and cerebral cortex transcriptomic changes of prenatal exposure of mouse neonates

Authors: *A. HAGHANI*\(^1\), J. I. FEINBERG\(^2\), K. LEWIS\(^3\), C. LADD-ACOSTA\(^2\), R. G. JOHNSON\(^1\), C. SIOUTAS\(^1\), C. E. FINCH\(^1\), D. B. CAMPBELL\(^4\), H. E. VOLK\(^2\), T. E. MORGAN\(^1\);  
\(^1\)USC, Los Angeles, CA; \(^2\)Johns Hopkins Bloomberg Sch. of Publ. Hlth., Baltimore, MD; \(^3\)Michigan State Univ., East Lansing, MI; \(^4\)Pediatrics and Human Develop., Michigan State Univ., Grand Rapids, MI

Abstract: Prenatal exposure to air pollutants is associated with higher risks of neurodevelopmental deficits and long-lasting cognitive and behavioral outcomes. Sex specific effects are apparent but little is known on the underlying mechanisms. Furthermore, biomarkers for prediction of future risk are needed. We approach these gaps by examining transcriptomic changes (RNAseq) of the blood and cerebral cortex of male and female mouse neonates that were prenatally exposed to traffic-related nano-sized particulate matter (nPM). In both sexes, cerebral cortex responded to nPM with altered expression of 540 genes, which included calcium and glutamate receptor signaling. Sex differences in nPM responses included 1,254 genes. WGCNA analysis revealed 3 modules of genes with directional divergent nPM response between male and females. Functional analysis by IPA showed nPM:Sex interactions for mitochondrial dysfunction, EIF2, mTOR, sirtuin, androgen, and estrogen receptor signaling. Psen1, mTOR, and NFE2L2 were among the top potential upstream regulators of nPM: Sex interactions. The blood transcriptome had much fewer responses to nPM (186 genes): coagulation and prothrombin pathways were among nPM responses that differed by sex. Several of these blood gene responses were highly correlated with cerebral cortex genes. Future validation of gene responses in human cord blood may provide biomarkers for air pollution-mediated neurotoxicity.


Poster

298. Mechanisms of Neurotoxicity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:/Poster #: 298.03/G36

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: P01 AG05536
Title: Cerebral cortex transcriptome responses to BPN-15606, a novel gamma secretase modulator

Authors: *C. D'AGOSTINO¹, A. HAGHANI¹, M. CACCIOTTOLO¹, C. SIOUTAS², R. E. TANZI³, T. E. MORGAN¹, S. WAGNER⁴, C. E. FINCH¹;
¹Leonard Davis Sch. of Gerontology, ²Dept. of Civil and Envrn. Engineering, Viterbi Sch. of Engin., USC, Los Angeles, CA; ³Massachusetts Gen Hosp, Harvard Med. Sch., Charlestown, MA; ⁴Dept. of Neurosciences, Univ. of California, La Jolla, San Diego, CA

Abstract: Gamma-secretase is a critical target for AD drug development because of its key role in amyloid-b42 (Aβ42) generation. We examined cerebral cortex transcriptome responses to BPN-15606, a recently developed GSM (Wagner 2017 PMID 28416568), in young male and female wild type mice (C57BL/6J, male and female). After treatment with 9 weeks of chow fed BPN-15606, RNAseq showed altered expression of 1031 genes, which included 576 (55%) sex-specific gene responses. Analysis of upstream regulators by IPA identified presenilin-1 (Psen1), a component of the γ-secretase complex, as the top upstream regulator in both sexes. Drug treatment altered expression of genes associated with oxidative phosphorylation and other mitochondrial functions, protein ubiquitination, and sirtuin signaling. The top sex-specific responses included immune responses. Sex differing genes were enriched for SAPK/JNK, ILK, PI3K/AKT and B cell receptor signaling. Mitogen protein kinases were among the top upstream regulators of this GSM:Sex interactions. These pathways are highly responsive to many AD risk factors including air pollution. Ongoing experiments examine the combined effects of air pollution and BPN-15606 on neurotoxicity.


Poster

298. Mechanisms of Neurotoxicity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 298.04/G37

Topic: C.07. Neurotoxicity/Inflammation/ and Neuroprotection

Support: R03DA045833
R03DA045350

Title: Characterizing methamphetamine (METH)-induced lethal toxicity in 5 days post-fertilization (dpf) zebrafish (Danio rerio) larvae
Abstract: Background: Methamphetamine (METH) is a psychoactive stimulant drug abused worldwide. METH neurotoxicity is well studied, but much less is known about the mechanisms underlying its lethal effects. METH neurotoxicity affects brain tissue monoamine levels, but the glutamatergic system is also involved and may also be involved in the acute lethal toxicity of METH. In response to efforts to circumvent legal restrictions on METH and other amphetamines, illicit use has turned to the β-ketone analogues of amphetamines. Even less is known about the potential lethal and toxic effects or mechanisms for these synthetic cathinones. To develop a high-throughput approach to the study of this diverse class of illicit drugs an approach has been developed using 5-day post-fertilization (dpf) Zebrafish (Danio Rerio) larvae. This initial report examines METH in this model. Methods: METH-induced lethal toxicity was examined in 5 dpf zebrafish larvae in a 96-well plate format. METH (500μM ~ 50mM) was administered by direct immersion of zebrafish larvae for 5 hours. The maximal non-lethal concentration (MNLC) and LC50 were determined. In mice, near-lethal doses of METH increase plasma levels of ammonia. We hypothesized that this may be an essential feature of METH-induced lethality. Ammonia excretion and internal ammonia concentrations were also measured. To examine the glutamatergic role in in METH-induced lethality, we co-administered drugs including MK-801 and GYKI 52466, as well as diazepam, SCH 23390, and raclopride with 50mM METH. Results: Exposure to non-lethal concentrations of METH caused a biphasic effect on heart rate (increase at low concentration, decrease at high concentration) and this effect was time-dependent. Exposure to the MNLC of METH increased ammonia excretion and exposure to the LC50 caused a decrease of internal ammonia, providing further evidence for a role of ammonia in METH-induced lethal toxicity. Furthermore, morphological changes were observed. METH caused sustained muscle contractions (distinct deflection of tail) in zebrafish larvae, suggesting that seizure may occur during the METH exposure (as observed in mice), further suggesting a role of glutamate in METH-induced lethality. In addition, co-administration of GYKI 52466 or raclopride significantly attenuated the METH-induced lethality in zebrafish larvae, suggesting not only glutamatergic systems but also dopaminergic systems are involved in this toxicity. Conclusions: METH caused lethal toxicity in 5 dpf zebrafish larvae and ammonia and glutamatergic systems are involved in this lethality. Continuing work will examine cathinones to determine if similar mechanisms are involved.

Disclosures: Y. Chen: None. A. Wisner: None. F. Williams: None. F.S. Hall: None.

Poster

298. Mechanisms of Neurotoxicity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program#/Poster #: 298.05/G38
**Topic:** C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

**Title:** Development and validation of a reproducible mouse model of intensive care unit delirium

**Authors:** M. ILLENDULA\(^1\), B. FERRARESE\(^2\), Z. ZUO\(^1\), *N. LUNARDI\(^1\);
\(^1\)Anesthesiol., Univ. of Virginia, Charlottesville, VA; \(^2\)Anesthesiol., Univ. of Padova, Padova, Italy

**Abstract:** Background: Intensive Care Unit (ICU) delirium is a form of acute brain failure characterized by fluctuating inattention, memory impairment and disorganized thinking. It affects up to 70% of elderly surgical ICU patients and is associated with increased length of hospital stay, morbidity and mortality, and steep healthcare costs. To date no treatment drugs are available, largely due to the lack of adequate animal models to study the underlying mechanisms. Thus, we set out to develop and validate a clinically relevant mouse model of delirium that faithfully recapitulates the peri-operative state by combining anesthesia, surgery, ICU sedation and sleep disruption, all of which are theorized to contribute to delirium pathogenesis. We hypothesized that Anesthesia/Surgery/ICU (A/S/I) would induce changes in mice behavior consistent with the clinical features of ICU delirium.

**Methods:** Eighteen month old, male mice (A/S/I: N=6, Control: N=6) were tested in the open field and Y maze 24 h before A/S/I. The following day, they were randomly allocated to A/S/I or control condition. A/S/I mice received 3 h sevoflurane anesthesia and laparotomy, then sedation with intraperitoneal propofol for additional 2 h. After recovering, they were subjected to 12 h of intermittent ICU sounds, bright light and cage rattling. Control mice were kept in a separate cage and did not receive A/S/I. Mice were tested in the open field and Y-maze at the end of ICU (0 h) and at 6, 12, 18 and 24 h. Differences between treatment groups were analyzed using the Mann-Whitney test. Data are expressed as percentage of baseline behavior and mean ± SEM.

**Results:** A/S/I decreased the number of entries in the Y maze novel arm at 0 h: 50.7% versus 124.3%, P=0.026, 6 h: 18.2% versus 85.0%, P=0.005 and 18 h: 20.33% versus 63.66%, P=0.015) compared to control. A/S/I did not significantly alter the number of entries in the novel arm at 12 h: 34.0% versus 51.17%, P=0.536 and 24 h: 15.66% versus 63.33, P=0.063. A/S/I did not affect the total distance traveled by mice in the open field test.

**Conclusions:** Laparotomy under sevoflurane anesthesia, followed by sedation with propofol and sleep disruption, impairs spatial memory in aged mice, a behavior that depends on the integrity of attention, memory and organized thinking. Such impairment is acute in onset, fluctuating, and independent of motor performance, and therefore consistent with the clinical hallmarks of ICU delirium.

**Disclosures:** M. Illendula: None. B. Ferrarese: None. Z. Zuo: None. N. Lunardi: None.
Poster

298. Mechanisms of Neurotoxicity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 298.06/G39

Topic: C.07. Neurotoxicity/Inflammation/ and Neuroprotection

Support: NIH Grant R01GM123746 (VJT/ST)
NIH Grant R01GM118197 (VJT)

Title: Neuroactive steroid CDNC24 is an effective gabaergic potentiator and a hypnotic that is not neurotoxic to the developing rat brain

Authors: *V. TESIC1, S. M. JOKSIMOVIC2, N. QUILLINAN3, K. KRISHNAN4, D. F. COVEY5, S. M. TODOROVIC6, V. JEVTOVIC-TODOROVIC7;
1Univ. of Colorado, Anschutz Med. Campus, Denver, CO; 2Anesthesiol., Univ. of Colorado, Anschutz Med. Campus, Aurora, CO; 3Anesthesiol., Univ. of Colorado, Aurora, CO; 4Washington Univ. in St. Louis, Saint Louis, MO; 5Dept Developmental Biol., Washington Univ. Sch. Med., Saint Louis, MO; 6Anesthesiol., Univ. of Colorado Anschutz Med. Campus, Aurora, CO; 7Anesthesiol., Univ. of Virginia Dept. of Anesthesiol., Charlottesville, CO

Abstract: Most currently used general anesthetics (GA) are potent positive modulators of postsynaptic \( \gamma \)-aminobutyric acid A (GABA\(_A\)) receptors and are invariably neurotoxic during early stages of brain development. Since causality between GABA\(_A\) potentiation and GA-induced developmental neurotoxicity has not been established, the question remains whether GABAergic activity is crucial for promoting/enhancing neurotoxicity. We have recently shown that a neuroactive steroid and T-type calcium channel blocker which lacks GABAergic activity, 3\(\beta\)-OH [(3\(\beta\),5\(\beta\),17\(\beta\))-3-hydroxyandrostane-17-carbonitrile], is an effective hypnotic with favorable safety profile when examined during critical stages of brain development. Using a structurally similar de novo synthesized neurosteroid analog that potentiates currents in recombinant GABA\(_A\) receptor, CDNC24 [(3\(\alpha\),5\(\alpha\)-3-hydroxy-13,24-cyclo-18,21-dinorchol-22-en-24-ol], we aimed to determine if GABA\(_A\) potentiation is the driving force in inducing neurotoxicity during development. Thus, we examined the neurotoxic potential of CDNC24 vis-a-vis clinically-used GABAergic intravenous anesthetic propofol at the peak of rat synaptogenesis. In addition to the morphological neurotoxicity studies of the subiculum and medial prefrontal cortex (mPFC), we assessed the synaptic physiology with the studies of pre- and postsynaptic effects of CDNC24 and propofol on inhibitory transmission in the developing subiculum. We investigated the hypnotic properties of CDNC24 in comparison with propofol by testing the loss of righting reflex (LORR) in post-natal day 7 Sprague-Dawley rats. We found that both CDNC24 and propofol caused dose-dependent hypnosis, with the resulting therapeutic index being surprisingly higher for CDNC24 when compared to propofol (>89.55 for CDNC24
and 23.1 for propofol). We have also shown that CDNC24, despite its GABAergic properties, does not cause developmental neurotoxicity when most vulnerable brain regions, the subiculum and mPFC, were examined. On the other hand, propofol induces significant developmental neurotoxicity compared to both its vehicle and the CDNC24. As expected, our studies of synaptic physiology showed that propofol exhibited mainly postsynaptic effects on sIPSCs (spontaneous post-synaptic currents) as evidenced by increasing sIPSC decay times. In contrast, CDNC24 had strong pre-synaptic effect in addition to a post-synaptic effect in an inhibitory synapse of the subiculum of rat pup, as evidenced by decreased frequency of sIPSCs. We propose that the lack of neurotoxicity of CDNC24 may be related to the suppression of GABA release in the subiculum during brain development.


Poster

298. Mechanisms of Neurotoxicity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 298.07/G40

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIGMS 5R01GM118197-13
NIGMS 5R01GM118197-13S
NIGMS 5R01GM123746-02
CU Medicine Endowment

Title: Properties of excitatory synaptic currents in CA3 neurons following neonatal ketamine exposure

Authors: *O. H. CABRERA, N. QUILLINAN, S. M. TODOROVIC, V. JEVTOVIC-TODOROVIC;
Anesthesiol., Univ. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: Objective
Developing neurons in the brains of infant rodents and non-human primates are susceptible to neuroapoptotic death by anesthetic drugs commonly used in human neonatal medicine. The apoptotic reaction is acute and widespread, which is thought to underlie chronic cognitive impairments observed in adult animals treated with anesthetics as neonates. Curiously, ketamine is not neurotoxic to developing hippocampus, yet causes severe hippocampus-dependent memory impairments in adulthood. Thus, acute neuroapoptosis may be sufficient, but not necessary, for long-term cognitive dysfunction, raising the possibility that anesthetics dysregulate cognitive function through non-neurotoxic mechanisms. Area CA3 of hippocampus
is resistant to several neonatal injuries, including anesthesia-induced neuroapoptosis, and a model region to study the intrinsic and synaptic properties of neurons surviving neonatal anesthesia-induced neuroapoptosis. To begin to dissect anesthesia effects on glutamate neurotransmission, we challenged neonatal mice with ketamine and recorded miniature excitatory postsynaptic currents (mEPSCs) seven days later.

**Methods**

Postnatal day (PND) 7 CD1 mouse pups of both sexes were randomly assigned to either 75 mg/kg ketamine or saline. 300 μm acute hippocampal slices were harvested following brief isoflurane anesthesia and transcardial perfusion with ice-cold artificial cerebrospinal fluid (ACSF) on PND14. Slices were incubated in ACSF at 37°C for 30 minutes then maintained at room temperature. CA3 mEPSCs were recorded in ACSF plus TTX and picrotoxin to block sodium and GABA currents, respectively, and using K-gluconate internal pipette solution. Neurons were voltage clamped at -70 mV, and frequency, amplitude, and decay of mEPSCs in CA3 neurons were quantified.

**Results**

CA3 neurons (n = 3) from PND14 mice treated with ketamine on PND7 had higher mean frequency of mEPSCs than neurons (n = 3) from saline-treated control mice. Thus, ketamine may alter presynaptic glutamate release or the number of excitatory synapses on CA3 neurons. Furthermore, we observed changes in postsynaptic parameters (mean amplitude and decay) suggestive of ketamine effects on recruitment of CA3 glutamate receptors or altered glutamate receptor subunit composition.

**Conclusions**

We report that brief ketamine exposure during infancy dysregulates pre- and postsynaptic parameters of glutamate neurotransmission in CA3 hippocampal neurons. Thus, anesthetics may compromise learning and memory in the absence of overt neuroapoptosis by interfering with the intrinsic and synaptic properties of neurons that subserve cognitive function.

**Disclosures:** O.H. Cabrera: None. N. Quillinan: None. S.M. Todorovic: None. V. Jevtovic-Todorovic: None.

**Poster**

**298. Mechanisms of Neurotoxicity I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 298.08/G41

**Topic:** C.07. Neurotoxicity/Inflammation/and Neuroprotection

**Title:** Isoflurane reduces brain-derived neurotrophic factor release with loss of dendritic spines

**Authors:** *J. PLATHOLI*¹, K. W. JOHNSON¹, R. WILLIAMS¹, F. S. LEE³, H. C. HEMMINGS, Jr.²;
Abstract: General anesthetics (GAs) modulate synaptic transmission by acting on multiple pre- and post-synaptic targets including neurotransmitter release, neurotransmitter receptors, and dendritic spine dynamics. Understanding how GAs destabilize dendritic spines is critical to identifying their reversible and durable effects on learning and memory. We hypothesized that isoflurane, a common GA, reduces release of brain-derived neurotrophic factor (BDNF), resulting in reduced dendritic spine size and number. We tested this in dissociated hippocampal neurons from wild-type and loss-of-function BDNF Val66Met knock-in mice that have reduced BDNF secretion. Isoflurane-induced changes in BDNF release dynamics were measured using the genetically encoded biosensor BDNF-pHluorin. Neurons with varying levels of BDNF secretion were used to determine the effect of reduced ambient BDNF levels on dendritic spine morphology and synaptic protein expression using time-lapse imaging and immunohistochemistry, respectively. Isoflurane attenuated BDNF release in a dose-dependent and reversible manner. This identifies a new molecular signaling pathway that might contribute to persistent changes in dendritic spine number and morphology following isoflurane exposure.


Poster

298. Mechanisms of Neurotoxicity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 298.09/G42

Topic: C.07. Neurotoxicity/Inflammation/ and Neuroprotection

Support: NIH Grant R42TR001270
CASIS Grant GA-2016-238

Title: Evaluating human nerve conduction velocity in vitro as a novel metric for assessing chemotherapeutics-induced peripheral neuropathy

Authors: *A. D. SHARMA1, L. MCCOY1, E. JACOBS1, M. TERRAL1, L. CURLEY1, M. J. MOORE1,2,  

Abstract: Preclinical models for assessment of toxicity and efficacy has consistently failed to deliver given that a high number (~90%) of clinical trials fail. Animal models do not translate to human success primarily because of the inherent difference in the biology of the different species, and a bridge which translates to human biology is highly desired. A great advancement
in this space has come in the form of physiologically relevant 3D biomimetic tissues using induced pluripotent stem cells (iPSCs)-derived neuronal cells which have greatly enhanced the predictive potential of preclinical assays. However, engineering 3D tissues relevant to the nervous system, especially peripheral nerves (PNs), is challenging because of the unique architecture and the necessity of bioelectrical conduction. Peripheral nerves are prone to injury because of their unprotected long axonal outgrowths throughout the body and a high surface area exposed to possible toxicants. In this study, we have engineered a human 3D nerve in vitro that supports axon growth analogous to PN anatomy and provides gold standard clinically relevant metrics such as nerve conduction velocity (NCV) and histological ultrastructure. Previously, these have only been obtainable using animal models. Taking advantage of commonly used 3D spheroid fabrication methods such as ultra-low attachment plates or hanging drops, we generated motor neuron-Schwann cells (coculture) spheroids or motor neuron only spheroids (monoculture) as controls. Both monoculture and coculture spheroids showed robust and dense axonal outgrowth of ~5mm which enabled the measurement of NCV, previously unrealized in an in vitro system. The population-level electrophysiological activity was seen in both culture types, with neuron-only spheroids producing a faster peak NCV of 0.18±0.04 m/s while co-culture spheroids had a peak NCV of 0.13±0.02 m/s. Schwann Cell migration, ensheathing, and myelination occurred in the co-culture spheroids as confirmed by S-100 immunostaining, and lamination seen in TEM micrographs. Initial experiments have used vincristine as a model chemotherapeutic drug, and we have successfully generated a dose-response curve (IC50 = ~5nM) demonstrating that chemotherapeutics drugs cause neuropathy in this in vitro model of human nerve. In summary, we have successfully engineered a 3D biomimetic model of human peripheral nerve in vitro which can provide clinically relevant metrics such as NCV and thus, can be used for modeling peripheral neuropathies and assessing chemotherapy-induced peripheral neurotoxicity.

**Disclosures:**  
**A.D. Sharma:** A. Employment/Salary (full or part-time); Full. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock options. **L. McCoy:** A. Employment/Salary (full or part-time); Full. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock options. **E. Jacobs:** A. Employment/Salary (full or part-time); Full. **M. Terral:** A. Employment/Salary (full or part-time); Full. **L. Curley:** A. Employment/Salary (full or part-time); Full. **M.J. Moore:** A. Employment/Salary (full or part-time); part-time. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock options, Patent holder.
Title: Activation of KCNQ channels prevents paclitaxel-induced peripheral neuropathy and associated neuropathic pain

Authors: J. LI, D. DANG, *Q. Y. YANG; NCBA, UTMB At Galveston, Galveston, TX

Abstract: Paclitaxel-induced peripheral neuropathy (PIPN) and associated neuropathic pain are the most common and serious adverse effects experienced by cancer patients receiving paclitaxel treatment. These effects adversely impact daily activities and consequently the quality of life, sometimes forcing the suspension of treatment and negatively influencing survival. Patients are usually at high risk of developing PIPN if paclitaxel induces acute pain, which strongly suggests that an acute increase in the excitability of nociceptors underlies the chronic alterations of PIPN. KCNQ/Kv7 channels are widely expressed in the primary sensory neurons to modulate their excitability. In the present study, we show that targeting KCNQ/Kv7 channels at an early stage is an effective strategy to attenuate the development of PIPN. We found that paclitaxel did not decrease the expression level of KCNQ/Kv7 channels in the primary sensory neurons as detected by qRT-PCR and Western blotting. However, retigabine, which is a specific KCNQ/Kv7 channel opener, significantly attenuated the development of PIPN, as shown by both morphologic and behavioral evidence. We also observed that retigabine had no obvious effect on the chemosensitivity of breast cancer cells to paclitaxel. While retigabine has been approved by the FDA as an anticonvulsant, our study suggests that this drug can be repurposed to attenuate the development of PIPN.

Disclosures: Q.Y. Yang: None. J. Li: None. D. Dang: None.
Poster

298. Mechanisms of Neurotoxicity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 298.11/G44

Topic: C.07. Neurotoxicity/Inflammation/ and Neuroprotection

Support: Consejo Nacional de Ciencia y Tecnología (CONACyT No. PN 2015-01-465 and INFR-280414)
Programa para el Desarrollo Profesional Docente PRODEP (213544)
CONACyT Fellowship Grants (No. 736339; No. 736004; No. 738774; No. 939505)
CONACyT INFR-281265

Title: Cyclohexane inhalation produces long lasting alterations in the hippocampal integrity in the adult mouse

Authors: *T. V. CAMPOS ORDONEZ¹, D. ZARATE-LOPEZ¹, N. E. IBARRA CASTAÑEDA², E. LIRA-DIAZ¹, J. GUZMAN-MUÑIZ², N. MOY-LÓPEZ², J. BURITICÁ³, O. GONZALEZ-PEREZ²;
¹Lab. of Neuroscience, Sch. of Psychology; Physiological Sci. PhD Program, School of Med., ²Lab. of Neuroscience, Sch. of Psychology, Univ. of Colima, Colima, Mexico; ³Ctr. de Estudios e Investigaciones en Comportamiento, Univ. De Guadalajara, Guadalajara, Mexico

Abstract: Cyclohexane (CHX) is an organic solvent commonly used as a drug-of-abuse. This drug increases the oxidative stress and glial reactivity in the hippocampus, which suggests that this brain region is vulnerable to CHX effects. This study aimed to establish the pathological alterations that occur in the Cornu Ammonis 3 (CA3) and Dentate Gyrus (DG) after a long-lasting exposure to CHX. We exposed CD1 mice to a recreational-like dose of CHX (~ 30,000 ppm) for 30 days and explored its consequences on the expression of c-Fos, NMDA receptor 1 (NMDAR1), neuropeptide Y (NPY), and apoptosis in the CA3 and DG subfields of the adult hippocampus. We analyzed the effects of CHX twenty-four hours after the last administration of CHX, we found a significant decrease in the number of c-Fos+ cells in the hippocampal CA3 and DG regions. This event coincided with an increased in NMDAR1 expression and apoptotic cells in the CA3 region. At day 13th without CHX, we found a persistent reduction in the number of c-Fos+ and TUNEL+ cells in DG. At both time points, the CHX-exposed mice showed a strong overexpression of NPY in the CA3 stratum lucidum and the hippocampal hilus. Altogether, this evidence reveals that CHX exposure provokes long lasting changes in the hippocampal subfield. These findings can help understand the deleterious effect of CHX into the adult hippocampus.

Poster

298. Mechanisms of Neurotoxicity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 298.12/H1

Topic: C.07. Neurotoxicity/Inflammation/Neuroprotection

Support: CONACyT Grant 241009

Title: Plastic changes in dendritic spines of 3xTgAD mice prefrontal cortex, after iAs exposure


1Inst. Mexicano del Seguro Social, Guadalajara, Mexico; 2Univ. Autónoma de San Luis Potosí, San Luis Potosí, Mexico; 3CIBO, IMSS, Guadalajara, Mexico; 4Neurobiol Desarrollo y Neurofisiologia, UNAM Campus Juriquilla, Queretaro, Mexico; 5Chem. Faculty, UASLP, San Luis Potosí, Mexico

Abstract: Worldwide, every year there is an increase in the number of people exposed to inorganic arsenic (iAs) via drinking water. Human populations present impaired cognitive function as a result of prenatal and childhood iAs exposure, while studies in animal models in similar conditions demonstrate neurobehavioral deficits accompanied by protein and enzyme alterations associated to Alzheimer’s disease (AD). In primary cultures of Tg2576 mouse brain, exposure to iAs promotes the release of Aβ and increases the activity of β-secretase, but morphological consequences of such biochemical changes are unknown. In order to determine whether iAs promotes plastic changes in spines of cortical neurons that could be related to the pathophysiological progression of AD, we used the 3xTgAD mouse model since it mimics the development of amyloid plaques, neurofibrillary tangles and behavioral dysfunction. Male 3xTgAD mice were divided into 2 groups: 1) control without arsenic; and 2) exposed to 3 ppm sodium arsenite (iAs-3xTgAD) in drinking water. Animals received the treatment from gestation until 2 or 4 months of postnatal age. We measured levels of Synaptophysin and PSD95, and we also quantified and characterized the number and morphology of dendritic spines in third-layer pyramidal neurons from the prefrontal cortex. PSD95 decreased and Synaptophysin increased in iAs-3xTgAD mice. iAs-3xTgAD also showed a decrease of spine density and of thin spines at 4 months, which was preceded by a transitory increase in the number of thin, stubby and wide spines at 2 months. These results strongly suggest compensatory plastic responses by cortical neurons to iAs exposure, which could be closely linked to neurodegeneration.
Poster

298. Mechanisms of Neurotoxicity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 298.13/H2

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Kynurenine pathway metabolites involved in lead toxicity

Authors: *D. RAMIREZ ORTEGA¹, P. OVALLE RODRIGUEZ¹, G. R. ROLDAN³, D. F. GONZALEZ ESQUIVEL¹, L. A. RAMOS¹, B. PINEDA OLVERA², V. PEREZ DE LA CRUZ¹; ¹Lab. de Neurobioquimica y Conducta, ²Lab. de Neuroinmunologia, Inst. Nacional De Neurologia y Neurocirugia "Manuel Velasco Suarez", Mexico City, Mexico; ³Lab. de Neurobiologia de la Conducta, Univ. Nacional Autonoma de Mexico, Ciudad de Mexico, Mexico

Abstract: Lead (Pb) is a neurotoxin. The immature brain is singularly sensitive to Pb neurotoxicity, compromising the cognitive and behavioral attributes which persist even later in adulthood. Several mechanisms have been implicated in the Pb cognitive impairment, including synapse function, redox homeostasis, calcium signaling, neurotransmitter synthesis and release, between others. In this context, kynurenine pathway (KP) could be altered since it is modulated by the redox environment. The KP metabolites have neuromodulatory and redox properties. Specifically, kynurenic acid (KYNA) is considered as a neuromodulator that have an important role in cognitive processes due it is an endogenous antagonist of NMDA and α7-nicotinic acetylcholine receptors. Another metabolite related with neurodegenerative process is 3-hydroxykynurenine (3-HK), which is a redox metabolite that at high concentration induces cell death. The aim of this work was to evaluate if the cognitive impairment induced by Pb intoxication was related with alterations in KP metabolites. Mice pups were exposed to Pb via their dams’ drinking water (lead acetate 500ppm) from PND1 to PND23. Control group received normal water. Then, from PND 21 both groups were given normal drinking water until 2 months of age. At PND 60 mice from both groups were subject to the locomotor activity test in open field and the long-term memory test through buried food probe. Different brain regions were obtained to quantify redox environment (reactive oxidative species (ROS), lipoperoxidation (LP) and cell viability), KYNA and 3-HK levels as well as kynurenine aminotransferase (KAT) and kynurenine monooxygenase (KMO) activities. Pb group showed a significant increase in the time looking for food, compared to controls (p<0.05) and showed a trend to hypoactivity. No changes were observed in ROS, and PL increased in hippocampus and cerebellum compared to...
controls. Cellular dysfunction was observed in all evaluated regions from Pb group vs. control. Both KYNA and 3-HK levels were increased (~2 to 4 times vs control) in all brain regions of Pb group. No change was observed in KAT activity. These results suggest that the increase in 3HK and KYNA levels could be another mechanism by which Pb induces cognitive impairment in adult mice.


Poster

298. Mechanisms of Neurotoxicity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 298.14/H3

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH Grant 2R25GM060507
NIH Grant P20MD006988

Title: Metabolomics uncovers key neurorestorative pathways after dietary omega-3 polyunsaturated fatty-acid supplementation in participants with type 2 diabetes

Authors: *A. DURAN¹, J. CÂMARA¹, L. SALTO¹, A. BASU¹, I. PEREZ-PAQUIEN¹, W. BEESON¹, A. FIREK², Z. CORDERO-MACINTYRE¹, M. DE LEÓN¹;

Abstract: Purpose: To determine the metabolomic impact of dietary omega-3 polyunsaturated fatty acid (PUFA) supplementation on neurorestorative pathways associated with antinociception in participants with type 2 diabetes. Methods: Forty volunteers with type 2 diabetes, with and without neuropathic pain symptoms, enrolled in the "En Balance-Plus" program. The program provided weekly nutrition-diabetes education and daily supplementation with 1,000 mg of docosahexaenoic acid and 200 mg of eicosapentaenoic acid over three months. The study assessed plasma samples from all participants at baseline and three months post-supplementation. Untargeted semiquantitative metabolomic analysis of plasma samples was measured by Metabolon. A bioinformatics approach was performed to elucidate if important neurorestorative metabolomic features and pathways were associated with supplementation. Results: A total of 659 compounds of known identity were classified using an untargeted metabolomics approach. Matched pairs t-test was used, p<0.05, to identify biochemicals that differed significantly between experimental groups (baseline and three months). An estimate of
the false discovery rate (q-value) was calculated to take into account the multiple comparisons in this study (a low q-value, q<0.10, is an indication of high confidence in the result). One hundred twenty-four compounds significantly changed from baseline; specifically, 74 increased while 49 biochemicals decreased in relative abundance. Random Forest classification of plasma samples collected at baseline and three months was 91% accurate in classifying samples. Further, taking several bioinformatic approaches, key features contributing to group separation were enriched with compounds of glycolipids, glucose, cysteine, methionine, and glutathione metabolism.

**Conclusion:** Global metabolic profiling was conducted to observe how dietary omega-3 PUFAs affected the metabolic phenotype of participants with type 2 diabetes (with and without neuropathic pain). High-level analysis of the data by Random Forest and p-value sorting pointed to changes in omega-3 PUFA metabolism, glycerolipid metabolism, cysteine, methionine, and glutathione metabolism, and fatty acid homeostasis.


**Poster**

**298. Mechanisms of Neurotoxicity I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program#/Poster #:** 298.15/H4

**Topic:** C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

**Support:** NIH P20-GM104932

**Title:** Effect of astrocytic insulin-like growth factor receptor deficiency on rodent behavior

**Authors:** *D. PRABHU, S. KHAN, K. BLACKBURN, E. HODGES, J. MARSHALL, N. M. ASHPOLE; Univ. of Mississippi, Oxford, MS

**Abstract:** The levels of Insulin-like Growth factor (IGF)-1 are altered in several neurological disorders, including stroke, epilepsy, traumatic brain injury, and neurodegenerative diseases. IGF-1 protects neurons and supports cognitive function. Neuron-specific IGFR KO animals show an impairment in spatial learning and memory. Supplementing rodents with IGF-1 is also shown to reverse some of the cognitive deficit associated with aging and neurodegeneration. A majority of studies have focused on the effects of IGF-1 regulating neurons, and not on astrocytes, despite the fact that it is the astrocytes that are the predominant cell type in the brain and highly express IGFR. This project aims to investigate the involvement of IGFR-R on the ability of astrocytes to regulate behavior. To investigate whether astrocytic IGFR is directly involved in influencing rodent behavior, we generated a mouse model of astrocytic IGFR
deficiency by crossing homozygous igfrf/f mice with iGFAP-Cre mice. To knock down IGFR after development, we induced Cre recombinase expression using tamoxifen. We then assessed the role of astrocytic IGFR in regulating behavior using a battery of behavioral assays which included Radial Arm Water Maze and Barnes Maze to examine spatial learning and memory, Elevated Plus Maze and tail suspension to examine anxiety and depression-like behavior respectively, open field and grip strength to assess overall locomotion and muscular strength respectively, and visual assessments. Our results indicate that astrocyte-specific IGFR knockout animals exhibit normal muscular strength, and locomotion, no anxiety or depression-like phenotypes. However, they do exhibit an impairment in learning and memory. Male KO animals show an increase in velocity and a decrease in path length during the acquisition phase of the Radial Arm Water Maze, compared to controls. In the Barnes Maze, female KO mice exhibit a higher path length and latency than the controls. To investigate the mechanism, we plan to explore the pathways responsible for glutamate handling and neurotransmitter cycling, as these are critical functions of astrocytes. Overall, this project will help uncover the role of IGFR in regulating the functions of astrocytes, and associated behavior.


Poster

298. Mechanisms of Neurotoxicity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 298.16/H5

Topic: C.07. Neurotoxicity/Inflammation/ and Neuroprotection

Support: This project was supported by a grant from the National Institute on Aging (R00AG047335)

Title: Inducing neuroinflammatory states in reactive glia associated with neurodegenerative disease progression

Authors: *Z. S. JORDAN1, K. THANGAMANI4, C. HUTCHINSON2, J. A. GOMEZ5, A. M. MAROOF3;
1Biol., 3Dept. of Biol., 2Univ. of Texas at San Antonio, San Antonio, TX; 4Univ. of Texas San Antonio, San Antonio, TX; 5The Univ. of Texas at San Antonio, San Antonio, TX

Abstract: Amyotrophic lateral sclerosis (ALS) is caused by the progressive degeneration of motor neurons (MNs) in the central nervous system (CNS), leading to paralysis and eventual death. The identification of familial ALS (fALS) gene mutations has led to the generation of transgenic mouse models which strikingly recreate the physiological and cellular phenotypes of human disease progression. Previous studies have co-cultured fALS mouse astrocytes with
human stem cell (SC)-derived MNs to create an in vitro model of MN toxicity, demonstrating that 1) toxicity of neurons is largely caused by soluble factors received and secreted by astrocytes, and that 2) astrocytic neurotoxicity is specific to motor neuron subtypes while other neuron subtypes are spared. In many studies, cytokines have been used to treat wildtype (WT) astrocytes and generate reactive astrocytes for modeling trauma and pathogenic disease states. Inflammatory modeling studies using bacterial endotoxins to stimulate immune cells in the brain have found that certain cytokines together are sufficient to cause astrocytes to become potently neurotoxic. However, this reactive astrocyte state is toxic to many subtypes of neurons. This broad neurotoxicity stands in contrast with the subtype-selective neurotoxicity observed in many neurodegenerative diseases, including ALS. Because reactive astrocytes have been identified at sites of MN degeneration in ALS, we therefore hypothesize that MN-specific neurotoxicity can be used as a functional readout for identifying the inflammatory factors which may induce MN degeneration from healthy astrocytes.


Poster

298. Mechanisms of Neurotoxicity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 298.17/H6

Topic: C.07. Neurotoxicity/Inflammation/and Neuroprotection

Support: Packard Center grant
          ALSA grant

Title: Nucleocytoplasmic transport factors suppress TDP-43 aggregation and toxicity in ALS/FTD disease models

Authors: *B. KHALIL1, G. FORTIN-NOUEL1, C. SMITH1, T. COMYN1, C.-C. CHOU2, W. ROSSOLL1;
          1Mayo Clin., Jacksonville, FL; 2Stanford Univ., Stanford, CA

Abstract: TDP-43 is a predominantly nuclear RNA-binding protein that undergoes abnormal cytoplasmic mislocalization, cleavage and aggregation in TDP-43 proteinopathies. In ALS, TDP-43 pathology is present in >97% of patients. Previously, we had used the proximity-dependent biotin identification (BioID) method to study the composition of pathological TDP-43 aggregates. This new approach has led to our discovery that C-terminal fragments of TDP-43 (TDP-CTF) abnormally associate with nucleoporins and transport proteins, which regulate the selective nucleocytoplasmic transport of macromolecules across the nuclear membrane (Chou et al., Nat Neurosci 2018). In this study, we also found that the importin karyopherin-β1 (KPNB1)
was able to reduce the formation of insoluble TDP-CTF inclusions. Here we present evidence that KPNB1 expression rescues TDP-CTF-induced cell death in neuronal cell culture. Disaggregation through KPNB1 predominantly occurs via its N-terminus, and flexibility of its α-helical HEAT repeats is needed for its function. Interaction with nucleoporins and nucleocytoplasmic transport regulator RAN is also required for KPNB1 to dissolve TDP-CTF aggregates. Interestingly, other importins and several peripheral nucleoporins also exhibit a potent effect on TDP-CTF inclusion formation, whereas exportins and scaffold nucleoporins mostly co-aggregate with TDP-CTF. Our data suggest that KPNB1-induced dis-assembly and/or prevention of TDP-43 aggregation is neuroprotective, suggesting new targets for future therapy development.

**Disclosures:** B. Khalil: None. G. Fortin-Nouel: None. C. Smith: None. T. Comyn: None. C. Chou: None. W. Rossoll: None.

**Poster**

**298. Mechanisms of Neurotoxicity I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 298.18/H7

**Topic:** C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

**Support:** CU Health Research Cluster Grant (CU-59-010-HR), Chulalongkorn University

**Title:** Effects of prenatal methamphetamine exposure on neuronal morphology and synaptogenesis in hippocampal primary cell culture

**Authors:** *H. BENYA-APHIKUL*¹, T. SOOKSAWATE¹, P. CHETPRAYOON², V. PONGRAKHANANON¹, R. RODSIRI¹;
¹Dept. of Pharmacol. and Physiol., Fac. of Pharmaceut. Sciences, Chulalongkorn Univ., Bangkok, Thailand; ²Nanosafety and Risk Assessment, Natl. Nanotechnology Ctr., Bangkok, Thailand

**Abstract: Introduction:** Methamphetamine (MA) is one of the most commonly health issue in women of childbearing age. Neurotoxicity of MA results from excitotoxicity and oxidative stress generated from the redundant dopamine auto-oxidation. MA abuse during pregnancy produces the deleterious consequences in prenatal MA-exposed children including cognitive impairment. Hippocampus is the most critical brain area accounting for learning and memory processes. Hippocampal formation can be influenced by substance used during prenatal developmental processes including neuron differentiation, neurogenesis and synaptogenesis. Therefore, adverse effect of MA on hippocampus might be responsible for learning and memory impairment.

**Objective:** This study aimed to determine the effect of prenatal MA exposure on neuronal morphology and synaptogenesis and its mechanisms including brain-derived neurotrophic factor
(BDNF)-Tropomyosin receptor kinase B (Trk-B) pathway. **Methods:** Pregnant mice (C57BL/6JNc, n = 4/group) were treated once daily with either MA (5 mg/kg, s.c.) or 0.9% saline solution on gestation day 8-15. Primary hippocampal culture was prepared from fetus at gestation day 16.5. The morphology of axon and dendrite branching was determined at 5 days in vitro (DIV5) by immunocytochemistry staining. Pre-synapse and post-synapse were stained using synapsin I and postsynaptic density protein 95 (PSD95) marker, respectively. The number of pre-synapse and the co-localization of pre- and post-synapse were quantified from DIV14 cells. Moreover, BDNF, Trk-B and cAMP response element binding (CREB) protein expressions were determined using western blot analysis. **Results:** The axon length and diameter were significantly decreased in MA-treated group (p<0.0001). However, MA exposure had no effect on the number of dendrite branching. The number of presynaptic sites and co-localization between pre- and post-synapse in MA group were also decreased significantly compared to control (p<0.0001). In addition, prenatal MA exposure reduced Trk-B expression (p<0.005), while BDNF level and CREB transcription factor were unaltered. **Conclusion:** Prenatal MA exposure impacts axon morphology and synapse formation in primary hippocampal cell. This malformation is related to Trk-B receptor down-regulation, which involved neuronal development and synaptic plasticity. These alterations might explain the learning and memory impairment in prenatal MA-exposed children. Further study will be focused on the molecular pathway of Trk-B receptor including PI3K-Akt and PLC-CaMK pathway which involved with axonal growth and Trk-B synthesis.

**Disclosures:** H. Benya-Aphikul: None. T. Sooksawate: None. P. Chetprayoon: None. V. Pongrakhananon: None. R. Rodsiri: None.
pollutants, such as polychlorinated biphenyls (PCBs) and the pesticide dieldrin are proposed risk factors for these neurological conditions. Recent evidence suggests these agents disrupt dopamine homeostasis in dopaminergic cells. While these organochlorine compounds have been phased out of use, they persist in the environment and bioaccumulate in humans. Altering dopamine homeostasis can yield elevated levels of the monoamine oxidase metabolite of dopamine, 3,4-dihydroxyphenylacetaldehyde (DOPAL), which is toxic and highly reactive towards proteins. Our goal here is determine if PCB-52 or its major human metabolite, 4-OH PCB-52, and dieldrin alter levels of dopamine and/or its metabolites including DOPAL, investigate oxidative stress induced by these agents, and finally to identify proteins modified by the reactive dopamine metabolite, DOPAL. Protective mechanisms against DOPAL include carnosine, a dipeptide aldehyde scavenger. Results show that 4-OH PCB 52 is toxic to dopaminergic PC12 and N27 cells at concentrations less than that of PCB-52 (less than 25µM) at 24hrs. Both PCB-52 and 4-OH PCB-52 were found to increase mitochondrial and whole cell reactive oxygen species in dopaminergic N27 cells.

**Disclosures:** B.S. Cagle: None. R.A. Crawford: None. H. Lehmler: None. J.A. Doorn: None.

**Poster**

298. Mechanisms of Neurotoxicity I

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 298.20/H9

**Topic:** C.07. Neurotoxicity/Inflammation/ and Neuroprotection

**Support:** R00AG047335

**Title:** Mouse mutant SOD1 glia cause hyperexcitability of induced cortical projection neurons

**Authors:** *J. A. GOMEZ*, Z. S. JORDAN, A. M. MAROOF;

*1Biol., 3Dept. of Biol., 2Univ. of Texas at San Antonio, San Antonio, TX*

**Abstract:** Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by progressive loss of both cortical “upper” motor neurons and spinal “lower” motor neurons. The vast majority of ALS research has focused on the degeneration of spinal motor neurons (sMNs). However, cortical projection neurons (CPNs) are also known to undergo selective degeneration, progressing before, or after the sMNs exhibit signs of apoptosis. Using induced pluripotent stem cells (iPSCs) we isolate CPNs expressing GFP regulated by the FEZF2 locus. We next paired CPNs expressing GFP with either mouse wild-type glia or mouse hSOD1 glia. Our results show CPNs co-cultured with hSOD1 glia have a lower survival rate after 10 days. Using multi electrode arrays (MEA), we also show that CPNs co-cultured with hSOD1 glia have a higher firing frequency when compared to CPNs co-cultured with wild-type glia.
Together this suggest that CPNs are also vulnerable to the neurodegenerative progression of ALS.

Disclosures:  
J.A. Gomez: None.  
Z.S. Jordan: None.  
A.M. Maroof: None.

Poster  
298. Mechanisms of Neurotoxicity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 298.21/H10

Topic:  
C.07. Neurotoxicity/Inflammation/ and Neuroprotection

Title: An adenosine augmentation compound (J4) attenuates abnormal tau phosphorylation in a cell model of tauopathy

Authors:  
*P.-Y. CHUANG¹, C.-P. CHANG¹, Y.-F. LIAO², Y. CHERN¹;  
¹Inst. of Biomed. Sci., ²Inst. of Cell. and Organismic Biol., Academia Sinica, Taipei, Taiwan

Abstract: Alzheimer’s disease (AD) is one of the most common progressive neurodegeneration diseases characterized by accumulation of amyloid plaques and tau aggregates, which leads to cerebral dystrophy and cognitive impairment. Adenosine is an important neuromodulator that regulates bioenergetic network in the brain, and the dysfunction of its homeostasis is often discovered in several neurological diseases such as AD and Huntington’s disease (HD). Results from this laboratory demonstrated that an inhibitor of a nucleoside transporter (ENT1), designated J4, is able to ameliorate several detrimental effects in an AD-like mouse model of tauopathy (THY-Tau22). Chronic-treatment with J4 normalized spatial memory deficiency, synaptic plasticity impairment, hyperphosphorylated Tau and abnormal kinase activities in the hippocampus of THY-Tau22 mice. However, the mechanism underlying the beneficial effect of J4 on tauopathy remain elusive. In the present study, our data showed that a SH-SY5Y neuroblastoma cell line (SH-SY5Y-Tau-P301L), which expresses human Tau (hTau) that carries a P301L mutation and fuses to a reporter (GFP), is an excellent cell model to study tauopathy. We first demonstrated that SH-SY5Y-Tau-P301L cells expressed ENT1 and adenosine receptor 2A (A2AR), two components of adenosine homeostasis. Consistent with what we have observed in the hippocampus of THY-Tau22 mice, expression of mutant hTau-GFP protein did not change the transcript levels of these two proteins. The induction of hTau-GFP protein in SH-SY5Y enhanced the levels of hyperphosphorylation and conformational change of hTau, which can be normalized by J4. These results demonstrated that SH-SY5Y-Tau-P301L is a suitable model to study the regulation of Tau hyperphosphorylation. The underlying mechanism for the J4-mediated adenosine augmentation in SH-SY5Y cells will be discussed.

Keywords: Alzheimer’s disease, tauopathy, adenosine, ENT1

Disclosures:  
P. Chuang: None.  
C. Chang: None.  
Y. Liao: None.  
Y. Chern: None.
Title: Pathological upregulation of adenosine A2A receptors exacerbates memory deficits in a mouse model of Alzheimer's disease

Authors: *D. BLUM\(^1\), V. GOMEZ-MURCIA\(^1\), K. CARVALHO\(^1\), C. MERIAUX\(^1\), R. CAILLIEREZ\(^1\), M. DUMOULIN\(^2\), S. BEGARD\(^1\), M. WISZTORSKI\(^3\), I. FOURNIER\(^3\), N. DEGLON\(^4\), A. BEMELMANS\(^5\), M. HAMDADE\(^1\), E. FAIVRE\(^1\), L. BUE\(^1\);\(^1\)Univ. Lille, Inserm, CHU Lille, UMR-S 1172 - JP Arc, LabEx DISTALZ, Lille, France; \(^2\)Univ. Lille, Lille, France; \(^3\)Univ. Lille, Inserm, U1192-PRISM, Lille, France; \(^4\)Dept. of Clin. Neurosciences, Lab. of Neurotherapies and Neuromodulation (LNTM), Lausanne Univ. Hosp., Lausanne, Switzerland; \(^5\)CNRS, CEA, Paris-Sud Univ., Univ. Paris-Saclay, Neurodegenerative Dis. Lab. (UMR9199), Fontenay-aux-roses, France

Abstract: Alzheimer’s Disease (AD) is characterized by memory loss, underlined by synaptic impairments promoted by both amyloid and Tau lesions. Various studies pointed out that chronic caffeine consumption reduces Alzheimer’s Disease (AD) risk and associated cognitive deficits. These protective effects are thought to be ascribed to the blockade of adenosine A2A receptors (A\(_2\)ARs), known to be crucial for synaptic tuning. Interestingly, A\(_2\)ARs are found abnormally upregulated in neurons of AD patient’s brains in correlation with the development of cognitive deficits, suggesting a link between neuronal A\(_2\)AR dysregulation and memory impairments. We recently demonstrated (SfN2018) that neuronal A\(_2\)AR upsurge potentiates Tau pathology-induced synaptic loss in a Tauopathy model, through the activation of the C1q complement. Here, we aimed at elucidating the pathophysiological impact of a chronic neuronal A\(_2\)AR upsurge in a transgenic mouse model of amyloidogenesis (APP/PS1dE9). To this aim, we crossed APP/PS1 mice with our newly developed transgenic TRE-A\(_2\)A strain, carrying the mouse A\(_2\)AR under the control of a Tet-responsive-element promoter. This led rise to four genotypic groups: WT, APP, WT/TRE-A\(_2\)A and APP/TRE-A\(_2\)A. At 3m of age, all the animals were bilaterally injected in the
hippocampus with an AVV2/5-CBA-ttA allowing the preferential overexpression of ttA transactivator in neurons, and then the neuronal A2A receptor upsurge in TRE animals. At 6m of age, a time APP mice do not display major deficits, behavioral evaluations revealed that A2A receptor overexpression strongly worsened spatial memory impairments of APP animals without significantly altering neither amyloid burden (plaques, Abeta levels) nor neuroinflammatory markers (IL1b, CD68, C1q, Trem2, Chemokines…). Importantly, using mass spectrometry-based high-throughput proteomics, we identified significant modifications in the molecular profile of APP/TRE-A2A vs. APP mice, with a notable downregulation of a large cluster of synaptic proteins. These data support that pathological upregulation of A2A receptors in the neurons of AD patients contribute to synaptic and cognitive impairments promoted by lesions, paving the way for a therapeutic use of A2A receptor antagonists.


Poster

299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 299.01/H12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA AMP-AD U01AG046170 and R01AG062355 (SRS and MEE) NIH R01DK117504 (SRS) BrightFocus A2018253F (MA) BrightFocus A2016508S (SRS) ADRC Grant P50 AG005138 (MA) Alzheimer's Drug Discovery Foundation (SRS) Cure Alzheimer’s Fund (SRS and MEE)

Title: VGF-derived peptide TLQP-21 modulates microglial function through C3aR1 signaling pathways and reduces neuropathology in 5xFAD mice

Authors: *M. AUDRAIN¹, F. EL GAAMOUCH¹, W.-J. LIN⁵, N. BECKMANN², C. JIANG³, S. HARIHARAN³, P. HEEGER⁴, E. E. SCHADT², S. E. GANDY¹, M. E. EHRLICH¹, S. R. SALTON³;
¹Neurol., ²Genet. and Genomic Sci., ³Neurosci., ⁴Med., Icahn Sch. of Med. at Mount Sinai, New York City, NY; ⁵Sun Yat-Sen Univ., Guangzhou city, Guangdong Province, China
Abstract: Multi-omic studies conducted by the NIH AMP-AD partnership led to the identification of VGF as a major driver of Alzheimer’s disease (AD) and reduced VGF expression has been significantly correlated with mean amyloid plaque density, Clinical Dementia Rating (CDR) and Braak scores. Moreover, the VGF-derived C-terminal peptide TLQP-21 (named by its four N-terminal amino acids and length) has emerged as a novel target of interest in pathological conditions such as obesity, amyotrophic lateral sclerosis, neuropathic pain, and AD. TLQP-21 activates the complement C3a receptor-1 (C3aR1), which in the central nervous system, is expressed on neurons, astrocytes, and microglia. Despite recent interest in the roles of the complement system and microglia in AD pathogenesis and progression, TLQP-21 function in this context remains poorly understood. Here, we show that TLQP-21 treatment increases motility and phagocytic capacity in the microglial cell line BV2 and in primary wild-type murine microglia, but not in C3aR1 knock-out primary microglia, which under basal conditions have impaired phagocytic function compared to wild-type. Using RNA sequencing of isolated primary microglia, we observed similar differentially expressed genes (DEGs) induced by treatment with TLQP-21 or C3a superagonist (C3aSA). No DEGs were observed in C3aR1 knock-out or wild-type microglia treated with the mutant peptide TLQP-R21A (which does not activate C3aR1). Most of these C3aSA and TLQP-21 DEGs were linked to cell migration and proliferation. Because proliferation of microglia around amyloid plaques is a major feature observed in AD brains and because VGF is decreased in the CSF of AD patients, intracerebroventricular (icv) TLQP-21 was chronically delivered to 5xFAD mice for 28 days via implanted osmotic pump, resulting in a significant reduction of amyloid plaques, associated dystrophic neurites, and microgliosis. Taken together, our results provide molecular and functional evidence suggesting that VGF and TLQP-21 may modulate microglia function and neuropathological progression in the 5xFAD mouse model of AD.


Poster

299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 299.02/H13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: POIAG14449

Title: Frontal cortex neuroinflammatory alterations during the progression of Alzheimer's disease
Authors: *M. MORENO-RODRIGUEZ, M. NADEEM, S. PEREZ, E. MUFSON; Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Chitinase 3-like proteins (CHI3L1 and CHI3L2) are markers of inflammation in several neurodegenerative diseases, including Alzheimer’s disease (AD). While studies have demonstrated that cerebrospinal fluid CHI3L1 protein levels are increased in pre-clinical and prodromal AD, no studies have examined changes in Chitinase like protein levels in the cortex during the early stages of AD. The present study evaluated levels of both CHI3L1 and CHI3L2 and neuroinflammatory-related markers (Iba1, C1q, GFAP, NPTX2 and CD44) in the frontal cortex (FC) of people who died with an antemortem clinical diagnosis of non-cognitive impairment (NCI, n=15), mild cognitive impairment (MCI, n=15), mild/moderate AD (mAD, n=12) and severe AD (sAD, n=11) using immunoblot and immunohistochemical techniques. CHI3L1-immunoreactive (ir) astrocyte number was significantly increased in FC and white matter (WM) in sAD compared to NCI. On the other hand, GFAP and Iba1-ir cell number was increased only in WM in MCI compared to NCI. Western blot analyses revealed significantly lower FC CHI3L2 levels, while CD44 was increased in sAD. No significant differences for CHI3L1, GFAP, C1q and NPTX2 protein levels were detected between clinical groups. Strong significant correlations were found between FC CHI3L1 and Iba1-ir cell number in WM and CHI3L1 and C1q protein levels in the early stages of AD. C1q and Iba1, CHI3L2 and CD44 and GFAP and CD44 protein levels were associated with disease progression. CHI3L1 and Iba1 cell number were significant associations with episodic memory and perceptual speed performance. These data suggest that CHI3L1 and CHI3L2 changes in the FC occur in later stages of AD. On the other hand, we found GFAP-ir astrocytes and Iba1-ir microglia are increased in FC WM in prodromal AD suggesting that these neuroinflammatory responses occur prior to CHI3L1 expression in astrocytes. The role of this pro-inflammatory markers requires further investigation.

Disclosures: M. Moreno-Rodriguez: None. M. Nadeem: None. S. Perez: None. E. Mufson: None.

Poster

299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 299.03/H14

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Role of MS4A4A M159V in myeloid cell response in Alzheimer's disease

Authors: *K. P. MACPHERSON, G. E. LANDRETH; Stark Neurosci. Res. Inst., Indiana Univ. Sch. of Med., Indianapolis, IN
Abstract: An accumulating body of work strongly suggests Alzheimer’s disease (AD) is the result of a failing complex biological network of protein clearance, neuronal health, and neuroinflammation. However, the specific mechanisms at play are still under investigation. GWAS studies identified risk loci in genes now known to be substantial contributors in the etiology and response to AD pathology in the CNS; however, some risk-associated genes, such as MS4A4A, remain under-investigated. Our analysis of public data sets shows MS4A4A gene expression is upregulated in AD prefrontal cortex and isolated microglia, CNS-resident myeloid cells. In addition, our preliminary data shows primary human blood-derived monocytes, have increased expression of MS4A4A as compared to total blood cell populations. Together this data suggests MS4A4A may play a role in myeloid cell function and specifically in microglial response to AD. A recent study suggests MS4A4A plays a role in directing the recycling of KIT to the plasma membrane and away from early endosomes in human mast cells, derived from myeloid cell lineage. However, the function of MS4A4A in relationship to AD pathology and the specific role in microglial cell function is unknown. In AD microglia are crucial regulators of plaque compaction and neuroinflammatory response, dependent upon the expression of TREM2 (triggering receptor expressed in myeloid cells 2). A recent report establishes a link between the SNP rs6591561, a missense variant within MS4A4A (p.M159V), with increased AD risk and decreased levels of soluble TREM2 in CSF. In addition, our preliminary data shows a trend for increased MS4A4A expression in human TREM2+ vs TREM2- blood-derived monocytes. In this study, we assess the role of MS4A4A and MS4A4A M159V in the function of human microglia and regulation of TREM2-dependent response to AD inflammatory stimuli. Our preliminary studies with THP1 macrophages show MS4A4A expression is significantly decreased by M1 differentiating stimuli, INFγ and LPS, and significantly increased, 3-4 fold, by M2 differentiating stimulus IL-4 but not TGFβ. Studies are ongoing to assess the specific role of MS4A4A and MS4A4A M159V in myeloid cell trafficking, phagocytosis, and the relationship to Trem2 production and processing.


Poster

299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 299.04/H15

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Pioneering Funding Award funded by Cure Alzheimer’s Fund (CAF; H.C)

Title: Neurodegenerative microglial activation exacerbated by astrocytes-driven oxidative stress and proinflammation in a human Alzheimer’s disease brain model
Abstract: Alzheimer’s disease (AD), the most common cause of dementia, leads to neuronal damage and deterioration of cognitive functions in aging brains. Recently, both astrocytes and microglia acquiring reactive properties are involved in the AD progression. However, there are challenges to discover molecular and intercellular mechanisms of astrogliosis and microgliosis in AD progression due to the lack of appropriate model systems that accurately reflect AD-related immunity in human brains. We previously presented effective human AD brain models by tric culturing human APP neurons, astrocytes, and microglia in a 3D microfluidic platform, which closely reconstructs key aspects of Aβ, p-tau signature, and neuroinflammatory. Here, the AD model was employed to clarify the molecular mechanisms of crosstalk between astrocytes and microglia contributing to neurotoxic inflammation in AD environments. We found the increased reactivity of astrocytes in response to Aβ producing excessive H₂O₂ (5.7 fold) and proinflammatory cytokines (IL-6, IFNγ, TNFα) compared to astrocytes in healthy models. The promoted neuronal damages in the co-cultured AD models were confirmed by increased phosphorylated tau (pTau) expression (2.2 fold) and LDH level (3.6 fold) compared to healthy models. The addition of either H₂O₂ scavenger or astrocyte-specific MAO-B inhibitor effectively prevented the tauopathy and neurodegeneration validating that the major neuro toxic factor was the oxidative stress from the reactive astrocytes. To investigate the involvement of microgliosis in the AD progression, we added microglia to the AD models and found the elevated neurotoxic effects by observing increased pTau expression (1.3 fold) and decreased cellular viability (1.3 fold) compared to healthy models. Further single culture study with microglia was performed to explore the potential underlying mechanisms of microgliosis, which may exacerbate the neurodegeneration in AD. We found that synergistic effects of H₂O₂ and IL-6, found in reactive astrocytes in AD models, on M1 microglial polarization (6.5 fold). In addition, the combined treatments of H₂O₂ and IFNγ triggered NO production (2.0 fold) from microglia. Currently, we are examining the intercellular pathways of astrocyte-driven microgliosis that promote neuronal damages in AD brains, which may offer a promising target for drug discovery in AD.

Support: NIH Grant 1R01AG057555-01A1

Title: Repurposing drugs for modulating microglia mediated neuroinflammation in Alzheimer's disease

Authors: *S. ALOE1, O. CHAUDRY1, L. XIE2, P. A. SERRANO3, P. ROCKWELL1, M. E. FIGUEIREDO-PEREIRA1;
1Dept. of Biol. Sci., 2Dept. of Computer Sci., 3Dept. of Psychology, Hunter Col. of the City Univ. of New York, New York, NY

Abstract: Alzheimer’s disease (AD) is a progressive neurodegenerative disorder and is the sixth leading cause of death in the United States. One of the hallmarks of AD is chronic neuroinflammation caused by overactive microglia, the resident immune cells of the CNS. The inability to reduce this inflammatory response leads to chronic neuroinflammation. AD drugs have a 99.6% failure rate and because of the difficulty and costliness of creating novel drugs, the aim of our study is to repurpose existing drugs for potential treatment of AD. A bioinformatics approach identified three drugs with potential targets in the neurodegenerative pathways: Diazoxide (DZ) a potassium channel activator, Ibudivlast, a phosphodiesterase inhibitor, and RG2833, an HDAC (histone deacetylase) inhibitor. Upon drug treatment, human microglia (HMC3) were analyzed for viability (MTT assay) and cytokine secretion (ELISA). DZ showed no significant cytotoxicity, while Ibudivlast was toxic only at the highest concentration tested and RG2833 showed a significant increase in microglia viability. Microglia were analyzed for secretion of two pro-inflammatory cytokines, IL-6 and IL-1β, and two anti-inflammatory cytokines, IL-13 and TGFβ. Ibudivlast treatment revealed a change in the microglia cytokine profile. At 10 µM, Ibudivlast increased the levels of IL-13, while at 100 µM, the highest concentration tested, Ibudivlast raised the levels of IL-6, a pro-inflammatory, neurotoxic cytokine. These data complement the MTT results. The predicted target of Ibudivlast is IRAK1, which is upstream of cytokine production. Down regulation of this kinase could be playing a role in the observed change in cytokine secretion. Based on these results and those of other studies, we are assessing the potential therapeutic effects of Ibudivlast and RG2833 in a transgenic rat model of AD. Examining whether these drugs modulate the release of neurotoxic and neuroprotective cytokines by microglia has potential for treating neuroinflammation in AD.


Poster

299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 299.06/H17

Topic: C.02. Alzheimer's Disease and Other Dementias
Title: The expression changes of lncRNA associated with miRNA-101a in 5XFAD

Authors: *Y. CHOI¹, Y. LEE², D. LEE¹²;  
¹Seoul Natl. Univ. Hosp., Seoul, Korea, Republic of; ²Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: The long non-coding RNAs (lncRNA) are non-protein coding transcripts longer than 200 nucleotides and have been reported to regulate gene and protein expression. In addition, lncRNA is involved in various diseases including Alzheimer’s disease. In order to understand changes in early-stage AD before cognitive impairment, we isolated RNAs from hippocampus and analyzed lncRNAs at 10- and 20-week-old 5XFAD initiating amyloid beta production and senile plaque deposition and differentially expressed 212 lncRNAs among 16,251 lncRNAs were selected between 10- and 20-week-old 5XFAD compared to wild type mice. We focused on lncRNA X associated with miRNA-101a which is involved in the regulation of APP. To investigate the relationship between lncRNA X and miRNA-101a, the expression level of lncRNA X was measured in mouse primary astrocyte and microglia treated with LPS. In results, the lncRNA X expression was significantly down-regulated under astrocytes and microglia activation and negatively correlated with miRNA-101a expression. We found a change of lncRNA X expression in inflammatory conditions and the relationship between lncRNA X and miRNA-101a. We need to confirm the level of APP expression and the function of lncRNA X in the inflammation. Understanding the expression and function of lncRNA X in inflammatory conditions can confirm the potential of lncRNA X as a diagnostic and therapeutic target for early-stage AD.

Disclosures: Y. Choi: None. Y. Lee: None. D. Lee: None.

Poster

299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 299.07/H18

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Improved Alzheimer's disease models using neuronal and microglial live-cell analysis in 2D and 3D

Authors: *S. L. ALCANTARA¹, J. RAUCH², G. LOVELL¹, L. OUPICKA², J. TRIGG¹, A. OVERLAND², M. BOWE², N. HOLTZ², E. ENDSLEY², C. SCHRAMM², D. APPLEDORN², D. TREZISE¹, T. DALE¹;  
¹BioA Applications, Sartorius (Essen Bioscience), Welwyn Garden City, United Kingdom; ²BioA Applications, Sartorius (Essen Bioscience), Ann Arbor, MI
Abstract: Development of more translational in vitro Alzheimer’s disease (AD) models will benefit understanding of neuro-pathology. To enable improved AD models, and to obtain a greater insight on the role of microglia in disease processes, we have developed techniques for long term measurements of cell health, morphology and function in 2D and 3D using live-cell analysis.

Healthy (hN6) and AD patient (hAD2) derived (Axol, Cambridge, UK) neuro-progenitor cells were seeded, differentiated, and matured for up to 90 days and monitored throughout using an IncuCyte® S3 for Neuroscience. After 14 d post differentiation in mono-culture, hAD2 iPSC-derived neurons yielded lower neurite outgrowth compared to the hN6 (neurite length of 80 ± 2.9 or 48 ± 2.6 mm/mm² for hN6 or hAD2, respectively, mean ± SEM; 48 replicates). In a 3D spheroid model, culturing in ultra-low-attachment (ULA) plates, hAD2s demonstrated enhanced spheroid growth (63 ± 7 or 119 ± 4 % change for hN6 or hAD2, respectively; 2-5 replicates).

The impact of AD related peptides (Tau and Aβ₁₋₄₂) on neuronal health and function was quantified using nuclear labeled SH-SY5Y cells or rat primary cortical neurons in mono- or co-cultured with rat primary astrocytes. Tau and Aβ₁₋₄₂ peptides induced a time- and concentration-dependent neuronal toxicity, with a reduction in neuronal number, neurite length and activity measured by calcium signalling.

Phagocytosis by microglia of pHrodo® labeled aggregated peptides was quantified as an increase in fluorescence following entry into acidic intracellular compartments. Rapid engulfment of aggregated Aβ₁₋₄₂ peptide was observed in mouse BV-2 cells (fluorescent area 18.1 ± 1.2 mm/mm² at 48 h, 3 replicates). Little or no engulfment was observed in undifferentiated SH-SY5Y cells or when the Aβ₁₋₄₂ was not aggregated. hiPSC-derived monocytes (Axol) were differentiated for 14 d into microglia. Addition of pHrodo® labelled aggregated Aβ₁₋₄₂ (3.7 - 100 µg/ml) yielded concentration-dependent phagocytosis (maximum fluorescent area: 2.88x10⁵ ± 0.02 µm² at 24 h for 33 µg/ml peptide, 2 replicates). To elucidate the involvement of scavenger receptors in peptide uptake, CD204 and CD36 antibodies known to block scavenger receptors A and B, respectively, were added with aggregated Aβ₁₋₄₂ or E.coli bio-particles®. Whilst the CD204 antibody inhibited the uptake of both reagents, CD36 selectively attenuated Aβ₁₋₄₂ peptide engulfment.

Taken together, these data show that long term monitoring, alongside combining multiple readouts from advanced cellular models, has the potential to deliver greater biological insight into neurological disorders, therefore contributing to drug discovery.

Poster

299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 299.08/H19

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Bioinformatics analysis of TILRR regulated gene activation patterns demonstrates primary effects on the PI3K-AKT-mTOR pathway and links with Alzheimer’s disease and inflammation

Authors: B. POYNTER¹, *P. R. HEATH², E. QWANSTROM³, J. COOPER-KNOCK²; ¹Neurosci. and Infection, Immunity & Cardiovasc. Dis., ²Neurosci., ³Infection, Immunity and Cardiovasc. Sci., Univ. of Sheffield, Sheffield, United Kingdom

Abstract: Toll-like and IL-1 receptors (TIR) are key regulators of immunity and inflammation and activated by ligand binding and co-receptor association. Aberrant control of the systems impacts progression of multiple diseases with an underlying inflammatory component, these include but are not limited to Alzheimer’s and other neurodegenerative diseases. Ligand-induced stimulation of the interleukin-1 receptor, IL1R1 is amplified by its co-receptor TILRR (toll-like and IL-1 receptor regulator), leading to marked changes in NF-KB and gene activity(1). Recent studies demonstrate that TILRR is highly expressed at sites of dysregulated NF-KB activity and inflammation and show a central role for TILRR in disease(2). In order to determine gene expression alterations caused by changes in TILRR expression; and to link these gene expression changes to described biological functions such as inflammation, microarray analysis was carried out. RNA samples from spleen and blood monocytes of TILRR/- and wild-type C57BL/6J mice were harvested, treated and applied to Affymetrix MOE430_2 arrays. Data was normalized and differential expression calculated using PUMA(3). WGCNA(4) was used to divide genes into modules with similar expression. Pearson correlation coefficients were calculated for the association between module expression and TILRR status. Significantly correlated modules (p<0.05) were functionally characterized using enrichR(5). Bioinformatics analysis, comparing microarray data from blood and spleen samples from wild type and TILRR knockout mice, identified alterations in expression of gene modules functionally associated with PI3-AKT signalling including the genes; BACE 1, ITCH, CCR2 and CALM 3. Results from the bioinformatics analysis were confirmed by qPCR, which demonstrated changes in the range of the identified genes in the TILRR KO relative to levels in wild type mice. 1. Zhang X, et al (2010) J. Biol. Chem. DOI:10.1074/jbc_M109.073429 2. Smith S.A., et al. (2017) JACC bts. DOI:10.1016/j.jacbts.2017.03.014 3. Pearson R, et al (2009) BMC Bioinformatics DOI:10.1186/1471-2105-10-211 4. Langfelder P et al (2008) BMC Bioinformatics DOI:10.1186/1471-2105-9-559 5. Chen EY et al (2013) BMC Bioinformatics DOI:10.1186/1471-2105-14-128
Disclosures:  B. Poynter: None. P.R. Heath: None. E. Qwanstrom: None. J. Cooper-Knock: None.

Poster

299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 299.09/H20

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01AG055059

Title: Role of pyruvate kinase M2 in microglia-mediated neuropathology in Alzheimer’s disease

Authors: *Y. LIU1, G. C. DOS SANTOS, Jr.2, J. C. RYU1, R. BRUSCHWEILER2, L. BRUSCHWEILER-LI2, S. O. YOON1;
1Dept. of Biol. Chem. and Pharmacol., 2Dept. of Chem. and Biochem., Ohio State Univ., Columbus, OH

Abstract: Growing evidence suggests that brain glucose hypometabolism is an early phenotype that is detectable long before cognitive decline is manifested, and continues in patients with Alzheimer’s disease (AD). Together with the finding of the brain insulin resistance in AD patients, it suggests that disruption of the brain metabolism may contribute to the development of AD pathology. In support, we have published that amyloid-beta (Aβ) peptides disrupt metabolic homeostasis in a mouse model of AD, 5XFAD. To investigate what types of metabolic changes occur in AD, we performed a longitudinal NMR-based metabolomics study using the serum, urine and brain samples from 1-, 3-, and 6-month-old 5XFAD mice. Our analyses demonstrated that serum lactate levels decreased significantly at 6-month-old 5XFAD mice compared to control mice. In tumors, lactate level is increased due to the Warburg effect that results from aberrant expression of pyruvate kinase M2 (PKM2). We asked whether the changes in serum lactate levels also involved dysregulation of PKM2 expression and function in AD. Indeed, we found that the levels of active PKM2 tetramers were significantly increased in cortical lysates from AD patients as well as 6-month-old 5XFAD mice compared to respective age-matched control samples. In addition, PKM2 was primarily expressed in CD68+ inflammatory microglia, and its expression increased after LPS treatment in vitro and in vivo, both in the control and 5XFAD mice. PKM2 activation appears to facilitate phagocytic activity of microglia, as pharmacological activation of PKM2 with 50 uM DASA-58 increased phagocytosis of oligomeric Aβ in rat primary microglia. On the other hand, inhibiting PKM2 with a pharmacological inhibitor, shikonin at 10 uM, blocked phagocytosis of both Aβ and control dextran beads. These results together suggest that PKM2 may be involved in microglial phagocytic activity, perhaps by altering metabolic state of microglia.

Poster

299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 299.10/H21

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 2017R1A2B4002861

Title: Lysine demethylase 4 inhibitors block TNF alpha-induced adhesion protein expression in human brain microvascular endothelial cells

Authors: *S. A. JO¹, J.-H. KO²;
¹Dankook Univ., Cheonan, Korea, Republic of; ²Cheonana, Chungnam Cheonan, Korea, Republic of

Abstract: Lysine demethylase (KDM) proteins are histone demethylases which remove methyl groups from lysine residue of histone. KDMs have been studied in the cancer research as epigenetic modulators, but not much reports regarding to brain diseases are found. Increase of adhesion proteins in vascular endothelial cells contributes to immune cell migration through the blood-brain barrier although their role in pathogenesis of brain diseases is not well understood. Our previous studies demonstrated that the TNFα-induced increase of intracellular adhesion protein1 (ICAM1) is regulated by histone modification at lysine 9 (H3K9me2 and H3K9me3) in human brain microvascular endothelial cells (HBMVECs), and KDM4 is responsible for demethylation of H3K9. Thus, in the present study, we examined the effect of two KDM4 inhibitors, a broad spectrum KDM4 inhibitor (ML-324) and a KDM4A and KDM4B-specific inhibitor on TNFα-induced adhesion protein expression. The results demonstrated that both inhibitors blocked more effectively an increase of vascular cell adhesion molecule 1 (VCAM-1) than ICAM1 induced by TNFα. In addition, these compound inhibited the TNFα-induced neutrophil adhesion and infiltration. Thus, KDM4 inhibitors could have the potential as a therapeutic drug for brain diseases. The present study was supported by a research grant of the National Research Foundation of Korea (NRF2017R1A2B4002861).

Disclosures: S.A. Jo: None. J. Ko: None.
**Poster**

**299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 299.11/H22

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Title:** Development of a systems biology platform to enable drug discovery for neuroinflammatory Alzheimer's disease

**Authors:** *L. H. MARTENS, R. NUNES, K. C. LARSON, M. MARCONI, C. D. DEJESUS, T. MILLER, J. M. LEVENSON, B. TATE; Tiaki Therapeut., Cambridge, MA

**Abstract:** Neuroinflammation is a pathological hallmark of multiple CNS degenerative diseases, including Alzheimer’s disease. Genetic and transcriptomic studies have identified microglial dysfunction as a key driver of the chronic neuroinflammation that is associated with neurodegenerative disease. Microglia, the resident CNS innate immune cells, also have non-innate immune cell functions during CNS development and in the adult brain, demonstrating that neuron-glial interactions are required for normal brain function and health. This has led to the hypothesis that modulation of adult microglial function may be a potential therapeutic strategy to protect the aging, diseased brain. One challenge in the field of neuroinflammation is authentic modeling of the complex CNS biology present in vivo, including critical neuron-glial interactions. Tiaki Therapeutics has developed a neuroinflammation systems biology platform based on an adult mouse ex vivo brain slice assay, which permits the longitudinal analysis of all CNS cell types within their authentic matrix and inter-cellular environment. A neuroinflammatory signature was established using transcriptomes from the whole slice, which encompasses all CNS cell types, and isolated microglia. Early, dynamic gene changes equilibrate and result in a stable, chronic neuroinflammatory state that can be monitored using transcriptomic and protein techniques. Bioinformatic approaches demonstrate a significant correlation between the neuroinflammatory gene expression signature observed in the brain slice assay to gene expression data from Alzheimer’s disease patients. Tiaki’s platform is uniquely positioned to provide longitudinal transcriptomic and protein signatures for CNS health and disease, as well as biomarkers for target engagement and compound efficacy. We have identified targets that, when manipulated, alters the neuroinflammatory signature and benefit neuronal health. Our systems biology platform enables the discovery and development of disease-modifying therapeutics against microglial targets for Alzheimer’s disease.

**Disclosures:** L.H. Martens: A. Employment/Salary (full or part-time); Tiaki Therapeutics. R. Nunes: A. Employment/Salary (full or part-time); Tiaki Therapeutics. K.C. Larson: A. Employment/Salary (full or part-time); Tiaki Therapeutics. M. Marconi: A.
Abstract: A prominent pathway within the innate immune system involves a three-protein complex known as the NLRP3 inflammasome. This complex is comprised of NLRP3, apoptosis-associated speck-like containing a CARD (ASC), and procaspase-1, an enzyme in its inactive form. Activation of the NLRP3 inflammasome results in the generation of active caspase-1 via autoproteolytic cleavage of the pro-form of the enzyme. Ultimately, this facilitates the production of mature interleukin-1β (IL-1β), an inflammatory cytokine. The inflammasome is highly regulated and its activation has been implicated in a variety of inflammatory disorders including Alzheimer’s disease (AD). One feature of AD pathology is the accumulation of amyloid beta peptides (Aβ) in the brain. This event causes a mobilization of microglial cells to the site and a localized inflammatory response. Data from our laboratory and others have shown that the NLRP3 inflammasome is activated and IL-1β is produced when microglia are exposed to, and internalize, Aβ. Yet, the mechanism of activation is not fully understood. Thus, we sought to investigate direct interactions between Aβ and the NLRP3 inflammasome proteins. Two Aβ-inflammasome interaction paradigms were utilized, 1) Aβ treatment of primary murine microglia and 2) solution interactions of Aβ with recombinantly-expressed NLRP3 inflammasome proteins. For both paradigms, co-immunoprecipitation strategies were employed. Full-length and truncated NLRP3, ASC, and procaspase-1 proteins were expressed in the ExpiCHO-S mammalian cell system. The proteins were purified with affinity and size-exclusion chromatography. Immunoblot analysis revealed both monomeric and oligomeric forms of each protein. In vitro experiments in which Aβ42 protofibrils were incubated with recombinant ASC suggested a direct interaction between the two proteins. Lysates prepared from primary microglia
exposed to Aβ42 protofibrils were immunoprecipitated with NLRP3 and ASC antibodies separately. SDS-PAGE separation and probes of the subsequent transfer membranes with multiple antibodies confirmed an NLRP3-ASC interaction and indicated a likely interaction with between Aβ42 protofibrils and ASC and potentially with NLRP3. In sum, these findings will provide new insights on the convergence of AD and this vital inflammatory pathway and may also provide information on potential targets for therapeutic intervention.

Key Words: Alzheimer’s disease, amyloid beta, inflammasome, caspase-1, cytokine


Poster

299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 299.13/H24

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Neuroprotective role of quinic acid and its derivatives in phytohaemagglutinin (PHA)-induced Aβ toxicity in SH-SY5Y cells

Authors: *K. RAFI¹, S. HUSSAIN², S. FAIZI², S. SIMJEE²;¹

Abstract: Alzheimer’s disease (AD) is a progressive neurodegenerative disorder, pathologically characterized by extracellular insoluble Aβ plaques and intracellular neurofibrillary tangles. Neuroinflammation and oxidative stress are involved in the onset and progression of AD. The production of inflammatory mediators, ROS and RNS causes synaptic dysfunction that is responsible for memory decline in AD. Therefore, the therapeutic options based on anti-inflammatory and anti-oxidant therapy are of important concern in targeting AD. The aim of this study was to develop a suitable in vitro model of Aβ deposition and to determine the role of novel neuroprotective agents for their therapeutic role thus targeting the disease at early stage or delay its onset. Our preliminary studies were conducted using PHA to observe plaques formation that releases cytokines upon stimulation. SH-SY5Y cells were incubated with PHA at the concentrations 5-40 µg/ml for 24 hours. After incubation, cells were observed using phase contrast microscopy. Oxidative stress was analyzed using 2',7'-dichlorodihydrofluorescein diacetate at concentrations of PHA 5-40 µg/ml by fluorimetric method. Immunocytochemistry was conducted to confirm the presence of Aβ plaques after stimulation with PHA. RT-qPCR was performed to analyze the expression of inflammatory markers (TNF-α, IL-1β, iNOS, P38-α and P38-β) and α and β-secretase genes involved in plaques formation. Morphological analysis of PHA stimulated cells showed that 5 µg/ml PHA did not cause any prominent changes in cellular morphology, while 10, 20 and 40 µg/ml concentrations caused visible aggregation when
compared with unstimulated cells. Oxidative stress analysis of PHA stimulated cells demonstrated increased ROS levels upon stimulation with significant increase at 10 µg/ml of PHA. Later, immunocytochemistry analysis at 10 µg/ml PHA concentration showed significantly increased expression of Aβ in stimulated cells. Quantitative gene expression analysis revealed that expression of inflammatory markers and β-secretase gene was increased in PHA-treated cells. Based on our findings, we suggest that PHA is involved in oxidative stress induced Aβ production. Further we have screened different compounds for their neuroprotective effect to use for the treatment of PHA induced AD symptoms and neurodegeneration. Preliminary, quinic acid and its derivatives were found to increase cell viability as compared to control cells. Next, we will use these compounds to check whether they are effective in reducing PHA induced Aβ generation and also, we will determine the molecular mechanism of action involved in the functioning of these compounds.

Disclosures: K. Rafi: None. S. Hussain: None. S. Faizi: None. S. Simjee: None.

Poster

299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 299.14/H25

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA 1R01AG059639
NIH/NINDS 1K22NS092688
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Indiana Alzheimer Disease Center pilot project
Research Supplement to Promote Diversity in Health-Related Research (PA-18-906)

Title: Early astrocytic alterations and loss of TREM2 are associated to cerebral amyloid angiopathy

Authors: *X. TAYLOR¹, P. CISTERNAS¹, Y. YOU², A. PERKINS³, Y. YOU⁴, S. XIANG⁵, J. ZHANG⁶, A. OBLAK⁶, R. VIDAL⁷, C. A. LASAGNA-REEVES⁸;
**Abstract: Background:** Cerebral Amyloid Angiopathy (CAA) is typified by the cerebrovascular deposition of β-amyloid and has a close molecular relationship with Alzheimer’s disease (AD), but remains clinically distinct. Vascular amyloid accumulation is identified in an estimated 85-95% of individuals with AD, positioning CAA as one of the strongest vascular contributors to age-related cognitive decline. Several genome-wide association studies (GWASs) have shown linkage of genes involved in the immune system and AD pathogenesis. Based on this, numerous efforts have been focused on understanding the contribution of the neuroinflammation associated with parenchymal amyloid-plaques to neurodegeneration in AD. Nevertheless, there is still no clear understanding of the mechanisms underlying the contribution of neuroinflammation associated to vascular amyloid deposition to neurodegeneration in CAA related dementias.

**Methods:** We used a model of CAA in the form of a transgenic mouse for Familial Danish Dementia (Tg-FDD) that accumulates AD amyloid in the vasculature, to study the contribution of neuroinflammation to neurodegeneration associated to CAA. We performed RNAseq, imaging, protein and Nanostring analysis to establish if the immune system could play a preponderant role in the pathogenesis of CAA related dementias.

**Results:** We demonstrate that our transgenic mouse model for CAA exhibit severe astrogliosis at early stages of vascular amyloid deposition in comparison to microgliosis and display a shift in astrocytic profiling to A1-like neurotoxic astrocytes. Analysis of neuroinflammatory genes by NanoString technologies also revealed a significant downregulation in genes associated to astrocytic function, lipid metabolism and immunity. Remarkably, our RNAseq analysis revealed immune risk-factors associated to AD, such as ABCA7, TREM2 and CX3CL1, dysregulated in the context of CAA. Importantly, biochemical analysis has revealed significantly decreased expression of TREM2, an important modulator of neuroinflammation and significant LOAD risk factor.

**Conclusion:** In addition to demonstrating the effect of cerebrovascular amyloid on lipid metabolism in the Tg-FDD model, our results suggest that astrogliosis and astrocytic loss of function is an early event in CAA and how known immune risk factors for AD could play an important role in CAA pathogenesis. Therefore, new therapies in CAA-related dementias should be focused on the influence of this system.

Title: Role of TLR signaling pathways in systemic inflammation and Alzheimer's disease

Authors: *J. YANG, B. DIDIER, L. WISE, K.-I. FUKUCHI;
Univ. of Illinois Col. of Med. at Peoria, Peoria, IL

Abstract: Obesity, diabetes, hypercholesterolemia, infection and aging are strong risk factors of Alzheimer’s disease (AD) and associated with chronic/systemic inflammation and activation of toll-like receptor (TLR) signaling. We and others demonstrated that activation of TLR signaling can modulate the initiation and progression of AD pathophysiology. Two distinct pathways, myeloid differentiation factor 88 (MyD88) and toll/interleukin-1 receptor domain-containing adaptor inducing interferon-β (TRIF), are involved in TLR signaling. We previously demonstrated that MyD88 deficiency (MyD88KO) decreases the brain Aβ load in an AD mouse model (TgAPP mice). However, the involvement of MyD88 and/or TRIF in the increased AD risk associated with chronic/systemic inflammatory diseases is unclear. In this study, ten-month-old MyD88KO and MyD88 wild-type TgAPP mice were subjected to weekly intraperitoneal injection of lipopolysaccharide (LPS 0.5 mg/kg) for three months to mimic chronic/systemic inflammation. PBS was similarly injected as a control. Consistent with our previous observations, less Aβ accumulation in the brains of MyD88KO TgAPP mice was found compared with MyD88 wild-type TgAPP mice. After LPS administration, plasma TNF-α and IL-6 levels significantly increased in MyD88 wild-type TgAPP mice, compared with MyD88KO TgAPP mice. LPS administration significantly increased IBA1-immunoreactive areas in the brains of MyD88 wild-type TgAPP mice, compared with PBS. However, there is no significant difference in IBA1-immunoreactive areas between LPS- and PBS-treated MyD88KO TgAPP mice. Similarly, LPS administration increased insoluble Aβ42 in the cortex as well as Aβ40 and Aβ42 in the CSF in LPS-treated MyD88 wild-type TgAPP mice, but not in LPS-treated MyD88KO TgAPP mice. Hippocampal RNA-Seq analysis revealed that 131 genes are altered (26 genes down-regulated and 105 genes upregulated) in LPS-treated MyD88 wild-type TgAPP mice and that 192 genes are altered (19 genes down-regulated and 173 genes upregulated) in LPS-treated MyD88KO TgAPP mice, compared with PBS-treated controls. Currently we are investigating the signaling pathways potentially involved in the AD progression in response to chronic/systemic inflammation by bioinformatics.

Funded in part by NIH AG053719, AG054937 and AG056862.

Key words: Alzheimer’s disease, inflammation, macrophage, microglia, MyD88

Microglial activation and cognitive decline in a new rat model of Alzheimer disease

Alzheimer disease (AD) is characterized by amyloid plaques, neurofibrillary tangles and generalized cortical neuronal loss. It is believed that cerebral amyloid produce the neurotoxic events in the dementia. However, the precise mechanism by which the early accumulation of amyloid-B (Aβ) peptides leads to cognitive decline remains unknown. For this reason, the development of animal models has been a priority to understand pathogenic mechanisms and therapeutic strategies. We have established a new model of Alzheimer’s disease in rat to evaluate cognitive decline and neuroinflammation. We injected amyloid-B (1-42) into the hippocampus to establish AD model rats. The male Sprague-Dawley rats at 3 months of age were divided into a control group (saline solution), an AD model group low-dose group (0.5 µg/µl Aβ) and an AD model group high-dose group (1 µg/µl Aβ). Fourteen days later the reference memory was evaluated by the Morris water maze to examine the cognition in this model of Alzheimer-induced cognitive impairment. Then, the rats were sacrificed and examined the plaque deposition and gliosis using histology and immunohistochemistry. Samples were immunostained for Iba-1 (microglia), glial fimbillary acidic protein (GFAP, astrocytes), visualized using diaminobenzidine tetrachloride (DAB) and sections counterstained with haematoxylin. Ratstrack plugin of ImageJ was used to process recorded videos of the Morris water maze. Alzheimer’s disease was successfully induced by injected of AB peptide in this new model. The microglial activation was reflected by increased levels of microglial markers Iba-1 and the cognitive impairment was evidenced in the AD model group low-dose group (0.5 µg/µl Aβ) and AD model group high-dose group (1 µg/µl Aβ)(p<0.05). Differences were found in reference memory and changes histopathologicals. These results show that the injection of beta amyloid 1-42 causes cognitive impairment or memory loss, which makes this new animal model very interesting allowing us to continue with research in the pharmacological field.

Poster

299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 299.17/H28

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA Grant AG059710
         NIH/NIA Grant AG056032
         Department of Veteran Affairs Merit Award BX001875

Title: Periodontal disease modifies the neuroinflammatory process in a mouse model of Alzheimer’s disease

Authors: *A. KANTARCI1, C. TOGNONI2,3, W. YAGHMOOR1, A. MARGHALANI1, D. STEPHENS1, J.-Y. AHN2,3, I. CARRERAS2,3,4, A. DEDEOGLU2,3,5;

Abstract: Plaque-associated microglia (PAMs) may both provide a protective barrier between Aβ and neurons and thus preventing neuronal dystrophy and play a significant role in Alzheimer’s disease (AD)-associated pathological changes. Chronic infections, such as periodontitis (PD), which increase the overall “set point” of systemic inflammation in individual patients, could modify the neuroinflammatory process. We tested the impact of PD on AD-like pathologies in 5xFAD mouse model and the role of PAMs. Fifteen male 5xFAD and 15 non-transgenic littermate 8-month old mice were used. Ligature-induced PD was established for 4 weeks. The bone levels and osteoclasts around the teeth were quantified macroscopically and histologically. Aβ40 and Aβ42 levels and inflammatory cytokines and chemokines were measured in prefrontal cortex. Double-label fluorescent analysis of thioflavin-S (ThS) and Iba1 was used to measure PAMs. 5xFAD mice at baseline had higher levels of bone loss than WT littermates; ligatures significantly increased bone loss with no significant differences between groups. Both Aβ40 and Aβ42 were increased in 5xFAD mice with PD; only the insoluble Aβ42 was significantly increased. The topographical distribution of Aβ42 immunostaining in cortex, hippocampus, and dentate gyrus (DG) showed Aβ42 plaques within L. 4/5 of the cortex, hippocampus, and DG regions of the 5xFAD mice with experimental PD. PD resulted in significantly increased levels of Iba1 immunostaining in WT mice. Microglia cells in 5xFAD mice showed a dense and robust Iba1 staining presenting enlarged cell body and reduced branching characteristic of the activated phenotype of microglia in AD brains. There was a
statistically significant decrease in Iba1 immunostaining in the DG region in 5xFAD mice with PD. In parallel, the % area occupied by Iba1 positive particles bigger than 400 µm² significantly decreased and there was a decreased percentage of Iba1+ staining within the immediate proximity of ThS positive plaques in 5xFAD mice with PD indicative of decreased PAMs. 5xFAD mice had significantly lower levels of IL-1β, KC, and IL-10 compared to the WT mice. The data collectively demonstrated that experimental PD leads to an increased neuroinflammatory process in brains of non-AD mice while resulting in an aberrant regulation of inflammation in 5xFAD mice. These results suggest that PD may prime microglia to a significantly activated state that may be detrimental to cognition and increase vulnerability to additional immune challenges or diseases and PAMs may be a crucial link between the systemic chronic inflammation induced by periodontitis and accelerated AD pathology.


**Poster**

**299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 299.18/H29

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Support:** Alzheimer Association Grant, NIRG

**Title:** Gut microbiome alterations and bacteria derived components in Alzheimer's disease

**Authors:** *A. CATTANEO*¹, S. PROVASI², N. LOPIZZO³, C. PARIANTE⁴;
¹IRCCS Fatebenefratelli, Brescia, Italy; ²Biol. Psychiatry Lab., IRCCS Ctr. Fatebenefratelli, Brescia, Italy; ³Biol. Psychiatry Unit, IRCCS Fatebenefratelli Ctr., Brescia, Italy; ⁴King's Col. London, London, United Kingdom

**Abstract:** Alzheimer Disease is a neurodegenerative disease which is characterized by the presence of beta-amyloid plaques and neurofibrillary tangles. Another important feature characterizing these patients is represented by enhanced inflammatory status both in the periphery, reflected by high levels of pro-inflammatory cytokines and also at central levels as indicated by enhanced activation of microglia. However, it is still not clear where this inflammation comes from. Recently, the gut microbiome and gut bacteria have been proposed to play a central role in the development of this inflammatory status as well as in the pathogenesis and progression of the illness. Here we wanted to identify a possible signature of the gut microbiome composition in association with AD pathology and to investigate the possible role of the gut microbiome in modulating inflammatory status of these patients. In particular we tested whether alterations in the abundance of specific gut microbiome taxa could be associated with an
enhanced peripheral proinflammatory status, tested both in term of peripheral pro-inflammatory cytokines as well as in term of bacteria derived components such as the Lipopolysaccharide (LPS) and Short Chain Fatty Acids (SCFAs). We investigated the composition of the gut microbiome by 16s sequencing in 20 controls and 50 AD patients and the blood levels of pro- and anti-inflammatory cytokines by using Meso Scale Discovery platform and of short chain fatty acids (SCFAs). AD patients showed higher levels of NLRP3, CXCL2, IL-6, IL-1b and LPS (all p<0.05) as compared to controls. We also found a specific microbiome profile in AD patients as compared to controls, where specific taxa at genus levels, including Blautia correlated negatively with the abundance of IL-1beta and NLRP3 and other taxa such as Escherichia/Shigella correlated positively with IL-1beta, NLRP3 and LPS. We also found a negative correlation between blood levels of LPS and butyrate and acetate (p<0.05). Our results suggest that the presence of a specific microbiome signature in AD patients may lead to the passage into circulation and then into the brain, of immune systems mediators as well as bacteria components, such as LPS, but also pro-inflammatory cytokines, contributing to the pathophysiology of AD.

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Poster

299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 299.19/H30

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 5T32GM008151-34

Title: The astrocyte circadian clock regulates amyloid beta deposition and degradation

Authors: *C. MCKEE1, B. V. LANANNA2, J. BASAK3, E. S. MUSIEK4;
1Washington Univ. In St Louis, St Louis, MO; 2Washington Univ. in St Louis, Saint Louis, MO; 3Washington Univ. in St Louis, St Louis, MO; 4Neurol., Washington Univ. In St. Louis, Saint Louis, MO

Abstract: An emerging link between circadian clock function and processes of neurodegeneration has indicated a critical role for the molecular clock in brain health. We have previously reported that disruption in core clock genes induces long-term activation of both microglia and astrocytes. Elevated activation of glia has important implications in chronic neurodegenerative conditions such as Alzheimer's disease (AD). Our goal is to investigate how the circadian clock functions in astrocytes to regulate inflammation, proteostasis, and neuronal support in the context of disease. To address these questions, we generated mice with astrocyte-specific knockout of the master circadian clock gene Bmal1, which renders astrocytes
transcriptionally arrhythmic. We report here that astrocyte-specific deletion of Bmal1 influences astrocyte activation state, lysosomal enzyme expression, and uptake and degradation of amyloid-beta peptide (Aβ). Bmal1-deficient astrocytes upregulate proteins and enzymes involved in Aβ metabolism and may degrade Aβ faster in vitro. In vivo, astrocyte-specific clock deficiency in the APP/PS1 transgenic mouse model of AD increases activation of astrocytes around Aβ plaques while also altering the accumulation of Aβ into plaques. We are now investigating underlying pathways linking the astrocytic clock to Aβ metabolism by glia in order to define how the molecular clock regulates pathogenesis in AD. This work will help elucidate mechanisms by which the clock in astrocytes influences long-term neuronal health and neurodegenerative disease.

Disclosures:  C. McKee: None. B.V. Lananna: None. J. Basak: None. E.S. Musiek: None.

Poster
299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 299.20/H31

Topic: C.02. Alzheimer's Disease and Other Dementias

Support:  NFSC Grant 31530089
         CAS Grant XDPB10

Title: TNFR2 overexpression in astrocytes attenuates Alzheimer-like pathology in Alzheimer's disease mouse models

Authors:  D. Li, *Z. Chen, Y. Shen;
         Univ. of Sci. and Technol. of China, Hefei, China

Abstract: TNFα signaling pathway plays essential roles in Alzheimer’s disease (AD) pathology. TNFα has two receptors, TNFR1 and TNFR2, which mediate different effects on cell fate. Our previous work has demonstrated that TNFR2 expression was reduced in human AD brains. Moreover, we have also found that TNFR2 deletion in APP23 mice accelerated plaque formation and microglial activation. Thus, it is a potential therapeutic strategy to reverse AD pathology by up-regulating TNFR2 levels. To test this hypothesis, we have generated astrocyte-specific TNFR2 transgenic mouse model and crossed it with APP transgenic mouse models to evaluate the pathological changes. Amyloid β plaque deposition was significantly decreased in cortex and hippocampus of astrocyte-specific TNFR2 overexpression AD mice than of control mice. Furthermore, reduced BACE1 activity and necroptosis were also found in these mice. Together, we conclude that TNFR2 up-regulation of astrocytes could ameliorate Alzheimer-like pathology.

Disclosures:  D. Li: None. Z. Chen: None. Y. Shen: None.
Title: Evaluation of inflammatory signaling in a novel GABA\textsubscript{B} receptor knockout mouse model

Authors: *A. M. LEISGANG\textsuperscript{1}, A. M. SALAZAR\textsuperscript{1}, A. A. ORTIZ\textsuperscript{2}, J. W. KINNEY\textsuperscript{1}; \textsuperscript{2}Psychology, \textsuperscript{1}Univ. of Nevada Las Vegas, Las Vegas, NV

Abstract: Alzheimer’s disease (AD) is a neurodegenerative disorder that is characterized by a learning and memory deficits. Pathologically, AD is characterized by the presence of three core features, beta-amyloid plaques (A\textbeta), neurofibrillary tangles (NFT), and chronic neuroinflammation. The activated immune response has been demonstrated to exacerbate both A\textbeta and NFT pathology. Additional data support a connection between A\textbeta and NFT formation that may be mediated by the inflammatory response, making investigations of inflammation a central target in AD. Activated microglia, the resident immune cells in the CNS mediate the immune response via the production of several pro-inflammatory cytokines. Mechanisms capable of modulating the immune response has considerable promise as potential interventions in AD. Microglia express a number of receptors, including the GABA\textsubscript{B} receptor. In the present study, we investigated if the loss of GABAergic signaling on microglia impacts the inflammatory response and AD related deficits. As alterations have been demonstrated in gamma amino butyric acid (GABA) in AD, a change in microglia function may contribute to neuroinflammation in AD. We have developed a novel GABA\textsubscript{B} knockout that is restricted to glial populations that were evaluated for alterations in inflammatory signaling following poly I:C, as well as AD related pathology. Our data indicate the loss of GABA\textsubscript{B} on microglia alter inflammatory signaling.

Poster

299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program#/Poster#: 299.22/H33

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Natural Science Foundation of China 81671248

Title: BACE1 expression and function in T cells from mice and human

Authors: *L. DAI, Y. SHEN;
Univ. of Sci. and Technol. of China, Hefei, China

Abstract: BACE1, an asparatic-acid protease, also known as beta-site APP cleaving enzyme1 (BACE1), is a therapeutic target for Alzheimer’s disease (AD) due to its neuronal activities of generating amyloid β peptide (Aβ), which is the major component of amyloid plaques in the brain. Interestingly, however, our recent studies accidentally discovered BACE1 abundant expression in T-cells when we were searching biomarkers from the blood of AD patients, suggesting unknown functions of BACE1 in the peripheral immune system. Why and how does BACE1 express in T cells? If we inhibit BACE1 in clinic for AD treatment, do BACE1 inhibitors have side effects that affect the immune system? These are very critical to uncover physiological properties of BACE1 which has been targeting in the brain and also are important to avoid any possible side effects in the immune system. Therefore, study of BACE1 in the T cells is an exciting and brand new area to explore. To investigate the function of BACE1 in T cells, we used the BACE1 knockout mice and also used BACE1 inhibitors to explore the effect of BACE1 knockout or inhibition on T cell function. We found that not only inhibition of BACE1 would inhibit T cell activation, but also we discovered that TCR signaling induces BACE1 expression and causes functional alterations. This observations suggest that BACE1 do play physiological roles in the immune system and might involved in immune diseases. Importantly, these findings will provide guidance for BACE1 inhibitor design to avoid any possible side effects.

Disclosures: L. Dai: None. Y. Shen: None.
Gene expression analysis of neuroinflammation markers in aged neurodegenerative disease transgenic mouse models

**Authors:** *B. A. JENKINS*¹, T. A. DAY³, D. C. AIREY¹, F. D. TINGLEY, III⁴, L. K. THOMPSON¹, M. L. HAYASHI²;

¹Eli Lilly & Co., Indianapolis, IN; ²Eli Lilly & Co., Windlesham, United Kingdom; ³Neurosci Discov Res., Eli Lilly & Co./Llc, Indianapolis, IN; ⁴Eli Lilly and Co., Indianapolis, IN

**Abstract:** Growing evidence implicates neuroinflammation as a significant contributor to neurodegenerative disorders, however, the understanding of the relationship between inflammation and disease progression remains unclear. We profiled the gene signature of several neurodegenerative disease mouse models to gain an improved understanding of the neuroinflammatory state within a given model, and to help guide preclinical pharmacodynamic assessment of therapeutic intervention.

Microglia, astrocyte, cytokine, and other pro-inflammatory markers were measured by microfluidic qPCR in the hippocampus, cortex and cerebellum of the transgenic mouse models: J20, APP NL-G-F knock-in, PDAPP, A53T and rTg4510 (n=3-6 each). Animals were assessed at ages where pathology had already been established. All models were compared to age-matched, wild-type mice of an appropriate genetic background. Samples were tested in duplicate for gene expression using Fluidigm DeltaGene assays (133 genes) using the Biomark HD system. A53T, PDAPP, and rTg4510 mouse cortical and hippocampal tissues showed robust gene expression increases for multiple neuroinflammatory markers profiled, relative to wild-type controls. This included markers of microglia proliferation (Aif1, Trem2, Itgam and Csf1r) and activation (Ctse, Cst7, Itgax and Clec7a). These models also displayed increased expression of genes for cytokine signaling, lysosomal activation and astrocyte signaling. In contrast, the J20 and APP NL-G-F knock-in models showed less prominent gene expression changes. Ccl subfamily cytokine markers were notably increased, while astrocyte expression was unchanged. Brain regional differences in neuroinflammation signatures were noted within and across the models, and cerebellar tissue showed no significant gene expression changes relative to wild-type controls.

In summary, all models assessed had unique neuroinflammatory signatures with differences in regional expression and fold-changes relative to wild-type controls. Cortical and hippocampal tissues showed an enhancement of cytokine signaling and a robust increase in microglia activation markers.
Disclosures:  **B.A. Jenkins:** A. Employment/Salary (full or part-time); Eli Lilly & Company. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly & Company.  **T.A. Day:** A. Employment/Salary (full or part-time); Eli Lilly & Company. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly & Company.  **D.C. Airey:** A. Employment/Salary (full or part-time); Eli Lilly & Company. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly & Company.  **F.D. Tingley:** A. Employment/Salary (full or part-time); Eli Lilly & Company. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly & Company.  **L.K. Thompson:** A. Employment/Salary (full or part-time); Eli Lilly & Company. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly & Company.  **M.L. Hayashi:** A. Employment/Salary (full or part-time); Eli Lilly & Company. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly & Company.

**Poster**

**299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 299.24/H35

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Support:** Funding from Charles and Helen Schwab Foundation (DMH)
Funding from the Tau Consortium (DMH)
Funding from the JPB Foundation (DMH)

**Title:** Impact of R47H variant of TREM2 on tau pathology and neurodegeneration in Alzheimer's disease

**Authors:** *M. GRATUZE*¹, C. E. LEYNS², R. HOYLE³, E. KIM³, M. MANIS³, M. COLONNA⁴, J. D. ULRICH⁵, D. M. HOLTZMAN⁵;
¹Washington Univ. in St Louis, St Louis, MO; ²Neurosci., Merck, Boston, MA; ³Washington Univ. Sch. of Med., St Louis, MO; ⁴Dept Neurol., ⁵Washington Univ., Saint Louis, MO; ⁶Neurol. Dept., Washington Univ. of St Louis, Saint Louis, MO

**Abstract:** Alzheimer’s disease (AD) is characterized by extracellular plaques composed of Aβ peptide and intraneuronal neurofibrillary tangles composed of tau protein. Beyond tau and Aβ, evidence suggests that neuroinflammation plays an important role in AD pathogenesis. Rare variants in the microglial-expressed Triggering receptor expressed on myeloid cells 2 (*TREM2*) gene increase AD risk 2-4 fold. Compared to the role of TREM2 in influencing Aβ pathology,
few studies have examined the function of TREM2 on tau pathology. Previously our lab showed that TREM2 deficiency attenuated neuroinflammation and neurodegeneration in the PS19 mouse model of tauopathy. Given the emerging role for microglia in tau pathology and tau-mediated neurodegeneration, it will be important to sort out the role of TREM2 in the context of pure tauopathy. To this end, we investigated the impact of the most prominent AD-associated TREM2 variant (R47H) on tau pathology and tau-mediated brain damage using BAC transgenic mouse models expressing human TREM2CV (common variant) or TREM2R47H variant on a TREM2 KO background crossed with the PS19 mice, a model of pure tauopathy. The hypothesis was that TREM2R47H results in a partial loss of function that leads to a lower microglial inflammatory response and protects against tau-dependent neurodegeneration. Using immunohistochemical analysis, we first confirmed that TREM2R47H-expressing PS19 mice have reduced inflammatory glial activation in the piriform cortex compared to TREM2CV mice. Surprisingly, no change was observed on tau pathology or tau-dependent neurodegeneration between PS19 mice expressing TREM2R47H or TREM2CV. These results suggest that the degree of glial inflammation that is suppressed by the TREM2R47H in the presence of tau pathology is not enough to attenuate neuronal loss and brain atrophy. Whether TREM2R47H affects other events relevant to tau-mediated pathogenesis will be important to sort out.

Disclosures: M. Gratuze: None. C.E. Leyns: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CEGL is listed as inventors on a patent licensed by Washington University to C2N Diagnostics on the therapeutic use of anti-tau antibodies. R. Hoyle: None. E. Kim: None. M. Manis: None. M. Colonna: None. J.D. Ulrich: None. D.M. Holtzman: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); DMH is listed as inventors on a patent licensed by Washington University to C2N Diagnostics on the therapeutic use of anti-tau antibodies. Other; DMH co-founded and is on the scientific advisory board of C2N Diagnostics, LLC. C2N Diagnostics, LLC has licensed certain anti-tau antibodies to AbbVie for therapeutic development., DMH is on the scientific advisory board of Proclara and Denali and consults for Genentech, Eli Lilly, and AbbVie..

Poster

299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Research Grants Council of Hong Kong (16149616, 16100418, and 16102717)
The Area of Excellence Scheme of the University Grants Committee (AoE/M604/16)
The S.H. Ho Foundation
Title: Small molecule rhynchophylline inhibits immune/inflammatory responses in a transgenic mouse model of Alzheimer's disease

Div. of Life Science, Mol. Neurosci. Center, and State Key Lab. of Mol. Neurosci., The Hong Kong Uni Sci and Tech, Hong Kong, China

Abstract: Alzheimer’s disease (AD) is a neurodegenerative disease characterized by cognitive dysfunction. One of its pathological hallmarks is the accumulation of amyloid-beta oligomers, which substantially contributes to the synaptic dysfunction and mediates the immune/inflammatory responses in the AD brain. We previously demonstrated a novel role of the signaling of EphA4, a receptor tyrosine kinase, in mediating amyloid-beta-mediated hippocampal synaptic dysfunction. Furthermore, we identified that a small molecule, rhynchophylline, can effectively block EphA4-dependent signaling and restore the synaptic deficits in the APP/PS1 transgenic mouse model of AD. Moreover, rhynchophylline ameliorates the amyloid pathology and microgliosis in the cerebral cortex in APP/PS1 mice. Here, to understand the mechanisms by which the reduction of EphA4 signaling ameliorates the pathological effects of AD progression, we performed microarray analysis to examine the transcriptome changes in the cerebral cortex in APP/PS1 mice treated with rhynchophylline. Gene ontology analysis and the top upregulated genes revealed that innate immunity and neuroinflammatory responses are activated in APP/PS1 mice. Accordingly, rhynchophylline ameliorated the upregulation of biological processes such as cytokine secretion as well as the expression of genes (e.g., spp1 and Igax) that are associated with immune/inflammatory responses in the cerebral cortex in APP/PS1 mice. Thus, our results collectively suggest that EphA4 signaling is a critical signaling pathway that mediates the immune responses in AD, making it a novel target for AD therapeutic development.


Poster

299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 299.26/H37

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: the BK21-plus education program provided by the National Research Foundation of Korea.
National Research Foundation of Korea (NRF) grant, funded by the Korean Government (800-20190161)
Title: Non-invasive *in vivo* imaging of inflammasome activation enables rapid and spatiotemporal detection of Alzheimer’s disease

Authors: *E. YANG*¹, Y. KO², J. LEE¹, N. JANG¹, Y. JEON¹, J.-W. YU³, N.-H. CHO¹, I.-C. KWON², H.-S. KIM¹;

Abstract: Alzheimer’s disease (AD) is the most common form of dementia among the elderly and the number of individuals with AD continues to increase. To develop effective therapeutics in AD, many scientists have been focusing on the development of novel drugs. However, the development of drugs has been known to fail. This failure could possibly be attributed to the administration of the treatment at a late stage in the disease progress. Thus, it is important to establish early diagnosis methods which can predict the risk of AD for effective prevention and treatment. Inflammasome plays a critical role in diverse inflammatory disorders, such as AD. Inflammasome can be activated by various pathogenic insults and induces secretion of IL-1β after cleavage by active caspase-1, an executing protease of the inflammasome complex. Therefore, direct imaging of active caspase-1 *in vivo* may provide enormous advantages for diagnosis, drug discovery, and therapeutic monitoring in AD. Here, we developed an activatable fluorescence probe, comprised of caspase-1-specific cleavable peptide bridging a near-infrared fluorescence dye and a quencher, for visualization of active caspase-1. This novel caspase-1 probe is biocompatible and can be efficiently delivered into cells and tissues, and specifically emit fluorescence upon caspase-1 activation as assessed *in vitro* and *in vivo* inflammatory conditions. We demonstrated efficient *in vivo* imaging of caspase-1 activation in AD. Significant fluorescence emitted from the inflamed sites, as well as their draining lymph nodes, can be detected by macroscopic imaging analysis within 30 min after systemic injection of the probe. This novel synthetic probe could be applied for efficient and rapid detection of caspase-1 activity in a spatiotemporal way by non-invasive imaging and revolutionize the current paradigm for diagnosis and therapeutics in AD.


Poster

299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 299.27/H38

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Neuronal SphK1 acetylates COX2 and contributes to pathogenesis in a model of Alzheimer’s disease
Authors: *H. JIN*\(^1\), J. LEE\(^2\), S. HAN\(^2\), K. PARK\(^2\), I. JUNG\(^2\), H.-J. KIM\(^3\), J.-S. BAE\(^2\);
\(^1\)Col. of Vet. Med., \(^2\)Dept. of Physiology, Sch. of Med., Kyungpook Natl. Univ., Daegu, Korea, Republic of; \(^3\)Dept. of Neurology, Col. of Med., Hanyang Univ., Seoul, Korea, Republic of

Abstract: Although many reports have revealed the importance of defective microglia-mediated amyloid β phagocytosis in Alzheimer’s disease (AD), the underlying mechanism remains to be explored. Here we demonstrate that neurons in the brains of patients with AD and AD mice show reduction of sphingosine kinase1 (SphK1), leading to defective microglial phagocytosis and dysfunction of inflammation resolution due to decreased secretion of specialized proresolving mediators (SPMs). Elevation of SphK1 increased SPMs secretion, especially 15-R-Lipoxin A4, by promoting acetylation of serine residue 565 (S565) of cyclooxygenase2 (COX2) using acetyl-CoA, resulting in improvement of AD-like pathology in APP/PS1 mice. In contrast, conditional SphK1 deficiency in neurons led to reduction of SPMs secretion and abnormal phagocytosis similar to AD. Overall, these results reveal a novel mechanism of SphK1 pathogenesis in AD that leads to defective microglial phagocytosis due to impaired SPMs secretion, and suggests that SphK1 in neurons has acetyl-CoA dependent cytoplasmic acetyltransferase activity towards COX2.


Poster

299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 299.28/H39

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ZEN-15-321311

Title: Interleukin 1 beta mediated inflammation in a tau depositing mouse model

Authors: *A. S. SOLIMAN*\(^1\), M. M. ALHADIDY\(^1\), D. J. FINNERAN\(^1\), K. R. NASH\(^3\), D. G. MORGAN\(^2\), M. N. GORDON\(^4\);

Abstract: Alzheimer disease is a neurodegenerative disease and the leading cause of dementia. Its pathological hallmarks comprise extracellular insoluble aggregates of amyloid β called plaques and intracellular insoluble aggregates of tau protein called tangles. Furthermore, neuroinflammation is an early event in the disease trajectory that may initiate or propagate
Interleukin-1β (IL-1β) is a primary proinflammatory cytokine that has been shown to be implicated in tau phosphorylation and synaptic loss. Hence, we hypothesize that IL-1β-mediated neuroinflammation induces tau pathology and cognitive impairment by stimulating tau phosphorylation and aggregation. Herein, we study IL-1β mediated inflammation in the rTg4510 mouse model and investigate the therapeutic benefit of interleukin 1 receptor antagonist (IL-1Ra).

Temporal changes in IL-1β-mediated inflammation were explored. We quantified mRNA levels of IL-1β and IL-1Ra in the posterior cortex of 2, 4, 6, 9, and 14 mo. rTg4510 by real-time PCR. In addition, western blot and ELISA analyses were performed for IL-1β, IL-1 receptor, IL-1Ra, and the downstream effectors as inducible nitric oxide synthase 2 (iNOS) and nuclear factor κβ p65, as well as kinases known to phosphorylate tau. One-way ANOVA followed by Tukey’s post hoc was used for statistical analyses. We observed low IL-1β mRNA levels at 2 and 4 mo. of age followed by a non-significant increase at 6 mo. In contrast, mRNA levels of IL-1β at 9 mo. showed a 9- and 7-fold increase compared to 2 and 4 mo. respectively (p<0.05). Surprisingly, IL-1β mRNA level at 14 mo. decreased by 30% compared to 9 mo. Western blot analysis of iNOS showed a similar time course. On the other hand, IL-1Ra protein showed a more linear pattern of increase, with a non-significant 2-fold increase at 9 mo. compared to 4 mo. with larger increases at 14 and 16 mo.

Second, IL-1β mediated inflammation was suppressed by overexpressing IL-1Ra. Transfection of cultured cells demonstrated expression and secretion of recombinant DNA constructs. Transfected IL-1Ra blocked IL-1β induced inflammation in vitro using a HEK-IL-1β blue reporter cell line. Effects of recombinant AAV9 overexpressing IL1-Ra on IL1-β-mediated inflammation and tau pathology will be examined after injection into rTg4510 hippocampus and anterior cerebral cortex. These experiments highlight the role of IL-1β-mediated inflammation in tau pathology and the therapeutic potential of IL-1Ra.


Poster

299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 299.29/H40

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIEHS R01 ES024331 AARF-16-440554

Title: Deletion of hematopoietic cell kinase exacerbates amyloid-beta deposits, pre-synaptic protein loss and microglial dysfunction in Tg2576 mice
**Authors:** S.-L. LIM¹, D. N. TRAN², C. CHEN², M. KITAZAWA¹;  
¹Univ. of California, Irvine, Irvine, CA; ²Univ. of California, Merced, Merced, CA

**Abstract:** Mounting evidence has shown that microglia plays a key role in modulating neuroinflammation and neuropathology, hence cognitive outcomes in Alzheimer’s disease (AD). Recent studies in animal models unveil not only neuroprotective role of microglia by effectively containing and clearing amyloid-beta (Aβ) plaques, but also its potentially detrimental role to synapses and neurons during the disease course. These differences are in part derived from its activation and biological status, cellular signaling and subsequent gene expression profiles. Hematopoietic cell kinase (Hck) is a member of the Src family tyrosine kinases which mediate immunoreceptor-induced phagocytic activation in microglia upon Aβ stimulation. We hypothesize that inactivation of Hck impairs microglial phagocytosis and Aβ clearance, leading to the accelerated buildup of toxic Aβ species and cognitive decline in the mouse model. We have previously shown that the activation of murine microglial/macrophage cells (BV-2) phagocytic activity by Aβ oligomers stimulation was mediated by the Src family tyrosine kinases pathway, and inactivation of the kinases significantly attenuated the phagocytic capacity of BV-2 cells. We have also demonstrated that genetic ablation of Hck in Tg2576 (Tg/Hck-KO) mice resulted in cognitive deficit when compared to age-matched Tg2576, Hck-KO, and wild-type mice as assessed by Morris water maze (n = 9-13). In this study, we quantitatively analyzed Aβ neuropathology in these mice by MSD, western blot and immunohistochemical staining. In correlation with the behavioral study, we found significant reduction of pre-synaptic synaptophysin intensity at the CA3 and overall hippocampal regions in the Tg/Hck-KO mice when compared to Tg2576 mice. However, the genetic ablation did not change post-synaptic PSD95 in these brain regions. While full-length APP and APP processing remained unchanged among all groups, soluble forms of Aβ38 and Aβ40 were substantially elevated and insoluble forms of Aβ38, Aβ40 and Aβ42 were augmented in the Tg/Hck-KO mice. In accordance, 4G8+ Aβ plaque burden was elevated in the Tg/Hck-KO mice with significant reduction of 4G8+ Aβ plaques internalized in the CD68+/Iba1+ microglial phagolysosomes. This denotes an impairment of microglial Aβ phagocytic activity owing to Hck deficiency in Tg2576 mice. In addition, significantly higher levels of iNOS and pSyk were expressed in CD11b+ microglial clusters in Tg/Hck-KO mice, signifying induction of inflammatory response upon Hck deletion. Our data suggest that Hck prevents AD neuropathology by promoting microglial phagocytic capacity and suppressing microglial pro-inflammation.

**Disclosures:** S. Lim: None. D.N. Tran: None. C. Chen: None. M. Kitazawa: None.

**Poster**

299. Alzheimer's Disease: Neurotoxicity, Inflammation, and Neuroprotection

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 299.30/H41
Abstract: Emerging evidence indicates that innate immune response is not only involved in the progression of Alzheimer’s disease (AD) but also in driving glia cells to clean aggregated Aβ peptides in the brain. The inflammatory response in brain is not only regulated by the local immune response but also the peripheral system. Gut-brain axis usually involves the intestinal microbiota, the immune system, the enteric nervous system (ENS), and the central nervous system (CNS), which displays a complex multidirectional crosstalk. However, whether regulating the intestinal microbiota or the intestinal immune response ameliorates the impairments of Alzheimer’s disease is remained unclear. Here, we utilized Drosophila AD model, the Aβ42 transgenic fly, and found that pan-neuronal expression of Aβ42 caused gene expression changes within the IMD signaling pathway, an innate immune pathway of Drosophila, in the brain and gut compared to the control fly. Downregulated the expression of AttacinA (AttA) either in neurons or gut rescued the Aβ-induced learning deficit. Collectively, overexpression of Aβ42 modulates innate immune response both in the brain and gut. Genetic manipulation of downstream AMPs expression may possess possibility to reverse Aβ-induced deficits.

Disclosures: T. Hsieh: None. H. Chiang: None.

Poster

300. Traumatic Brain Injury: Models, Mechanisms, and Recovery

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 300.01/H42

Title: Investigating the relationship between intestinal immune responses and amyloid-beta induced deficits

Authors: *T.-C. HSIEH¹, H.-C. CHIANG²;
¹Inst. of Basic Med. Sci., ²Dept. of Pharmacol., Natl. Cheng Kung Univ., Tainan, Taiwan

Title: Characterizing a scalable blunt-force traumatic brain injury model and the injury-induced regeneration in the adult zebrafish

Support: NSF GRFP DGE-1313583 JTH
The Center for Stem Cells and Regenerative Medicine
The Center for Zebrafish Research
**Authors:** *J. HENTIG, Jr*, M. LAHNE, K. CLOGHESSY, D. R. HYDE; 
Biol. Sciences, Ctr. for Stem Cells and Regenerative Med., Univ. of Notre Dame, Notre Dame, IN

**Abstract:** Traumatic Brain Injuries (TBI) affect 60 million people annually worldwide and contribute to other neurological disorders, such as cognitive decline and stroke. Our goal is to develop a blunt force TBI model that is inexpensive, rapid, and recapitulates the injury seen in humans, in a model organism that exhibits neuronal regeneration. Unlike traditional TBI rodent models, zebrafish potentially fit all these criteria. We modified the well-characterized blunt force TBI method, the Marmarou weight drop, for adult zebrafish. Our modified Marmarou weight drop resulted in a reproducible and scalable mild, moderate, and severe TBI that shares key pathophysiological features found in human TBI, with subdural/intracerebral hematomas, cerebral edema, seizures, visual disruption, and cognitive impairments. Cell death was examined using a TUNEL assay, where we observed a gradient of TUNEL-positive cells across the cerebellum and optic tectum that radiated outward from the impact site, with TBI severity proportional to cell death. To examine the extent of cell death, the TUNEL assay was paired with HuC/D and GFAP immunostaining to label neuronal and glial cells, respectively. We found significantly more neuronal HuC/D-positive cells were TUNEL-positive (p<0.0001, n=9) than GFAP-positive glial cells (n.s., n=9). Following severe injury, there was a significant decrease in optical acuity measured by optical kinetic response (OKR) over 3-5 days post-injury (dpi), which returned to near pre-injury conditions by 1-month post-injury (p<0.01, n=10). OKR was not significantly decreased in mildly injured fish (n.s., n=10). In response to injury, there was a significant increase in cell proliferation across the brain. At 60 hours post-injury, we observed increased cell proliferation, EdU labeling, at the posterior mesenphalic lamina (p<0.05, n=15), mitotic periventricular grey zone (PGZ, p<0.01, n=15), and the cerebellar crest, where nestin-positive cells colabelled with EdU (p<0.01, n=15 EdU, n=5 nestin/EdU). At 7dpi, all three regions possessed EdU/HuCD-colabeled cells in the PGZ and granular region of the cerebellum. Thus, we developed a TBI model that reproducibly mimics various key aspects of human TBI pathology that exhibited neuronal cell death followed by robust cell proliferation and neuronal regeneration. We will continue to examine these neuronal progenitors, their induction, migration, and differentiation through molecular, genetic, and bioinformatic approaches.

**Disclosures:** *J. Hentig: None. M. Lahne: None. K. Cloghessy: None. D.R. Hyde: None.*

**Poster**

**300. Traumatic Brain Injury: Models, Mechanisms, and Recovery**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 300.02/H43

**Topic:** C.10. Brain Injury and Trauma
Title: The olfactory bulb of zebrafish regenerates and recovers by 21 days after an excitotoxic focal lesion

Authors: *E. CALVO-OCHOA, C. A. BYRD-JACOBS; Biol. Sci., Western Michigan Univ., Kalamazoo, MI

Abstract: Unlike mammalian brains, which have a limited capacity to repair following damage, zebrafish effectively repairs brain lesions and constitutively generates new neurons throughout the lifespan, originating from sixteen neurogenic niches. Thus, zebrafish is an excellent model for studying brain repair, regeneration and neurogenesis mechanisms. The olfactory bulb, which contains neurogenic areas, presents extensive neuroplasticity mechanisms to adjust to swiftly changing environmental factors. It has been established that the olfactory bulb of zebrafish recovers after damage to the peripheral olfactory organs. However, the repair and recovery mechanisms of the olfactory bulb following direct injury have not been studied. In this work we establish a new paradigm of focal excitotoxic lesion in the olfactory bulb of zebrafish and investigate the recovery and remodeling of the lesioned olfactory bulb in time. We used adult zebrafish of both sexes and produced a unilateral focal excitotoxic lesion in the right olfactory bulb by injecting 1 µl of 15mM quinolinic acid (QA), while the unlesioned left bulb served as internal control. Next, we assessed several markers of bulbar damage, repair and morphological remodeling following 1, 7, 15, and 21 days post lesion (dpl). For this, we performed histological, immunohistochemical, stereological and morphological analyses of olfactory bulb sections. Our results show that a QA focal excitotoxic lesion greatly damages the olfactory bulb and causes the following: 1) reduction of olfactory bulb volume; 2) severe tissue damage; 3) neuronal death by apoptosis and necrosis; and 4) disruption of olfactory glomerular morphology. We also report that by 21 dpl, lesioned olfactory bulbs are repaired and restored to unlesioned levels: 5) olfactory bulb volume recovers; 6) lesioned tissue presents extensive morphological remodeling; 7) neuronal density is restored; and 8) glomerular structure is reestablished. These results are the first to report a time-course characterization of the regeneration and recovery of the lesioned olfactory bulb in zebrafish, increasing our current understanding of mechanisms of adult brain regeneration and repair following injury.

Disclosures: E. Calvo-Ochoa: None. C.A. Byrd-Jacobs: None.

Poster

300. Traumatic Brain Injury: Models, Mechanisms, and Recovery

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 300.03/H44
**Topic:** C.10. Brain Injury and Trauma

**Support:** Department of Army, U.S. Army Research Office WP911F-17-2-0222

**Title:** Ex-vivo modelling of traumatic brain injury using porcine brain tissue

**Authors:** *B. HOFFE*, A. MAZURKIEWICZ, T. PIEHLER, R. BANTON, O. PETEL, M. R. HOLAHAN;

1Dept Neurosci., 2Dept Mechanical and Aerospace Engin., Carleton Univ., Ottawa, ON, Canada; 3US Army Res. Lab., Aberdeen Proving Ground, MD

**Abstract:** Traumatic brain injury (TBI) is one of the more common forms of injury, affecting millions of individuals around the world each year. Many of these injuries occur in sports events or military operations. The movement of the brain and the resulting forces have been shown to create high amounts of strain within the brain that may differ between axonal tracts and cortical cell layers. Post-mortem analyses of human brain tissue have shown a pattern of pathological outcomes in midline structures and the apex of certain sulci as opposed to the regions adjacent to the gyri. Given that the cingulate sulcus runs adjacent to the corpus callosum, this region may be of particular vulnerability after a traumatic brain injury. Rodents may not be the most appropriate model for TBI-associated pathology as occurs in humans due to their lack of gyrification. In the current work, we explored the utility of a larger, more gyrified pig brain to better represent the dynamical forces the brain experiences during an impact and the biological outcomes. Pig heads were collected from an abattoir and brains were removed within 90 minutes. Whole brains were placed into cold ACSF for 1 hour. After this, 5 mm coronal slabs were collected and subjected to a drop impact from a height of 0.9m at 4m/s. Adjacent slabs from the same brain were used as controls and not subjected to the drop impact. Following impact, the impacted slabs were placed back into bubbling ACSF for 1 hour. One control and one impacted slab were placed into 4% paraformaldehyde and stored at 4°C. The other two slabs (one impacted and one control) were flash frozen using absolute ethanol and dry ice. Fixed tissue slabs were sectioned with a cryostat at 60μm and sections were immunohistochemically stained for microtubule associated protein 2 (MAP-2) and neuronal nuclear protein (NeuN). The MAP2 staining intensity in the cortex appeared lighter in the impacted brains than the controls. This was clearly visible in the sulcus region. The NeuN stain paralleled what was seen with the MAP2 stain. Images showed less NeuN staining in the impacted sections within the sulcus compared to the control sections. These findings shed light on the immediate neural changes that might occur within the sulcus of the brain during an impact using an ex vivo pig model of TBI.

**Disclosures:** B. Hoffe: None. A. Mazurkiewicz: None. T. Piehler: None. R. Banton: None. O. Petel: None. M.R. Holahan: None.
**Poster**

**300. Traumatic Brain Injury: Models, Mechanisms, and Recovery**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 300.04/H45

**Topic:** C.10. Brain Injury and Trauma

**Support:** UAB Department of Surgery

**Title:** Aberrant signal transduction in a novel model of rotational acceleration traumatic brain injury

**Authors:** *A. UMFFRESS¹, D. EPSTEIN¹, A. CHAKRABORTI², A. NATARAJAN³, K. HENSLEY⁴, J. A. BIBB²;
²Surgery, ¹Univ. of Alabama at Birmingham, Birmingham, AL; ³Univ. of Nebraska Med. Ctr., Lincoln, NE; ⁴Univ. of Toledo Med. Ctr., Toledo, OH

**Abstract:** Traumatic Brain injury (TBI) is a prevalent problem with 1.7 million TBI’s occurring annually in the United States. In these traumatic brain injuries (TBIs) the head undergoes rapid acceleration and deceleration, resulting in both linear and rotational forces that deform the brain and damage circuitry. While both linear and rotational forces can cause insult, the brain is more sensitive to rotational motion, which introduces shear and tensile forces resulting in diffuse axonal injuries, microvascular insults, or blood brain barrier compromise that develop into more severe or long-lasting effects with less recovery. It is critical that we understand the mechanisms of rotational acceleration-induced TBI. However, there are few reports of reliable or established methods to induce rotational brain injury in preclinical animal models. In order to understand this prevalent problem, we have developed a novel model of mild rotational acceleration TBI. We have characterized this injury model at acute and prolonged time points following repeated mild TBI showing reactive gliosis, calpain protease activation, and neurotoxic signal transduction. We have identified aberrant cyclin dependent kinase 5 (cdk5) activity as a principal perpetrator in cellular death in TBI following glutamatergic excitotoxicity. The Cdk5-activating cofactor, p35 is a prime target for calpain. Upon activation, calpain cleaves p35, producing the truncated cofactor p25. The resulting Cdk5/P25 holoenzyme engenders aberrant activity and phosphorylates substrates that mediate neurotoxicity. We screened a library of potential aberrant Cdk5 inhibitors and have selected compounds to test in vivo following TBI. This research is the first step in understanding how rotational TBIs are mediated by aberrant signal transduction, the mechanisms involved, and how treatments targeting this aberrant signaling may be used to improve outcomes for brain injury patients.

**Disclosures:** A. Umfress: None. D. Epstein: None. A. Chakraborti: None. A. Natarajan: None. K. Hensley: None. J.A. Bibb: None.
Poster

300. Traumatic Brain Injury: Models, Mechanisms, and Recovery

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 300.05/H46

Topic: C.10. Brain Injury and Trauma

Support: NIH/NINDS RO1 NS098740
The Miami Project to Cure Paralysis

Title: D-amino acid transporters regulate synaptic damage following traumatic brain injury

Authors: *S. A. TAPANES¹, E. J. PEREZ¹, D. T. BALU²,³, D. J. LIEBL¹;
¹Dept. of Neurolog. Surgery, Univ. of Miami Miller Sch. of Med., Miami, FL; ²Dept. of Psychiatry, Harvard Med. Sch., Boston, MA; ³Translational Psychiatry, McLean Hosp., Belmont, MA

Abstract: Traumatic brain injury (TBI) is a multi-faceted ailment resulting from an insult to the head that disrupts the homeostasis of the brain and leads to a variety of deficits and symptoms ranging in severity, from changes in personality to loss of memory or consciousness. Many of these deficits are a result of synaptic damage in addition to changes in neurotransmitter levels. Recent evidence points to D-serine, an endogenous co-agonist for N-methyl-D-aspartate (NMDA) receptors in the hippocampus, as a contributor to synaptic dysfunction and behavioral deficits within the first week after TBI in areas distal to the epicenter of injury that are spared of cell death. D-serine is formed from L-serine by the enzyme serine racemase (SRR), transported mainly by two amino acid transporters (Slc7a10 and Slc1a4), and degraded by the astrocyte-specific enzyme D-amino acid oxidase (DAAO). In naïve (uninjured) conditions, D-serine is mainly synthesized and released by neurons. Following TBI, expression of SRR switches from neurons to astrocytes and results in synaptic dysfunction. I hypothesize that this is the result of a buildup of astrocytic D-serine and increased tonic release from Slc1a4 transporters that contributes to synaptic dysfunction. To investigate this hypothesis, we will utilize 2-4 month C57Bl/6 male mice to first determine a temporal expression profile of D-serine, SRR, Slc1a4, Slc7a10, and DAAO in the hippocampus. mRNA expression of Slc1a4 is significantly increased within the first week following injury, whereas SRR remains constant and DAAO is significantly decreased before restoring to baseline by 7 days post injury (dpi; n=4/group in triplicate). Further, we are analyzing the effect of pharmacologically inhibiting Slc1a4 or Slc7a10 with the small molecule inhibitors L-4-Chlorophenylglycine (L-4-CPG) or BMS-466442 (BMS), respectively, via intraventricular infusion. Following small molecule infusion, we examine fear-conditioned learning and memory (n=14/group) and dendritic spine morphology (n=6/group) at 7 dpi, where we have observed an increase in spine protrusion density and the proportion of mushroom shaped protrusions in CA1 pyramidal neurons following L-4-CPG administration.
compared to vehicle in TBI mice. To alleviate off-target pharmacological effects, we have developed transgenic mice to genetically ablate Slc1a4 in a cell-specific manner using the Cre-LoxP system. Together our studies support a role for D-amino acid transporters in regulating astrocytic D-serine release and synaptic damage after TBI.


Poster

300. Traumatic Brain Injury: Models, Mechanisms, and Recovery

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: 300.06/11

Topic: C.10. Brain Injury and Trauma

Support: NIH R01NS100793
ABRC ADHS14-000003606
VRP P1 2256030
PCH Mission support

Title: Sexual dimorphic dysregulation in the hypothalamic-pituitary-adrenal axis after brain injury

Authors: S. W. RIDGWAY1, Z. E. SWANN2, C. E. HAIR1, R. ROWE1, *T. C. THOMAS1; 1Child Hlth., Univ. of Arizona Col. of Med. Phoenix, Phoenix, AZ; 2Neurosci., Oberlin Col., Oberlin, OH

Abstract: Upwards of 50% of all traumatic brain injury (TBI) results in an endocrinopathy and persisting post-concussive syndrome. Despite these data, the influence of sexual dimorphisms on dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis following TBI have not been studied. The HPA axis regulates circulating glucocorticoid levels, in particular cortisol (corticosterone in rats; CORT), and is mediated by negative feedback through glucocorticoid (GR) receptors. We hypothesized that experimental diffuse TBI incites dysregulation of the HPA-axis, influencing circulating CORT as well as hypothalamic GRs and corticotrophin releasing hormone (CRH) gene expression. We predict that this dysregulation influences neuroinflammatory processes in regions of the brain that are critical for HPA axis regulation and do not demonstrate TBI-induced neuropathology. To test this hypothesis, hypothalamic CRH and GR gene expression, hypothalamic microglial morphology, and plasma CORT levels were evaluated at 7 days following diffuse axonal injury induced by midline fluid percussion injury in young, adult, male and female Sprague-Dawley rats (n=3-8 rats/group/sex). Scientists carrying out experimental assays were blinded to injury status. Extensive measures were implemented to control for stress and diurnal CORT regulation. GR gene expression in the hypothalamus was significantly decreased in injured compared to sham rats, where injury, not sex, accounted for...
26% of the variability, respectively (p<0.05). These data indicate that males and females both demonstrate some evidence of decreased levels of GR at 7 days post-injury. Decreased levels of GR coincided with lower circulating CORT levels in male rats. Preliminary skeleton analysis of microglial morphology in the paraventricular nucleus of the hypothalamus demonstrate morphological changes indicative of a neuroinflammatory response in male rats, further analysis is ongoing. CRH, GR, and microglial studies are exploratory, however, CORT levels in male rats confirm previously published results. While clinical trials indicate that cycling females are at risk for more post-concussive symptoms in comparison to men, prepubescent girls, and post-menopausal women, the mechanisms mediating these differences are unknown. This injury paradigm provides a potential model by which to study the interaction of axonal injury and sex hormones on HPA regulation and neuroinflammation.

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Poster

300. Traumatic Brain Injury: Models, Mechanisms, and Recovery

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 300.07/12

Topic: C.10. Brain Injury and Trauma

Support: Office of Naval Research Global-US Department of Defense

Title: Acute and subacute axonal, neuronal and behavioral changes following repetitive mild traumatic brain injury

Authors: H. ISMAIL1, L. NASRALLAH2, F. KOBEISSY3, *H. J. DARWISH4;

1Anatomy, Cell Biol. and Physiol. Sci., 2Hariri Sch. of Nursing, 3Biochem. Dept., 4Anatomy, Cell Biol. and Physiol. Sci. and Hariri Sch. of Nursing, American Univ. of Beirut, Beirut, Lebanon

Abstract: Introduction: Mild Traumatic Brain Injury (mTBI), represents 70% of all TBIs worldwide. Repetitive mTBIs; such as in football players and boxers, makes the person at higher risk of developing neuro-degenerative diseases in the future. Axonal injury and neuronal damage are assumed to contribute to cognitive deficits following r-mTBI. Phosphorylated Neurofilament Heavy (pNFH) is elevated post-TBI and linked to Alzheimer’s disease (AD). We hypothesize that in a mouse model of repetitive mild traumatic brain injury (r-mTBI), pNFH will be elevated and axonal and neuronal damage will be correlated with impaired learning and memory. Aims: 1. To measure the extent of axonal injury, by quantifying the amount of pNFH in the serum, following r-mTBI 2. To evaluate acute and sub-acute novel object and location recognition memory and spatial learning and memory, following r-mTBI 3. To assess for acute and subacute
neurodegeneration and astrogliosis post-r-mTBI

**Methods:** C57BL/6 mice (6-8 weeks) were randomly assigned into two groups: A sham group (n=23) underwent anesthesia and incision 3 times, once every 24 hours. And an experimental r-mTBI group (n=24) underwent 3 closed head injuries, using the closed controlled cortical impact model, once every 24 hours. All the animals were tested at 48 hours post-injury and half of them at 7 days post-injury. To assess learning and memory, novel object and location recognition memory test was conducted at 48 hours and 7 days. To explore their spatial learning and memory, Morris water maze test from day 3 to day 7 was performed. ELISA was used to quantify pNFH in the serum. Immunofluorescence staining against Fluorojade-C was used to measure neurodegeneration in the cortex and the Dentate Gyrus (DG). Immunohistochemistry staining against Glial Fibrillary Acidic Protein (GFAP) was used to examine the astroglial injury that follows the trauma. **Results:** We found significant recognition memory and spatial learning deficits at 48 hours that persisted up to 7-days post r-mTBI. Partial recovery of deficits was noted on the 7th-day post-injury. Neurodegeneration was consistently present in the cortex at 48 hours and 7 days post-injury. Neuro-degeneration in the DG was greater (double) at 48 hours than at 7 days post-injury. pNFH was detected in the serum of injured animals at 48 hours but not at 7 days. Axonal injury and neurodegeneration in the DG followed the same temporal pattern of the behavioral changes. **Conclusion:** We found neurobehavioral deficits, accompanied by axonal injury and neurodegeneration post-r-mTBI. All deficits were more prominent at 48 hours, although they diminished by the 7th-day post-injury, they did not resolve completely.

**Disclosures:** H. Ismail: None. L. Nasrallah: None. F. Kobeissy: None. H.J. Darwish: None.

**Poster**

**300. Traumatic Brain Injury: Models, Mechanisms, and Recovery**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 300.08/I3

**Topic:** C.10. Brain Injury and Trauma

**Support:** R21 AA020951

Office of Academic Affiliations VA Advanced Fellowship in Polytrauma/Traumatic Brain Injury Rehabilitation

the Kalmanovitz Center for CNS Repair

**Title:** The effect of binge alcohol and traumatic brain injury on hippocampal neural precursor cell proliferation and survival

**Authors:** *S. T. TON*, S.-Y. TSAI, J. Y. WU, J. P. GERLING, R. P. NOCKELS, G. L. KARTJE;

Abstract: We determined the effects of traumatic brain injury (TBI) and binge alcohol on neural precursor cell (NPC) responses in the dentate gyrus (DG) of the hippocampus. Rats underwent binge alcohol (3g/kg/day) by gastric gavage for 3 days prior to TBI. We assessed the DG NPC response by utilizing the proliferation marker BrdU along with other markers for neurogenesis such as Doublecortin. We found that TBI injury alone significantly increased DG proliferation 7 days post injury. However, a combined binge alcohol and TBI regimen resulted in decreased DG proliferation at 7 days post-TBI. Long-term survival of DG hippocampal cells was also assessed at 6 weeks post-TBI. We found that TBI did not affect the overall survival of BrdU+ cells, but binge alcohol reduced the number of BrdU+ cells that survived. Using an AAV2/4 viral vector to increase the expression of brain derived neurotrophic factor (BDNF), we are currently examining how this trophic factor may influence DG neurogenesis in relation to binge alcohol and TBI. Taken together, these results suggest that TBI and binge alcohol separately increased short-term DG proliferative responses but in combination, attenuated DG proliferation. However, binge alcohol alone decreased the number of surviving DG cells long term. These results point to important consequences for public health as alcohol misuse conditions such as binge drinking are currently on the rise.

Acknowledgements: Supported by the Office of Academic Affiliations VA Advanced Fellowship in Polytrauma/Traumatic Brain Injury Rehabilitation, National Institute on Alcohol Abuse and Alcoholism R21 AA020951 and the Kalmanovitz Center for CNS Repair.


Poster

300. Traumatic Brain Injury: Models, Mechanisms, and Recovery

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 300.09/I4

Topic: C.10. Brain Injury and Trauma

Support: NIAAA training grant T32AA007577
NIAAA fellowship F32AA026779
NIAAA fellowship F30AA026468
NIAAA research grant R01AA025792

Title: Predator odor exposure exacerbates traumatic brain injury outcomes in rats

Abstract: Traumatic brain injury (TBI) is widespread, undertreated, and leads to persistent neurological and neurobehavioral dysfunction. TBI frequently occurs following traumatic stressful events, like military combat, vehicular accidents, or domestic violence. Stress is associated with pathophysiological and behavioral changes that overlap with those produced by TBI. However, whether stress worsens TBI-related outcomes is not understood. This study tested the hypothesis that pre-TBI exposure to predator odor stress exacerbates TBI-induced physiological and neurobehavioral changes. Adult male Wistar rats all received a 5mm craniotomy above the left sensorimotor cortex. After 3-7 days recovery from surgery, rats received a 15-min bobcat urine exposure or no odor control treatment and 24 hours later received mild to moderate TBI (~2 atm) via fluid percussion or sham (anesthesia only) procedures. Physiological changes (respiratory rate, righting reflex, weight gain), neurological severity and neurobehavioral scores, and anxiety-like behavior (elevated plus-maze) were measured following injury. Stress+TBI animals exhibited decreased respiratory rate immediately following injury and decreased weight gain relative to sham controls. Stress+TBI animals also had increased neurological severity and neurobehavioral scores compared to stress or TBI alone 1 day post-injury. At 7 days post-TBI, stress+TBI animals exhibited elevated neurobehavioral scores and trends toward increased anxiety-like behavior relative to sham controls. These results suggest that pre-injury stress exposure may exacerbate post-TBI physiological and behavioral impairments, possibly delaying recovery from injury. Further investigation of neurobiological mechanisms underlying TBI outcomes that are worsened in stressed animals could help identify clinically relevant treatments for co-morbid TBI and stress-related pathology.

Abstract: Impairments in cognitive function up to one year and longer after traumatic brain injury (TBI) are observed in patients and recapitulated in experimental models of TBI. Previous work implicates neurotransmitter release deficits as a contributor to cognitive impairment after TBI, but additional work is warranted to better understand the mechanisms underlying this dysfunction. Neurotransmitter release is facilitated by the formation of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex. Cysteine-string protein alpha (CSPα) is a chaperone important for SNARE complex formation. We previously showed reduced SNARE complex formation and CSPα abundance after TBI. To test the importance of CSPα on neurobehavioral function and SNARE complex formation after TBI, CSPα heterozygous (CSPα HET) knock-out mice were utilized. We hypothesized CSPα HET mice exhibit reduced SNARE complex formation and exacerbated neurobehavioral dysfunction post-injury. CSPα HET and wild-type (WT) mice were subjected to controlled cortical impact (CCI) injury (1.8mm, 6 m/s, 150msec dwell) or sham surgery. Two cohorts were generated to test the effects of genetically reduced CSPα at 2 weeks (n=9-10/group) and 3 months (n=10/group) following CCI. In the 2wk cohort, motor performance on 1-5d post-injury revealed no differences between groups. Assessment of spatial acquisition in the Morris water maze task on 9-13d post-injury showed a significant injury effect (p<0.05), independent of genotype. Evaluation of spatial memory performance at 14d post-injury revealed no difference between groups. Immunoblotting revealed reduced hippocampal CSPα abundance (p<0.01) and reduced SNARE complex formation (p<0.05) in sham and CCI-injured CSPα HET mice compared to sham and CCI-injured WT mice at 2wk post-injury. In the 3mo cohort, a significant injury effect (p<0.05) was observed in spatial memory performance, independent of genotype. While genetic reduction of CSPα did not alter neurobehavioral function at 2wk or 3mo post-injury, immunoblot data revealed reduced SNARE complex formation. Future work will examine the efficacy of therapeutic interventions, dependent upon CSPα, to promoting SNARE complex formation.

Disclosures: S.W. Carlson: None. D. Edinboro: None. Y. Li: None. J. Henchir: None. C. Dixon: None.

Poster

300. Traumatic Brain Injury: Models, Mechanisms, and Recovery

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 300.11I6

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant HD061963
PA Department of Health Grant SAP 410-007-9710
Title: Sex differences in cognitive deficits following repetitive mild TBI in adolescent rats


Abstract: Sports-related concussions result in acute and chronic behavioral deficits in high school- and college-age athletes who participate in contact sports. Clinical data suggests that male and female athletes suffer different types of behavioral problems and exhibit a different trajectory of behavioral recovery after the concussion. In this study we compared the effect of single and repetitive mild traumatic brain injuries (TBI) in adolescent (35-40-day old) male and female Sprague-Dawley rats. Mild TBI was induced by subjecting anesthetized animals to an impact on the intact skull centered over the midline suture behind bregma (2.5mm depth at 5.5m/s velocity); sham-injured animals were surgically prepared but did not receive injury. On days 7-11 following injury/surgery, sham-and brain-injured animals were tested for working memory using the Morris Water Maze as previously described (Hamm et al., J Neurotrauma, 13 (1996) 317-323). Sham-injured male rats had significantly shorter latencies in the second trial compared to the first, whereas their brain-injured counterparts did not significantly differ in performance between the first and second trials on any of the test days suggestive of an injury-induced working memory deficit (p<0.02 between sham- and brain-injured rats). In contrast, both sham- and brain-injured female rats performed significantly better in the second trial than the first indicative of intact working memory. A separate group of male and female rats were subjected to 3 episodes of mild TBI (2.0mm depth at 5.5m/s velocity) every 3 days; sham-injured rats were anesthetized and surgically prepared on each of the “injury” days but did not receive impacts. On days 3 and 8, sham- and brain-injured male and female rats were tested for short-term working memory using the Novel Object Recognition (NOR) task. On day 3, male brain-injured rats spent significantly more time with the novel object compared to their sham-injured counterparts (p<0.05) suggestive of perseverative behaviors; no differences in NOR memory were observed between sham- and brain-injured rats on day 8 post-injury. Female brain-injured rats could not differentiate between the familiar and novel objects on day 3 post-injury suggestive of a deficit in NOR; by day 8, the brain-injured rats were similar to the sham-injured rats in the NOR test. Together these data suggest that either single or repetitive mild TBI result in cognitive deficits in the acute post-traumatic period. However, the nature and type of these deficits appear to be dependent on the sex of the animal and the type of test used.

Title: Pain (nociceptive) input increases lesion site hemorrhage after traumatic brain injury

Authors: *P. BEAN, M. K. HENWOOD, D. T. JOHNSTON, J. W. GRAU;
Psychological and Brain Sci., Texas A&M Univ., College Station, TX

Abstract: Traumatic brain injury (TBI) is a major cause of death and disability in the United States. Many of these injuries are the result of events such as car accidents, combat violence, and falls, and often result in additional injuries (polytrauma) that serve as a source of pain (nociceptive input). Previous work in our laboratory has shown that nociception is detrimental to recovery after spinal cord injury (SCI) and leads to increased lesion site hemorrhage. It is not known whether these findings generalize to other neurologic insults. The present study sought to determine whether nociceptive input expands the area of hemorrhage after TBI. Male Sprague-Dawley rats (N=12) weighing between 275-300 grams were given either a moderate traumatic brain injury in the right parietal region centered over the primary motor cortex, using the Leica One controlled cortical impact device (2mm impactor tip, 4 m/s, deformation depth 3mm), or underwent a sham surgery. A day later, the irritant capsaicin (3%, 50 ml) or vehicle was applied to the contralateral hindpaw via a subdermal injection. Behavioral tests, including cylinder test and catwalk gait analysis, were performed at zero and three hours post nociceptive input. Animals were then euthanized, perfused with 4% paraformaldehyde, and brain tissue was collected. Collected tissue was sectioned and stained with hematoxylin and eosin to quantify hemorrhage extent at the lesion site, and sections were imaged. Images were analyzed for hemorrhage, quantified by percent area of hemorrhage, by a blinded experimenter using Image J software. The results showed that nociceptive input increased the area of hemorrhage at the injury site (p < .05) and produced an acute disruption in behavior. Pain-induced hemorrhage was limited to the injured hemisphere. On-going work is examining the circumstances under which pain input fuels hemorrhage after TBI, how it affects behavioral function, and the neurobiological processes involved.

Abstract: It is estimated that there are 3.8 million sport related concussions per year, in the United States. Dizziness, headaches, balance abnormalities, and/or difficulties concentrating are common short-term symptoms lasting a few days after a concussion. Proper diagnosis and treatment of concussions or mild traumatic brain injury (mTBIs) are severely lacking because we do not fully understand the cellular and molecular effects that underlie these injuries. Our laboratory as well as others have shown that spreading depolarizations (SDs) are initiated by mTBI-like impacts (Bouley et al., 2018). Using a closed skull impact model, we have shown that these impacts result in concussion-like behavior that includes a prolonged period of immobility immediately following the impact. These periods of immobility are correlated with the presence of a SD but do not result in cell death or astrocyte activation. However, it remains unclear if the SD is associated with any short-term behavioral, cognitive, or attention deficit known to accompany these injuries. We utilized a home cage monitoring system and touchscreen based operant tasks test for behavioral, cognitive and/or attention deficits in the week following a mTBI-like injury. Data from our laboratory and others have shown that the SD produces a 30% to 40% reduction in cerebral blood flow that returns to baseline levels within about 90 minutes. Our preliminary data indicates that although the cerebral blood flow recovers to baseline levels within 90 minutes, it is highly variable in the days that follow. Studies are currently underway using a touchscreen based 5-choice serial reaction time tasks paired with a go/no go paradigm testing cognitive and attention deficits 4 hours post-impact and subsequent days. Overall, our data suggests that SDs may be associated with short-term behavioral, cognitive, and attention deficits in mTBIs.

**Poster**

**300. Traumatic Brain Injury: Models, Mechanisms, and Recovery**

**Location:** Hall A  
**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 300.14/19

**Topic:** C.10. Brain Injury and Trauma

**Support:** BrightFocus Foundation

**Title:** Deciphering the cerebrovascular link between traumatic brain injury and Alzheimer’s disease

**Authors:** *Y. WU*¹, J. ZENG²;  
¹Physiol. & Neurosci., ²USC, Los Angeles, CA

**Abstract:** Background: Alzheimer’s disease (AD) is an age-related progressive neurodegenerative condition manifesting amyloid plaque and neurofibrillary tangle formation, and cognitive impairment. Traumatic brain injury (TBI) is considered the most robust environmental risk the factor for Alzheimer’s disease. Piling evidence has demonstrated that TBI triggers multiple neurodegenerative cascades including axonal and dendritic damages, excitatory toxicity, neuroinflammation, and cell death, as well as cerebrovascular impairment, such as local edema, blood flow reduction and breakdown of the blood-brain barrier (BBB). More importantly, TBI also exacerbates certain pathological events that are specific to Alzheimer’s disease, including the brain over-production and accumulation of beta-amyloid. Brain deposition of amyloid and its associated pathologies are consequences of imbalanced amyloid production and clearance during aging. However, whether TBI impairs amyloid clearance pathways associated with brain vasculature has not been investigated. **Aim:** Investigating the relation between vascular impairment induced by TBI and amyloid-related pathogenesis in AD.  

**Research Strategy:** In our study, we established a mild TBI mouse model, which represents the majority of TBI survivors and recapitulates a body of symptoms including vascular damages and amyloid-related pathologies. And we assessed motor and cognitive impairment based on behavioral tests, determined brain clearance of Aβ and AD pathogenesis using in vivo microdialysis, biochemical analysis, ELISA and immunohistochemistry. **Results:** Our results showed that mild TBI induced BBB breakdown and cerebral blood flow reduction. Mild TBI also structurally altered the vascular basement membrane and perivascular space, which impaired brain clearance of β-amyloid (Aβ) through the vascular routes and accelerated amyloid pathologies and cognitive impairment in a mouse model of AD. Our data demonstrated the key role of vascular impairment in TBI pathogenesis and its link between TBI and Alzheimer’s disease, indicating that restoring vascular functions might be beneficial for patients with mTBI and might prevent the development of AD.

**Disclosures:** Y. Wu: None. J. Zeng: None.
**Poster**

300. Traumatic Brain Injury: Models, Mechanisms, and Recovery

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 300.15/I10

**Topic:** C.10. Brain Injury and Trauma

**Support:** Minnesota Spinal Cord and Traumatic Brain Injury Research Grant Program (Grant #128515), MN office of Higher Education, State of MN

National Research Service Award T32 DA007097, NIDA, NIH

**Title:** Long term spatial learning deficits after mild traumatic brain injury

**Authors:** *N. L. EMMITT*¹, V. D. KRISHNA², A. CRANE², M. CHROSTEK³, A. W. GRANDE⁴, W. C. LOW⁴, M. C.-J. CHEERAN¹;

¹Vet. Population Med., Univ. of Minnesota, St. Paul, MN; ²Stem Cell Inst., ⁴Neurosurg., ³Univ. of Minnesota, Minneapolis, MN

**Abstract:** The incidence of traumatic brain injury (TBI) in 2014 increased by 53% since 2006, with approximately 2.87 million people affected with TBI’s that led to hospitalization, ER visits, or death. Mild TBI accounts for 69-90% of all brain injuries. Though acute impairments after mild TBI are minor, long-term sequelae like increased risk of Parkinson’s Disease, Alzheimer Disease, and depression are evident. To investigate if inflammation due to mild TBI is linked to cognitive impairments, we designed experiments to evaluate the changes in neuroimmune responses to mild TBI in 9-week-old female C57bL/6 mice at 3, 7, 14 and 30 days post injury by flow cytometry. Mild TBI was induced with a controlled cortical impactor centered over the primary and secondary motor cortices (velocity=4m/s, depth=1mm, dwell=100ms). Motor deficits were assessed using a beam walk test at 3, 7, 14, and 30 days post TBI while spatial learning deficits were evaluated on a Barnes maze at 30 days post injury. Minor motor deficits were observed in mice with TBI on the beam walk test when precise foot placement was needed to cross the beam. This deficit resolved at 14 days post TBI. However, on the Barnes maze these animals showed spatial learning deficits both during the training period and probe trial. For the training periods in Barnes maze, mice were tasked with identifying an escape hole. On training day 4, mice that had TBI took longer to complete the maze and traveled larger distances to get there compared to sham injured mice. At the probe trial, where the escape hole is taken away, mice with TBI spent less time in the goal zone and much more time in the opposite zone than those with sham injury. The neuroinflammatory response was dominated by neutrophils and macrophages at 3 days post injury with activation markers Ly6c, MHCII and CD86 upregulated on macrophages. The numbers of infiltrating macrophages and neutrophils returned to levels in sham-injured mice by 7 days post TBI, though CD86 remained upregulated in the remaining macrophages through 30 days post TBI. Microglia were also activated at 3 days post TBI with
upregulation of CD86, but returned to control levels at 7 days post TBI. These results show that in mild TBI spatial learning deficits are present in mice even after key markers of neuroinflammation have returned to baseline levels. This suggests that though the initial injury causes mild motor deficits that resolve quickly, the long-term cognitive defects from a mild TBI are present after the initial inflammatory response subsides.

**Disclosures:** N.L. Emmitt: None. V.D. Krishna: None. A. Crane: None. M. Chrostek: None. A.W. Grande: None. W.C. Low: None. M.C. Cheeran: None.

**Poster**

300. Traumatic Brain Injury: Models, Mechanisms, and Recovery

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 300.16/I11

**Topic:** C.10. Brain Injury and Trauma

**Support:** R01NS37459
1I01RX000310-01

**Title:** The point mutation of the C152 site in ubiquitin C-terminal hydrolase L1 attenuates axonal injury and promotes functional recovery in mice after traumatic brain injury

**Authors:** *Z. MI1,4, H. LIU1,4, M. E. ROSE1,4, X. MA2,3,4, E. C. DIXON2,3,4, S. H. GRAHAM1,4; 1Neurol., 2Neurosurg., 3Critical Care, Univ. of Pittsburgh, Pittsburgh, PA; 4Geriatric Res. Educ. and Clin. Ctr., VA Pittsburgh Healthcare Syst., Pittsburgh, PA

**Abstract:** Diffuse axonal injury is a major component of the motor and cognitive sequelae of traumatic brain injury (TBI). Many acute treatment strategies have targeted neuronal cell death mechanisms after TBI, but these approaches have not been successfully translated. Ubiquitin C-terminal hydrolase L1 (UCHL1) is a multifunctional protein that is selectively expressed in neurons throughout brain at high levels. UCHL1 may play an important role in axonal and synaptic function. Preservation of axonal integrity and synaptic function play a pivotal role in restoration of motor and cognitive function after TBI. A variety of free radical and reactive lipids species are produced after TBI including cyclopentenone prostaglandin (CyPg) derivatives such as Δ12-PGJ2 and 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2). Binding of 15d-PGJ2 to UCHL1 unfolds the enzyme and results in protein aggregation. Mutation of the C152 cysteine of UCHL1, but not the other five cysteine residues in UCHL1, prevents the unfolding of the protein after CyPg treatment. To investigate whether the binding of CyPgs to the C152 site of UCHL1 is important in the pathogenesis of TBI, we constructed a knock-in mouse bearing a C152A mutation in UCHL1 (UCHL1-C152A). UCHL1-C152A or wild type (WT) mice were subjected to the controlled cortical impact (CCI) model of TBI and sacrificed at 1 and 7 d for amyloid precursor protein (APP) immunohistochemistry to detect axonal damage. Hippocampal samples
were collected for western blot detection of ubiquitin proteasome pathway (UPP) and autophagy markers 1 d after CCI, and motor testing was performed on days 1-5 post injury. Axonal injury was quantified by immunostaining with anti-APP antibody and counting axonal swellings, beads and bulbs in a 400 µm by 400 µm field in thalamus. There were significantly fewer morphologically injured axons in thalamus 24 h and 7 d after CCI in UCHL1-C152A mice than in WT controls. There was significantly reduced expression of K48-linkage ubiquitin (Ub) proteins in hippocampus of UCHL1-C152A mice than in WT controls, but no significant changes in K63-linkage Ub or poly-Ub proteins after CCI. The UCHL1-C152A mice had significantly improved motor performance on the beam balance test than WT after TBI; sham-operated controls exhibited no difference between groups. These results suggest that binding of reactive lipids to the C152 site inhibits the UPP in neurons and plays a significant role in axonal and dendritic injury, and motor dysfunction after TBI.


Poster

300. Traumatic Brain Injury: Models, Mechanisms, and Recovery

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 300.17/I12

Topic: C.10. Brain Injury and Trauma

Support: Grant to SRS from National Health and Medical Research Council

Title: Aged rats have an altered immune response and worse outcomes after traumatic brain injury

Authors: *M. SUN1, R. BRADY1, P. CASILLAS-ESPINOSA1, D. WRIGHT1, B. SEMPLE1, S. MCDONALD1, R. MYCHASIUK1, H. KIM3, C. SOBEY3, T. O'BRIEN1, A. VINH3, S. SHULTZ1; 1Monash Univ., Melbourne, Australia; 2The Univ. of Melbourne, Melbourne, Australia; 3La Trobe Univ., Melbourne, Australia

Abstract: Initial studies suggest that increased age is associated with worse outcomes after traumatic brain injury (TBI), though the pathophysiological mechanisms responsible for this remain unclear. Immunosenescence (i.e., dysregulation of the immune system due to aging) may play a significant role in influencing TBI outcomes. This study therefore examined neurological outcomes and immune response in young-adult (i.e., 10 weeks old) compared to middle-aged (i.e., 1 year old) rats following a TBI (i.e., fluid percussion) or sham-injury. Rats were euthanized at either 24 h or one-week post-injury to analyze immune cell populations in the brain and periphery via flow cytometry, as well as telomere length (i.e., a biomarker of neurological
health). Behavioral testing, as well as volumetric and diffusion-weighted MRI, were also performed in the one-week recovery rats to assess for functional deficits and brain damage. Middle-aged rats had worse sensorimotor deficits and shorter telomeres after TBI compared to young rats. Both aging and TBI resulted in worse cognitive function and reduced cortical volume. These changes occurred in the presence of fewer total leukocytes, fewer infiltrating myeloid cells, and fewer microglia in the brains of middle-aged TBI rats compared to young rats. These findings indicate that middle-aged rats have worse functional deficits, decreased cortical volume, and shorter telomeres after TBI than young rats, and this may be related to an altered neuroimmune response. Although further studies are required, these findings have important implications for understanding the pathophysiology and optimal treatment strategies in TBI patients across the life span.


Poster

300. Traumatic Brain Injury: Models, Mechanisms, and Recovery

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 300.18/113

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R01NS107853 AHA Grant 14SDG18730034

Title: Entinostat improves acute neurological outcomes after intracerebral hemorrhage

Authors: *S. SUKUMARI RAMESH, F. BONSACK; Pharmacol. and Toxicology, 1120 15TH STREET, Augusta, GA

Abstract: Intracerebral hemorrhage (ICH) or hemorrhagic stroke is a major public health problem with no effective treatment. Entinostat is a class 1 histone deacetylase inhibitor (HDACi) and in a cell-free system, Entinostat inhibited class 1 HDAC enzymes, HDAC1, HDAC2 and HDAC3 with IC50 of 0.46, 1.29 and 1.57 micromoles, respectively. Given the role of epigenetic mechanisms in the pathophysiology of ICH, we tested the hypothesis that Entinostat attenuates neurodegeneration and improves neurobehavioral outcomes after ICH. To address the hypothesis, we employed a preclinical mouse model of ICH and Entinostat was administered intraperitoneally one-hour post-ICH. Entinostat treatment augmented histone H3 acetylation in the ipsilateral striatum and significantly reduced the number of degenerating neurons and the expression of cleaved caspase-3 (a marker of apoptotic cells) after ICH in comparison to vehicle-treated controls, emphasizing the efficacy of HDAC inhibition in
attenuating neuronal death after ICH. Further, expression of proinflammatory microglial/macrophage marker, CD16/32 was remarkably reduced in Entinostat treated group in comparison to control. Of note, Entinostat treatment significantly improved hematoma resolution and acute neurological outcomes after ICH. Moreover, Entinostat significantly reduced hemin or thrombin-induced release of proinflammatory cytokines from a murine macrophage cell line, Raw 264.7. Altogether, the data implicate the potential of class1 histone deacetylase inhibitor, Entinostat in improving acute neurological function after ICH warranting further investigation.

Disclosures: S. Sukumari Ramesh: None. F. Bonsack: None.

Poster

300. Traumatic Brain Injury: Models, Mechanisms, and Recovery

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: 300.19/I14

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant HD061963
PA Department of Health Grant SAP4100079710
PA Department of Health Grant SAP4100077079

Title: Is oxytocin effective in ameliorating the social deficits of neonate traumatic brain-injured rats in adolescence and adulthood?

Authors: A. M. RUNYAN¹, D. LENGENL₂, *J. W. HUH³, R. RAGHUPATHI⁴;

Abstract: Social behavioral deficits are often an overlooked facet of traumatic brain injury (TBI) outcomes. New clinical data shows children who suffer a TBI develop social problems later in life. Here we investigated social outcomes in adolescence and adulthood after TBI in neonate rats. Male and female rat pups at post-natal day 11 were anesthetized and received an impact (5m/s, 3mm depth) over the left lateral hemisphere; sham-injured animals were surgically prepared but did not receive an impact. Social behavior, including sociability and social novelty were measured via a three-chamber test in adolescence (4 weeks post-TBI, 39-45 days old, sham N=15, injured N=20) and adulthood (8 weeks post-TBI, 67-75 days old, sham N=6, injured N=7). Sociability was assessed by the amount of time the test rat spent sniffing an empty cup in one chamber compared to a cup with a live rat in a separate chamber. In adolescence and adulthood both sham- and brain-injured rats spent more time with a live rat compared to an empty cup (p<0.001), indicative of intact sociability. Social recognition/novelty was assessed by the time spent sniffing a “familiar” rat in one chamber compared to a “novel” rat placed in a separate chamber. Adolescent and adult sham-injured animals spent more time sniffing the novel
rat over the familiar rat indicating intact social recognition/novelty. However, in adolescence brain-injured rats spent about equal time sniffing both the familiar and the novel rat, suggestive of a social recognition/novelty deficit. This phenotype changed in adulthood wherein brain-injured rats preferred to spend more time sniffing the familiar rat compared to the novel rat (p<0.05) suggesting a recovery in social recognition, but an impairment in social novelty. Interestingly, these behavioral changes occur in the absence of deficits in the novel object recognition test at both ages suggestive of the social component of these behaviors rather than a cognitive deficit. All behavioral effects were independent of sex. The neuropeptide oxytocin has been implicated in regulating social behavior. Intranasal administration of either oxytocin (20μg/kg) or saline in adolescent sham-injured animals did not affect their preference for the novel rat although oxytocin administration increased the overall sniffing time. Oxytocin administration to adolescent brain-injured rats improved their social recognition/novelty behavior. Ongoing work seeks to define the mechanism of action of oxytocin in regulating social behaviors in brain-injured animals.


Poster
300. Traumatic Brain Injury: Models, Mechanisms, and Recovery
Location: Hall A
Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM
Program #/Poster #: 300.20/I15
Topic: C.10. Brain Injury and Trauma
Support: Loyola University Department of Otolaryngology: Head and Neck Surgery
Title: Testosterone recovers chronic vestibular impairment following repeat mild traumatic brain injury

Authors: *A. SEGISMUNDO¹, E. WESTFALL¹, A. BOLDUAN¹, D. G. WALLACE², D. A. KOZLOWSKI³, E. B. STUBBS⁴, E. FOECKING¹;
¹Loyola Univ. of Chicago, Maywood, IL; ²Northern Illinois Univ., Dekalb, IL; ³Biol., DePaul Univ., Chicago, IL; ⁴Edward Hines Jr. VA, Worth, IL

Abstract: Traumatic brain injury (TBI) is defined as the impact of an external force to the head and has quickly become a major public health concern. Vestibular symptoms, one of the most common complaints following TBI, and hypogonadism occur acutely following a single TBI and may persist chronically. Vulnerable populations such as military personnel are at an increased risk for repeat mild TBIs (rmTBI), which has been shown to exacerbate impairment. Our lab has previously indicated a neuroprotective role of testosterone (T). Loss of T following TBI disrupts hormonal rhythms and prevents neuroplasticity that is required to repair damage at the time of injury, which may induce greater chronic vestibular impairment. Here, we first sought to create a
clinically relevant rmTBI animal model with chronic vestibular deficits. Using this model, we tested whether T can recover chronic vestibular dysfunction. 

TBIs were performed on Long Hooded male rats five times, each spaced 48 hours apart, using an electromagnetic controlled cortical impactor. Sham TBI animals did not receive impact. Animals were subdivided into castrated or gonadally intact, creating 4 separate groups. Castration was performed to remove endogenous T to simulate chronic hypogonadism in a subset of rmTBI patients. Sham surgery animals were incised but gonads were not removed. Vestibular dysfunction was detected by a battery of five behavioral tests. Tests were scored out of 2 points, with the medians of each test contributing to a mean total vestibular score. Higher scores indicated greater vestibular dysfunction. Animals were tested at 1 day, 1 week, and 1 month post rmTBIs to chronicle development of vestibular dysfunction. To test the therapeutic effects of T, half of the animals of each group received T replacement via subcutaneous silicon capsules in the nape of the neck five weeks post TBI #5.

At 1-month post TBI #5, a significant difference was found between all groups, with TBI intact and TBI castrated animals exhibiting the greatest vestibular deficiency scores. Following T treatment, vestibular scores did not improve at 1-week post treatment, but TBI T treated animals were insignificant compared to sham TBI animals at 1-month post treatment. Untreated TBI animals exhibited persistent chronic vestibular deficit at all time points. The therapeutic effects of T continued 6 months post TBI. Therefore, T replacement following TBI induced hypogonadism should be considered as a potential therapeutic strategy for recovery of chronic vestibular dysfunction.

We would like to thank the support of the Loyola University Department of Otolaryngology: Head and Neck Surgery for funding this research.


Poster

300. Traumatic Brain Injury: Models, Mechanisms, and Recovery

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 300.21/I16

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant NS084921

Title: Snake venom pre-conditioning attenuates neuroinflammation and intraoperative hemorrhage via PLA2/SLOX/LTC4 synthase cascade and PLA2/COX1/TXA2 cascade respectively in a surgical brain injury rat model

Authors: *Z. D. TRAVIS¹, P. SHERCHAN², W. KELLN³, W. K. HAYES³, A. COOPER⁴, J. H. ZHANG²;
Abstract: Inflammatory preconditioning is a mechanism in which exposure to small doses of inflammatory stimuli prepares the body against future massive insult by activation of endogenous protective responses. PLA2/5LOX/LTC4 synthase axis is an important inflammatory signaling pathway that has been noted to increase post-operative complications. *Pseudechis papuanus* venom contains 90.2% secretory PLA2 of its dry weight. The remaining 9.8% of the venom is composed of 3FTXs, SVMPs, CRISPs, and LAAO. Because of the protein composition, the venom causes neurotoxicity, hemolysis pulmonary inflammation, and edema. We investigated whether purified *P. papuanus* venom preconditioning (VPC) reduces surgical brain injury (SBI) induced neuroinflammation via activating the PLA2/5LOX/LTC4 synthase/LTE4 cascade as well as attenuating intraoperative hemorrhage by increasing platelet aggregation via PLA2/COX1/TXA2 cascade using a partial frontal lobe resection SBI rat model. Sublethal doses of venom were injected subcutaneously three consecutive days prior to SBI. We observed that VPC significantly reduced edema and improved neurological function 24 h and 72 h after SBI. Furthermore, our long-term study showed an increase in neurological function 30 days after SBI. The VPC regime also significantly reduced intraoperative bleeding, while the sublethal dose caused no skin inflammation at the injection site and no other toxic effects. These findings suggest that VPC reduces neuroinflammation, intraoperative hemorrhage, and improves neurological outcomes after SBI by activating the PLA2/5LOX/LTC4 synthase and PLA2/COX1/TXA2 cascade respectively. Purified PLA2 VPC may be a beneficial therapy to reduce post-operative neuroinflammation and intraoperative hemorrhage complications from brain surgeries.


Poster

300. Traumatic Brain Injury: Models, Mechanisms, and Recovery

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 300.22/117

Topic: C.10. Brain Injury and Trauma

Support: Loma Linda University Pediatric Epilepsy Research Foundation

Title: Melanocortin agonist reduces microglial activation and neuroinflammation following experimental traumatic brain injury

Authors: *L. SIEBOLD*\(^1\), J. ABDALA\(^2\), B. TONE\(^3\), B. BARTNIK-OLSON\(^4\), B. HOLSHOUSER\(^4\), J. FIGUEROA\(^1\), S. ASHWAL\(^3\), C. G. WILSON\(^2\);
Abstract: Traumatic brain injury (TBI) is a leading cause of mortality/morbidity and is associated with chronic neuroinflammation. Melanocortin receptor (MCR) agonists (e.g., ACTH or adrenocorticotropic hormone) that target MC3R/4R ameliorate inflammation and provide a novel therapeutic approach. Following TBI, quiescent microglia become activated resulting in anti and pro-inflammatory responses and morphological changes. We examined the effect of Cosyntropin (synthetic ACTH) administration on microglial activation in a rodent TBI model. We hypothesized that Cosyntropin administration would reduce inflammation and microglial activation following experimental TBI. We used a rodent model of CCI randomized to 4 groups: sham-vehicle, sham-Cosyntropin, CCI-vehicle, and CCI-Cosyntropin. Subcutaneous injections of Cosyntropin (West Therapeutic Development; Grayslake, IL) were given for 7 days following injury. Microglia activation was quantified based on morphology. Sectioned brains were immunostained (Iba1) and imaged followed by morphological quantification with FIJI and FracLac for ImageJ. Parameters included cell area, fractal dimension, circularity, and cell perimeter and density. Cytokine expression, including TNF-alpha, TGF-beta, IL1beta and IL-10, was compared between groups. CCI animals exhibited increased microglia in the lesion site with no difference in cell count between vehicle and treated. Microglia from CCI animals exhibited decreased cell perimeter and increased density and circularity compared to sham animals. Cosyntropin treatment altered CCI-induced microglia changes in cell area, cell perimeter, and cell density. Cosyntropin-treated CCI animals showed reduced morphological changes in microglia suggesting a reduced activation state. Cosyntropin may decrease microglial activation and neuroinflammation through melanocortin receptor signaling and result in improved functional outcome.

Disclosures: L. Siebold: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); West Therapeutic Development. S. Ashwal: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); West Therapeutic Development. J. Abdala: None. B. Bartnik-Olson: None. B. Holshouser: None. J. Figueroa: None. B. Tone: None. C.G. Wilson: None.

Poster

300. Traumatic Brain Injury: Models, Mechanisms, and Recovery

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 300.23/I18

Topic: C.10. Brain Injury and Trauma

Support: NIH R21-NS102991
          NIH R00-MH101234
Title: The effects of injury and exosome treatment on biophysical and structural properties of pyramidal neurons in primate premotor cortex

Authors: *M. MEDALLA*¹, W. CHANG¹, S. CALDERAZZO¹, V. GO¹, A. TSOLIAS¹, J. GOODLIFFE¹, D. PATHAK¹, S. E. BUSCH¹, D. DEALBA¹, D. L. ROSENE¹, B. BULLER², T. L. MOORE¹;
¹Anat. & Neurobio., Boston Univ. Sch. of Med., Boston, MA; ²Henry Ford Hlth. Syst., Detroit, MI

Abstract: Functional recovery and circuit reorganization after cortical injury such as stroke in humans is limited by chronic inflammation and oxidative stress. Altering immune and trophic modulators of neuronal plasticity is a viable therapeutic target to facilitate recovery after cortical injury, but the nature of neuroimmune interactions in the primate brain is not well understood. Our recent work has shown that systemic administration of exosomes derived from mesenchymal stem cells enhances recovery of fine motor function in a non-human primate model of cortical injury. Exosomes are circulating nanovesicles that mediate cell-to-cell signaling of peripheral and central immune cells, but the neural mechanisms of exosome-mediated enhancement of recovery after cortical injury is largely unknown. Using in vitro whole-cell patch clamp recording and filling in acute slices, we studied the biophysical signaling and structural properties of surviving pyramidal neurons in perilesional premotor cortex, 14-16 weeks post-injury. Compared to control neurons from non-lesioned brains, perilesional neurons from vehicle-treated brains were more excitable, having significantly higher action potential (AP) firing rates in response to depolarizing current stimuli (p < 0.05). However, in exosome-treated brains, this lesion-related increase in AP firing was less pronounced; AP firing rates were more similar to non-lesioned controls and significantly lower than the vehicle group at low amplitude depolarizing current injections (p < 0.01). Thus, exosome treatment reduces injury-related hyperexcitability in perilesional neurons by lowering AP firing without changing passive membrane properties, which were similar between groups. At the synaptic level, perilesional neurons had significantly lower excitatory, but higher inhibitory postsynaptic current frequency compared to non-lesion neurons. While no significant group difference in the density of excitatory postsynaptic spines was found, the exosome group exhibited a higher density of the inhibitory synaptic marker, VGAT, specifically those aposed to apical dendrites of perilesional neurons. Perilesional neurons in the exosome group also exhibited significantly more complex dendritic apical tufts compared to the vehicle group. These data suggest that cortical injury induces hyperexcitability and synaptic reorganization in surviving perilesional neurons, to presumably compensate for neuronal damage and deafferentation. Further, exosome treatment may normalize these injury-related changes to likely prevent chronic excitotoxicity and restore excitatory:inhibitory network balance.

Poster

300. Traumatic Brain Injury: Models, Mechanisms, and Recovery

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 300.24/I19

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant NS102991
NIH Grant NS076474
NIH Grant NS075156

Title: Mesenchymal derived exosomes enhance recovery of motor function in a monkey model of cortical injury

Authors: *T. L. MOORE¹, V. GO², M. A. PESSINA¹, B. G. E. BOWLEY¹, M. L. COVERT¹, M. MEDALLA¹, S. CALDERAZZO¹, Z. ZHANG³, M. CHOPP³, S. P. FINKLESTEIN⁴, A. G. HARBAUGH⁵, D. L. ROSENE¹, B. BULLER³;

Abstract: Exosomes are endosome-derived nano-vesicles that are active biological containers transporting nucleic acids and proteins between cells. The efficacy of exosomes harvested from mesenchymal stromal cells (MSCs) to enhance functional recovery and alter inflammation following cortical injury has been demonstrated in rodent and porcine models of stroke and traumatic brain injury. Building on this work, we have utilized exosomes harvested from non-human primate (NHP) bone marrow cells as a treatment in our rhesus monkey model of cortical injury. Ten aged female rhesus monkeys were trained to retrieve a small food reward on a task of fine motor function of the hand and then received a cortical injury to the hand representation in primary motor cortex. Monkeys were then randomly assigned to either the exosome treated or vehicle groups (n=5 each). Exosomes or vehicle were administered intravenously 24 hours and again 14 days post-injury and recovery of motor function was followed for 12 weeks. Post-injury performance on our task of fine motor function revealed significantly greater degree of recovery of function in monkeys treated with exosomes. Specifically, treated monkeys returned to pre-operative performance in terms of speed to retrieve food rewards and the quality of grasp patterns within the first 3-5 weeks after injury. At the completion of testing, monkeys were euthanized and brains were harvested for analysis of markers of inflammation. Using 60 um sections of brain tissue throughout the extent of the lesion, microglia were labeled with Iba1, P2Y12, a purigenic receptor important for motility in homeostatic ramified microglia, and LN3, an MHC II receptor upregulated with immune activation. Confocal imaging, 3D reconstruction, and Sholl analyses revealed that microglial process length was greater in exosome-treated
monkeys compared to vehicle-treated monkeys. Further, exosome treated monkeys expressed lower densities of LN3+ hypertrophic microglia. These data suggest that exosome treatment promotes greater microglial ramification and reduce immune-activated hypertrophic/amoeboid microglia subtypes. Taken together these findings demonstrate that exosomes significantly enhance recovery of function and play a role in reducing inflammation following cortical injury.


**Poster**

**300. Traumatic Brain Injury: Models, Mechanisms, and Recovery**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 300.25/I20

**Topic:** C.10. Brain Injury and Trauma

**Support:** NIH/NICHD Grant R37HD059288

**Title:** Electrophysiologic analysis of excitation/inhibition balance in the ventral hippocampus-medial prefrontal cortex circuit following traumatic brain injury

**Authors:** *K. M. BEST*¹,³, H. METHENY⁴, A. S. COHEN⁴,²;
¹Ctr. for Sleep and Circadian Neurobio., ²Dept. of Anesthesiol. and Critical Care Med., Perelman Sch. of Medicine, Univ. of Pennsylvania, Philadelphia, PA; ³Dept. of Nursing & Clin. Care Services, ⁴Dept. of Anesthesiol. and Critical Care Med., Children's Hosp. of Philadelphia, Philadelphia, PA

**Abstract:** Traumatic brain injury (TBI) affects more than 2.5 million people in the United States each year and is associated with long-term cognitive impairments. Although there are diverse inputs to the prefrontal cortex (PFC), the majority of innervation is thought to come from afferent projections from the hippocampus (HPC), which is known to be vulnerable to injury. Strong unidirectional, monosynaptic glutamatergic projections from the ventral HPC to all cortical layers of the medial PFC are implicated in various cognitive functions, including spatial working memory. Previously, we have shown that TBI leads to diminished cortical layer V field potentials evoked by layer II/III stimulation. This study examined the electrophysiological correlates of vHPC-mPFC circuit dysfunction after mild TBI in a mouse model of brain injury, lateral fluid percussion injury (LFPI). Under anesthesia, mild LFPI was induced in 6- to 8-week-old male C57/BL6 mice and electrophysiological experiments were performed 6 to 8 days after injury. For recording, mice were anesthetized and brains were quickly and carefully removed and placed in ice-cold oxygenated sucrose-containing artificial cerebrospinal fluid (aCSF). Slices
were cut at 300 µm thickness on a 10° agar ramp using a VT1200S vibratome in order to isolate projections from the vHPC to the mPFC according to published methods, and transferred to 33-35°C normal aCSF for at least one hour of incubation. Extracellular recordings of field excitatory post-synaptic potentials (fEPSPs) were obtained by placing a stimulating electrode in layer II/III of the prelimbic cortex and a recording electrode also in layer II/III. Recorded fEPSPs were evoked with a single stimulus (100 µs duration) ranging from 50 to 800 µA in 50 µA increments to generate input/output (I/O) curves. All experiments were carried out under protocols approved by the Institutional Animal Care and Use Committee. Data were analyzed with two-way repeated measures ANOVA with Sidak’s multiple comparison test in order to evaluate for effects by injury condition and stimulation intensity. Our results showed alterations in excitatory/inhibitory (E/I) balance in the vHPC-mPFC circuit at one week following TBI. Further research is needed to determine whether the observed changes in E/I balance in the mPFC affect performance on working memory tasks.


Poster

300. Traumatic Brain Injury: Models, Mechanisms, and Recovery

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 300.26/I21

Topic: C.10. Brain Injury and Trauma

Support: Jane and Aatos Erkko Foundation

Title: Cerebral dopamine neurotrophic factor in zebrafish brain repair after traumatic brain injury

Authors: *S. SHARIFULINA1, S. SEMENOVA2, Y.-C. CHEN2, P. PANULA2;
1 Neurosci. Center, HiLife, 2 Dept. of Anat., Univ. of Helsinki, Helsinki, Finland

Abstract: Cerebral dopamine neurotrophic factor (CDNF) has been shown to protect dopaminergic neurons against toxic damage, and to inhibit endoplasmic reticulum stress, neuroinflammation, and apoptosis. The role of CDNF in the regenerative ability of adult zebrafish brain after telencephalic stab wound injury was investigated using zebrafish cdnf mutant line by quantification of gene expression relevant to neuroregeneration after traumatic brain injury (TBI) and by behavioral assays. cdnf mRNA level, assessed by quantitative PCR at 1, 3, 7, 14 and 21 days after the lesion, was increased in the damaged wild type zebrafish telencephalon at 1 day post lesion (dpl). Neurotransmitter measurement by high-performance liquid chromatography showed increased dopamine, serotonin and noradrenaline levels in the whole adult WT zebrafish brain at 1 dpl after TBI. The level of dopamine in WT zebrafish telencephalon was increased at 1dpl. cdnf mutant zebrafish showed normal locomotor behavior.
without lesion and at 1 dpl after TBI. Novel tank diving test (NTDT) showed significant differences between cdnf KO and WT fish at 1 dpl in vertical NTDT exploration. Expression of genes related to neuronal differentiation, such as sox2 and notch1a, was significantly increased in the telencephalon of cdnf KO and WT fish after TBI. Expression of comta, involved in the inactivation of the catecholamine neurotransmitters, was decreased in cdnf WT zebrafish telencephalon after TBI but not in cdnf KO at 1dpl. The number of Fluoro-Jade C positive cells was 4-fold higher in cdnf KO telencephalon compared with cdnf WT telencephalon at 3dpl but not at 1dpl. These differences suggest the involvement of CDNF in zebrafish brain response to TBI.

Disclosures: S. Sharifulina: None. S. Semenova: None. Y. Chen: None. P. Panula: None.

Poster

300. Traumatic Brain Injury: Models, Mechanisms, and Recovery

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 300.27/I22

Topic: C.10. Brain Injury and Trauma

Title: Assessing the behavioral effects of proton irradiation and polyphenol supplementation on a high throughput fruit fly model of traumatic brain injury

Authors: *T. A. TOGASHI, A. D. TROFIMOVA, C. BARCENAS, W. HARDEMAN, M. HANNA, S. LOONG, M. WHITE, V. WATTS, G. A. NELSON, R. E. HARTMAN; Psychology, Loma Linda Univ., Loma Linda, CA

Abstract: We investigated the effects of traumatic brain injury (TBI) using a blunt force impact fruit fly model. The effects of polyphenols and irradiation were also assessed. The neuroprotective effects of polyphenols have been documented across various species (including flies). Irradiation has been shown to have deleterious effects across several species (including flies). However, both may induce “hormetic” effects, in which large doses are toxic, but smaller doses activate endogenous neuroprotective pathways and provide protection against subsequent injury.

We hypothesized that pretreatment with polyphenols and/or radiation would reduce TBI-induced behavioral deficits, and that irradiation after TBI would exacerbate its effects. We also predicted that TBI deficits would worsen over 7d. Flies were subjected to TBI or a control procedure, and some were irradiated (10Gy protons) 12hrs pre- or post-TBI. Half of the diets were supplemented with pomegranate juice containing ~24mg/ml ellagic acid throughout the experiment. Climbing behavior was measured 1d and 7d post-TBI, and 24hr activity levels were recorded throughout the week.

Flies that climbed higher tended to be more active. TBI flies demonstrated significantly impaired climbing performance, and the magnitude of the deficit slightly increased over 7d. TBI did not
impact overall locomotor activity, however. Irradiation slightly increased climbing height in the absence of TBI, but significantly reduced climbing height in TBI flies. Irradiation after TBI had a larger effect than irradiation before TBI. Polyphenol supplementation had no effect on climbing behavior. Both irradiation and dietary supplementation reduced 24hr activity levels. In summary, low levels of irradiation significantly exacerbated the effects of TBI, suggesting that further research is warranted. For example, human astronauts on long-term deep space missions will be exposed to proton irradiation with the potential for accidents leading to a TBI. Our data suggest that this fly model of TBI and irradiation produces consistent and long-lasting behavioral deficits that will provide useful behavioral and neuropathological targets for inexpensive and high-throughput testing of various therapeutic approaches (such as prophylactic dietary supplementation).


Poster

300. Traumatic Brain Injury: Models, Mechanisms, and Recovery

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 300.28/123

Topic: C.10. Brain Injury and Trauma

Title: Exploring the effects of prophylactic & therapeutic dietary polyphenol treatments on traumatic brain injury outcomes in Drosophila melanogaster

Authors: *B. E. TOLAN, A. D. TROFIMOVA, R. E. HARTMAN;
Psychology, Loma Linda Univ., Loma Linda, CA

Abstract: Traumatic brain injury (TBI) is a leading cause of neurological deficits and mortality worldwide. In the United States alone, an estimated 2.8 million TBIs were diagnosed in 2013. Neurocognitive deficits associated with TBI include impaired attention, inability to form visuospatial associations, and poor executive functioning. Common psychological consequences include depression, increased impulsivity, poor decision-making, and aggressive behavior. A complex, multimodal neurodegenerative process, TBI presents both primary and secondary injuries. Primary injuries are sustained upon impact, whereas secondary injuries can occur over time as cellular and molecular responses are triggered in reaction to the impact. These secondary injuries have been linked with neurodegenerative diseases later in life. Inflammatory activation plays a critical role in the progression of secondary injuries, and suppressing long-term inflammation may help to reduce neurodegeneration.

A cost efficient and widely available solution might be found in dietary plants containing high levels of bioactive phytochemicals with anti-inflammatory and antioxidant properties (e.g.,
polyphenols). Pomegranates, for example, contain punicalagin, an ellagittannin polyphenol that is hydrolyzed into ellagic acid (EA). Previous research from our laboratory has demonstrated that both pre- and post-treatment with dietary polyphenols can provide neuroprotection in a number of models and clinical settings. This study examined whether prophylactic (pre-) or therapeutic (post-) treatments provide more favorable outcomes in a Drosophila melanogaster (fruit fly) model of TBI.

Young adult wild-type flies (N = 500) were raised on standard media (control), media containing pomegranate juice (PJ), or media containing ellagic acid (EA) for 10 days, followed by a mechanical impact designed to induce TBI. The flies were then transferred into clean food vials and treatment was continued for 24 hours with flies previously raised in treatment transferred to standard media and flies raised on standard media transferred to treatments. After 24 hours, climbing behavior and activity levels were measured. Preliminary results suggest that both pre- and post-treatment were effective in reducing locomotor deficits associated with TBI, with PJ producing slightly more favorable results than EA, but polyphenol treatment overall more effective than a standard diet. Female flies recovered better and responded more favorably to polyphenol treatment than males. This suggests that both prophylactic and therapeutic treatments of polyphenols may be effective in treating TBIs.

**Disclosures:** B.E. Tolan: None. A.D. Trofimova: None. R.E. Hartman: None.

**Poster**

**300. Traumatic Brain Injury: Models, Mechanisms, and Recovery**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 300.29/I24

**Topic:** F.03. Neuroendocrine Processes

**Title:** Differential expression of estrogen-metabolizing enzymes following traumatic brain injury in adult male and female zebra finches

**Authors:** *K. A. DUNCAN;* Vassar Col., Poughkeepsie, NY

**Abstract:** Traumatic brain injury (TBI) induces neuropathological changes, cognitive deficits, and psychosocial dysfunction. Characterized by damage inducing neuroinflammation, this response can cause acute secondary injury that leads to widespread neurodegeneration and loss of neurological function. Estrogens decrease neuroinflammation and increase cell survival and neuroprotection and thus are a potential target for use following TBI. To further explore the relationship between estrogens and brain injury, we examined the expression of estrogen-metabolizing enzymes (EME) following TBI. EMEs are involved in both the initial conversion
and interconversion of estrogens from precursors. EMEs include aromatase, steroid sulfatase (STS), estrogen sulfotransferase (EST), and some forms of 17β-hydroxysteroid dehydrogenase (HSD). Aromatase (CYP19; estrogen synthase) has been studied extensively, but the role of the remaining EMEs following TBI has yet to be determined. Generally, EST and 17β-HSD2 play opposite roles to STS and 17β-HSD1 in the production of estrogens. Adult female and male zebra finches were given a unilateral penetrating injury directed toward the entopallium and collected 24 hours later. Brain tissue was collected for qPCR analysis and analyzed. Following injury, STS, which hydrolyzes biologically inactive estrogen, sulfates to produce active estrogens showed no difference in expression. 17β-HSD1, which is responsible for the interconversion of estrone (E1) and estradiol (E2) was upregulated following injury in both males and females. Inversely, 17β-HSD2, which is involved in inactivation of androgens and estrogens, was downregulated in females, but not in males following injury. These data suggest that increased expression of estrogens occurs at many levels beyond aromatase expression following injury and in a sexually dimorphic manner.

Disclosures: K.A. Duncan: None.

Poster

301. Peripheral Nerve Injury

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: 301.01/I25

Topic: C.10. Brain Injury and Trauma

Support: JSPS KAKENHI Grant Number JP18H04013
JSPS KAKENHI Grant Number JP17K10926

Title: The MAP1B phosphorylation sites specifically localized in the developing brain and in the regenerating peripheral nerves

Authors: *Y. ISHIKAWA¹², M. OKADA³², A. HONDA², Y. ITO², A. TAMADA²⁴, N. ENDO¹, M. IGARASHI²;

Abstract: The growth cone is an essential structure involved in formation and rearrangement of the neuronal network. Since phosphorylation is one of the most important post-translational modifications in all of the cells including neurons, we have focused on “phosphoproteomics”, which enables us to identify both the phosphorylation sites of proteins and their frequency simultaneously and help us understanding the comprehensive signaling pathways. For this reason, we performed phosphoproteomic analysis of the growth cone membrane (Kawasaki et
al., iScience, 2018) and identified 4,600 phosphorylation sites corresponded to 1,200 proteins. In this study, we featured two of the most abundant sites, Ser25 and Ser1201 of rat microtubule-associated protein 1B (MAP1B), which is a protein known to stabilize axonal microtubules and to be involved in axon formation. So far, phosphorylation of MAP1B is thought to be accumulated in the distal portion of growing axons, however, biological functions of the phospho-MAP1B remain to be clarified. The above two sites are highly conserved between rodents and human, suggesting that they may have some important functions. Here, we produced the phospho-specific antibodies (Abs) against these sites, respectively. The growing axons were preferentially labelled by these Abs in the cultured mouse cortical neurons, and in the embryonic mouse brain using immunohistochemistry. Western blotting analysis revealed that phosphorylation of these sites gradually decrease in postnatal periods. In the crushed sciatic nerve, we found that the immunoreactivity of each Ab was increased at the injury site and extended to the distal side over the injured point. In the transected nerve, the immunoreactivity was increased but remained to be stuck in the proximal segment; after the nerve suture, it was also detected even in the distal segment over the injured point. These results suggest that these phosphorylation sites are involved in neuronal development and in peripheral nerve regeneration.


Poster

301. Peripheral Nerve Injury

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 301.02/I26

Topic: C.10. Brain Injury and Trauma

Support: This work was co-funded by Science Foundation Ireland and Integra LifeSciences through TP27-1846B1 as part of the Advanced Materials and Bioengineering Research (AMBER) Centre

Title: An advanced nerve guidance conduit for repairing large peripheral nerve defects

Authors: *Z. KOCI1,3,4, A. J. HIBBITTS1,3,4, S. L. KNEAFSEY1,3,4, L. ZILIC1,3,4, G. CHEN2, C. T. BUCKLEY1,3,4, S. J. ARCHIBALD5, F. J. O'BRIEN1,3,4;

1Tissue Engin. Res. Group, Dept of Anat., 2Dept. of Physiol. and Med. Physics, Ctr. for the Study of Neurolog. Disorders, Micros, Royal Col. of Surgeons In Ireland, Dublin, Ireland; 3Trinity Ctr. for Bioengineering, Trinity Col. Dublin, Dublin, Ireland; 4Advanced Materials and Bioengineering Res. Ctr. (AMBER), Dublin, Ireland; 5Integra Lifesciences, Plainsboro, NJ
**Abstract:** Peripheral nerve injuries (PNI) affect millions of patients worldwide and cause motor and sensory dysfunction leading to reduced quality of life and increased healthcare costs. The primary treatment option for repairing large PNIs is to use patient’s own nerve graft – an autograft, which is limited by availability and donor site morbidity. In this study, we aim to prepare an off-the-shelf advanced nerve guidance conduit (NGC) with capacity to regenerate critical-sized PNI as effectively, but overcoming the associated limitations of utilising autografts. Advanced NGCs were composed of two phases – an outer tubular shell composed of collagen type I (Coll) and internal matrix composed of collagen type I, chondroitin-6-sulphate and a series of extracellular matrix derived molecules (Coll/CS/ECM). Different ratios of ECM molecules were tested for their neurotrophic potential in vitro using primary rat Schwann cells (SC) and a rat dorsal root ganglia (DRG) explant culture. Following this, two formulations: Coll/CS/ECM1 (termed NGC1) and Coll/CS/ECM2 (NGC2) were selected for an in vivo study in a very large (15 mm) critical-sized rat sciatic nerve defect model. The nerve regeneration potential was compared to a reversed autograft.

In vitro analysis showed that Coll/CS/ECM1 and Coll/CS/ECM2 conduits supported viability and proliferation of SCs and DRGs. These conduits also significantly up-regulated neuron-specific β-III tubulin as well as NGF and VEGF production by DRGs in comparison to Coll/CS conduits.

In vivo analysis showed that sensory and motor function recovery significantly improved over time in all animals. Notably, the response of the two NGCs to electrical stimulation was similar to the autograft group and no differences were seen in electrophysiological recordings in either NGC1 or NGC2 when compared to the autograft group. Groups implanted with NGC1 and NGC2 displayed similar compound nerve as well as compound muscle action potential recordings. Consistent with these results, no significant differences in muscle weight loss were observed between either NGC and autograft. In addition, most importantly, the number of myelinated axons within both NGCs was similar to autografts showing the ability of the ECM molecules to direct functional nerve growth across a large defect.

In summary, this study demonstrated that enrichment of a NGC with ECM derived molecules resulted in a biomaterial capable of repairing large PNIs to a level equivalent to an autograft indicating its potential as a new clinical therapy for repairing large nerve defects.

**Disclosures:**  
**Z. Koci:** A. Employment/Salary (full or part-time):; Integra LifeSciences (Plainsboro, NJ, USA) is partially funding the project. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; This work was co-founded by Science Foundation Ireland and Integra Lifesciences (Plainsboro, NJ, USA) through TP-27-1846B1 as part of the Advanced Materials and Bioengineering Research Centre. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); In kind support was received from Integra LifeSciences (Plainsboro, NJ, USA) in the form of raw materials and technical knowhow.  
**A.J. Hibbitts:** A. Employment/Salary (full or part-time):; Alan Hibbitts is part-funded by Integra LifeSciences. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); In kind support was supplied from Integra LifeSciences (Plainsboro, NJ, USA).  
**S.L. Kneafsey:** A. Employment/Salary (full or part-time):; Integra LifeSciences (Plainsboro, NJ, USA) is partially
funding the project. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; This work was co-founded by Science Foundation Ireland and Integra Lifesciences (Plainsboro, NJ, USA) through TP-27-1846B1 as part of the Advanced Materials and Bioengineering Research Centre. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); In kind support was received from Integra LifeSciences (Plainsboro, NJ, USA) in the form of raw materials and technical knowhow. L. Zilic: A. Employment/Salary (full or part-time); Integra LifeSciences (Plainsboro, NJ, USA) is partially funding the project. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; This work was co-founded by Science Foundation Ireland and Integra Lifesciences (Plainsboro, NJ, USA) through TP-27-1846B1 as part of the Advanced Materials and Bioengineering Research Centre. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); In kind support was received from Integra LifeSciences (Plainsboro, NJ, USA) in the form of raw materials and technical knowhow. G. Chen: None. C.T. Buckley: A. Employment/Salary (full or part-time); Integra LifeSciences (Plainsboro, NJ, USA) is partially funding the project. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; This work was co-founded by Science Foundation Ireland and Integra Lifesciences (Plainsboro, NJ, USA) through TP-27-1846B1 as part of the Advanced Materials and Bioengineering Research Centre. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); In kind support was received from Integra LifeSciences (Plainsboro, NJ, USA) in the form of raw materials and technical knowhow. S.J. Archibald: A. Employment/Salary (full or part-time); Simon Archibald is employed by Integra LifeSciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Integra LifeSciences. F.J. O'Brien: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; This work was co-founded by Science Foundation Ireland and Integra Lifesciences (Plainsboro, NJ, USA) through TP-27-1846B1 as part of the Advanced Materials and Bioengineering Research Centre. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); In kind support was received from Integra LifeSciences (Plainsboro, NJ, USA) in the form of raw materials and technical knowhow.

Poster

301. Peripheral Nerve Injury

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 301.03/I27

Topic: C.10. Brain Injury and Trauma
Title: Long term neurological effects of acute exposure to a low or mild dose of sarin surrogate

Authors: *K. THIBAULT*¹, S. FRANCOIS², J. KNOERTZER¹, R. BEL¹, G. DAL BO¹; ¹Dept. de Toxicologie et Risques Chimiques, ²Dept. Effets Biologiques des Rayonnements, IRBA, Bretigny sur orge, France

Abstract: The symptoms, neuropharmacological mechanisms and toxic consequences of high dose organophosphate (OP) intoxications have been well documented. However, very little attention has been given to the possible deleterious effects of sub-lethal doses. Previous experiments studying asymptomatic exposure to OP have found various effects on physiological and behavioural functions: modification of cardiac functions, alterations of spatial orientation and disruption of EEG patterns. The aim of this study is to evaluate delayed effects of low dose exposure of sarin surrogate (4-nitrophenyl isopropyl methylphosphonate, NIMP) on mice behaviour. Mice are treated subcutaneously with 0.5 (n=15) and 0.9LD50 (n=16) of NIMP. We analyse time-course of intoxication signs and determine a scoring scale of intoxication. Behavioural tests are realized up to 6 months post-intoxication, to test strength, mechanical and thermal sensitivity, memory and anxiety. Results show anxiety occurrence during the first months after intoxication which disappear 3 months post-intoxication. Interestingly, anxiety is again observed 6 month after intoxication in 0.9DL50 animals. Moreover, we also observed mechanical and thermal sensitivity regardless the dose of interest coupled with a persistant inflammatory state and blood formulation deterioration at long term. Our findings provide evidence that low OP exposure intoxication induced behavioural and physiological long term impairments.

Disclosures: K. Thibault: None. S. Francois: None. J. Knoertzer: None. R. Bel: None. G. Dal bo: None.

Poster

301. Peripheral Nerve Injury

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 301.04/128

Topic: C.10. Brain Injury and Trauma

Support: NIH RO1 NS092754 to R.J.G.
NYSCIRB Institutional Grant C32245GG to R.J.G.
NIH R21 NS101298 to M.O.
NSFGRP DGE-1744655 to D.L.P.

Title: Schwann cell response to sustained release of fingolimod from fibrous biomaterials
Authors: *D. L. PUHL*¹, J. L. FUNNELL¹, A. R. D'AMATO¹, J. BAO¹, D. MORONE¹, Y. PRESSMAN², A. E. HAGGERTY², M. OUDEGA²³⁴, R. J. GILBERT¹;

Abstract: The peripheral nervous system (PNS) shows more robust regeneration following injury compared to the CNS. Such differences are due, in part, to the presence of Schwann cells in the PNS. Upon injury, Schwann cells dedifferentiate from their myelinating phenotype, unwrap from axons, and differentiate into a regenerative phenotype.¹ Electrospun fibers are used to support axon regeneration after injury. Fiber scaffolds can provide extended and localized release of therapeutic molecules and contact guidance for directed axon growth. Aligned electrospun fibers, however, promote the expression of the myelinating phenotype in Schwann cells², which may reduce the efficiency of Schwann cells to support axon regeneration. Fingolimod – an FDA-approved drug used to treat multiple sclerosis - shifts Schwann cells towards a regenerative phenotype.³ In the present study, we fabricated fingolimod-loaded electrospun fibers to direct neurite extension, while also shifting the phenotype of Schwann cells towards their regenerative phenotype. Fingolimod-loaded, aligned, poly(lactic-co-glycolic acid) (PLGA) fibers were electrospun on a vertical electrospinner using a 12% w/w polymer/solvent solution. Fibers were imaged using scanning electron microscopy and fiber alignment, diameter, and density were characterized using FIJI software. Primary dorsal root ganglia (DRG), consisting of mainly neurons and Schwann cells, were isolated from P2 Sprague Dawley rats. Whole and dissociated DRG were cultured on the fingolimod-loaded fibers for 4 days or 12 hours, respectively. Cultures were immunostained and imaged using a confocal microscope. Whole DRG neurite extension was measured using FIJI software, and dissociated DRG neurite extension was assessed using Neurolucida neuron tracing software. Finally, Schwann cells were cultured on fingolimod-loaded fibers for 4 days, and qPCR was used to assess gene expression. Fingolimod-loaded electrospun fibers increased neurite extension 1.5-fold from primary whole DRG (n≥14) and 2.5-fold from dissociated DRG neurons (n≥30). To our knowledge, this is the first study showing that fingolimod delivered by aligned electrospun fibers modulates Schwann cell phenotype and supports directional neurite extension.


**Poster**

**301. Peripheral Nerve Injury**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 301.05/DP05/I29

**Title:** Improving sensory function by utilizing vagus nerve stimulation after peripheral nerve injury

**Authors:** *M. J. DARROW*¹, T. MIAN², M. TORRES², Z. HAIDER², E. MEYERS¹, M. ROMERO-ORTEGA¹, M. KILGARD², S. HAYS¹; ¹Biomed. Engin., ²Neurosci., Univ. of Texas At Dallas, Richardson, TX

**Abstract:** There are in excess of 50,000 peripheral nerve repair procedures performed each year in the USA. The vast majority of these injuries occur in the upper limb. Impaired tactile sensation is common after peripheral nerve injury (PNI) and is associated with poor functional outcomes. Currently there is no well-established method to restore tactile sensation following peripheral nerve injury. However, recent evidence suggests that sensory retraining therapy can provide some benefit. When delivered during physical therapy, vagus nerve stimulation (VNS) has shown promise in augmenting neuroplasticity and improving motor function after stroke. VNS is used to reduce seizure frequency in some patients with epilepsy with a 30 seconds on, 5 minutes off stimulation paradigm. Animal research indicates that brief, 0.5 second bursts of 0.8 mA VNS triggers release of norepinephrine and acetylcholine in the cerebral cortex, which can restore synaptic connectivity after brain injury and improve function if delivered during rehabilitative training. It is not yet known, whether brief bursts of VNS paired with tactile discrimination training can improve the recovery of sensory function. Recently, a human case study paired tactile training with VNS and improved sensory function in a chronic stroke patient. We describe a design to test the hypothesis that repeated pairing of VNS with tactile training will drive improvements in tactile sensory function following a peripheral nerve injury. We observed that Tactile + VNS significantly reduced mecanosensory thresholds versus Tactile Alone and were sustained for a substantial period of time following the cessation of the therapy. These data demonstrate a novel method for restoration of sensory function following PNI which is readily translatable to the clinic.
Abstract: To repair transected peripheral nerves, biomaterial scaffolds could replace autografts. In our previous experiments, we found that biodegradable metallic magnesium (Mg) filaments, placed inside poly(caprolactone) (PCL) nerve guides, were compatible with axonal regeneration
and Mg filaments can provide physical contact guidance for regenerating axons. Here, we hypothesized that adding a Mg$^{2+}$ solution at surgery (Mg ions are neuroprotective, anti-inflammatory and improve cell attachment), will further promote axonal outgrowth. Experimental gaps made in one sciatic nerve of adult Lewis rats were repaired by placing both nerve stumps in hollow silicone guides. Two experimental groups received single Mg filaments (99.9% pure Mg, d=250μm), touching both nerve stumps, inside guides filled with saline or a MgSO$_4$ solution. Other groups had conduits with no filaments +/- MgSO$_4$ (negative controls) or isografts (positive controls). After 6 weeks, dissected nerves and conduits were imaged via microCT to assess Mg degradation, treated with iodine to provide soft tissue contrast and re-imaged by microCT. In all cases, Mg filaments were biocompatible: tissues encapsulated and surrounded Mg filaments. Mg metal degradation did not differ between groups. In guides with saline and a Mg filament, only 2/6 animals showed continuous tissue strands across the gaps. But in all empty guides and guides with MgSO$_4$ + Mg filament, the number increased to 5/6 animals with tissue strands. The cross-sectional area of tissue in the strands was measured in microCT images. With Mg metal + MgSO$_4$, there was a lack of tissue buildup at the proximal ends and a smoother transition to the center, which suggests better integration of tissue with the filaments. Analysis was done on distal stump tissues fixed with osmium tetroxide, embedded in resin, sectioned and stained with toluidine blue. All groups had comparable axon density, size and myelination. However, extensive amounts of intracellular vacuoles were observed in all conditions except Mg metal + MgSO$_4$. This suggests that the combined treatment resulted in tissue health enhancement and perhaps better resolution of Wallerian degeneration. In vitro experiments showed that adding MgSO$_4$ to culture media increased the degradation rate of Mg filaments and increased the attachment of Schwannoma cells (RT4-D6P2T) to Mg filaments. Our data demonstrated that increasing the concentration of Mg$^{2+}$ at the time of Mg metal implantation had a beneficial effect on nerve repair using Mg metal filaments and this might occur in part via increasing cell attachment to the Mg metal.


**Poster**

**301. Peripheral Nerve Injury**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 301.07/I31

**Topic:** C.10. Brain Injury and Trauma

**Title:** Influence of pannexin1 knockout on peripheral nerve regeneration
Authors: E. OROZCO, S. ADACHI, H. P. MAKARENKOVA, *S. B. SHAH; 
1Orthopaedic Surgery, 2Orthopaedic Surgery and Bioengineering, UCSD, La Jolla, CA; 3Mol. Med., Scripps Res., La Jolla, CA

Abstract: Peripheral nerve injuries are prevalent following traumatic injuries to the extremities, and often have severe consequences. Current strategies for repairing severe nerve injuries are often unsuccessful, leaving patients with motor dysfunction, sensory loss, and chronic pain. Among the factors posited to influence nerve regeneration are rates of axonal outgrowth and the favorability of the regenerative environment surrounding the axon, including inflammation. Pannexins are ATP releasing channels that work in coordination with type P2 purinergic receptors. Pannexins are expressed in almost all cell types, regulating multiple cell functions such as cell regeneration, wound healing, and inflammation. Previous studies using Pannexin-1 Knockout (KO) mice show an increase in axonal density and outgrowth in cultured DRGs, and reduced neuroinflammation. Based on these roles, we hypothesize that reducing levels of Pannexin-1 will enhance nerve regeneration by increasing axonal outgrowth and reducing inflammation following traumatic nerve injury. In our study, we compared several structural and behavioral sensorimotor regenerative outcomes were measured bilaterally in 8-10 week old Pannexin-1 KO and WT mice after unilateral sciatic nerve crush injury, at 0, 1, 4, 8, and 10 weeks of recovery. Von Frey hair testing was used to assess mechanical sensitivity, and hind limb grip strength and rotarod testing were also performed to test motor function. Immunolabeling with SMI-31 antibody (anti-phosphorylated neurofilament subunit H) was used to assess axonal number and density proximal and distal to the injury. Preliminary findings (n=5/group) suggest that knockout of Pannexin-1 channels resulted in no significant difference in mechanical filament sensitivity or hind limb grip strength, with the ipsilateral limb stronger than contralateral in both groups. Pannexin-1 KO mice lasted ~40% longer on the rotarod compared to the WT mice (Log transformed mean±SEM; WT: 2.84±0.28; KO: 3.15±0.08). Morphological assessment of regenerated nerves indicates robust axonal regrowth in Pannexin KO nerves, and assessment of the neuro-inflammatory environment is being performed. Our study shows that Pannexin KO does not enhance sensory regeneration, but may enhance motor recovery after nerve crush injury. References: 1. Makarenkova HP et al. Front Physiol. 5:63 (2014). 2. Horton et al. Front Cell Neurosci. 11:365 (2017). 3. Makarenkova HP et al. J Inflamm Res. 11:273-288 (2018).


Poster

301. Peripheral Nerve Injury

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 301.08/132
Evaluating vagus nerve stimulation paired with rehabilitation for sensory and motor dysfunction after radial nerve injury

Authors: *K. S. ADCOCK¹, T. DANAPHONGSE², Z. HAIDER¹, M. TORRES¹, R. A. MORRISON¹, S. A. HAYS³, M. P. KILGARD¹;
¹Behavioral and Brain Sci., ²Bioengineering, ³Univ. of Texas at Dallas, Richardson, TX

Abstract: Peripheral nerve injury (PNI) is a leading cause of lifelong disability in the United States. Most injuries occur in the upper limb, with the radial nerve being the most commonly injured. While injury to other forelimb nerves have been characterized in rodents, the effects of radial nerve injury has not yet been studied. In this set of experiments, we aimed to characterize motor and sensory function in rats with radial nerve injury. Additionally, we assessed whether vagus nerve stimulation (VNS), a therapy that has shown to enhance the benefits of rehabilitation when paired with a motor or sensory event, can be delivered as a therapeutic intervention to improve sensory motor impairment in a model of radial nerve injury. To test this, animals underwent behavioral training on a motor task, and were then given a PNI in which the radial nerve was completely transected in the trained forelimb. Following 10 weeks of reinnervation, animals were assessed on motor performance and mechanical sensitivity. A subset of animals received VNS paired with motor rehabilitation. Preliminary data suggests that injury to the radial nerve causes a long lasting motor impairment and mechanical hypersensitivity. Previous studies have shown that VNS paired with motor rehabilitation has been successful in improving motor recovery in animal models of traumatic brain injury, spinal cord injury, and stroke. While a recent study has demonstrated that VNS paired with rehabilitation can improve sensory loss, it is unknown whether VNS can reduce sensory hypersensitivity. These studies could lead to the development of a novel adjunctive therapy that could restore motor and sensory function in patients with peripheral nerve damage.


Poster

301. Peripheral Nerve Injury

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 301.09/I33

Topic: C.10. Brain Injury and Trauma

Support: CNPq
FAPERJ
CAPES
Title: Inosine enhances the regeneration and anticipates the functional recovery after sciatic nerve crush injury in mice

Authors: F. S. S. CARDOSO, R. CARDOSO, B. S. RAMALHO, T. B. TABOADA, A. S. NOGUEIRA, A. B. MARTINEZ, *F. M. ALMEIDA; UFRJ, Rio de Janeiro, Brazil

Abstract: Trauma to the peripheral nervous system (PNS) results in loss of motor and sensory functions. After an injury, a complex series of events begins, allowing axonal regeneration and target reinnervation. However, this regenerative potential is limited by several factors such as age, distance from the lesion site to the target and severity of lesion. Many studies look for ways to overcome these limitations. Inosine, a purine nucleoside derived from adenosine, emerges as a potential treatment, due to its capacity to regulate axonal growth, neuroprotection and immunomodulation, contributing to motor recovery. However, no studies demonstrated their effects on PNS. C57/Black6 mice were submitted to sciatic nerve crush and received intraperitoneal injections of saline or inosine (70 mg/kg), one hour after injury and daily for one week. To evaluate axonal regeneration and functional recovery, electroneuromyography, Sciatic Function Index (SFI), rotarod and pinprick tests were performed. Our results showed that the inosine group presented a higher number of myelinated fibers and a large amount of fibers within the ideal G-ratio. In addition, the results of electroneuromyography showed greater amplitude of the compound muscle action potentials in the first and second weeks, suggesting anticipation of regeneration in the inosine group. We also observed in the inosine group, motor and sensory neurons survival, reduction in the number of macrophages and myelin ovoids in the sciatic nerves, and an early recovery of motor and sensory functions. Thus, we conclude that the use of inosine accelerates axonal regeneration promoting an early recovery of motor and sensory functions.


Poster

301. Peripheral Nerve Injury

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 301.10/I34

Topic: C.10. Brain Injury and Trauma

Title: Single-unit recording reveal increased conduction delay and reversible conduction block in peripheral nerve axons undergoing tensile stretch

Authors: *N. GANESHBABU1, T. GUO2, L. CHEN2, B. FENG2; 1Physiol. and Neurobio., 2Biomed. Engin., Univ. of Connecticut, Storrs, CT
Abstract: Background: Peripheral nerves are mechanically stretched in physiological conditions. However, excessive stretch of peripheral nerves can occur during trauma or surgery. Previous biomechanical studies indicate no apparent histological damages to peripheral nerve axons with elongation less than 15%. The impact of nerve tension on action potential transmission has been mainly studied by whole-nerve recordings of compound action potentials (CAP), indicating progressive reduction of CAP amplitude and increase of conduction delay. But the exact effect of nerve tension on action potential transmission at the level of an individual axon is not well understood. In this study, we developed a novel ex vivo setup that allows robust single-unit recordings from mouse sciatic nerve axons while delivering computer-controlled tensile stretch to the sciatic nerve trunk. Methods: From C57BL/6 mice, sciatic nerves were dissected from the L3, L4, and L5 entry points to the spinal cord to the distal branches of sural, common peroneal, and tibia nerves. The proximal L4 spinal nerve and the distal common peroneal branch were tied with a fine silk suture at ~20 mm apart and placed in a custom-built tissue chamber perfused with oxygenated Krebs solution. The L3, L4, and L5 spinal nerves were pulled into the adjacent recording chamber filled with mineral oil. The L4 spinal nerve was pinned down and the distal peroneal branch stretched by 60-second steps from 0.5 to 5 mm in 0.5 mm increments (Aurora Scientific, 300D). Action potentials were evoked by a suction electrode from the distal end of the sural nerve and recorded at individual axons from the split filaments of the L5 spinal nerve by a platinum-iridium wire electrode. Results: Conduction delay showed instantaneous increase at the onset of stepped stretch and further progressive increase during the 60-sec step in both myelinated A- and unmyelinated C-fibers. Removal of stretch led to instantaneous but slight reduction in conduction delay. Complete conduction block was observed with excessive stretch. In some axons, the conduction block was reversed after removal of stretch. Conclusions: Action potential transmission in peripheral axons can be significantly affected even by 2-3% of tensile stretch, resulting in instantaneous increase in conduction delay. Excessive stretch can lead to reversible transmission block. The current study provides a solid experimental foundation to enhance our biophysical understanding of stretch-induced peripheral neuropathy.

Disclosures: N. Ganeshbabu: None. T. Guo: None. L. Chen: None. B. Feng: None.

Poster

301. Peripheral Nerve Injury

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 301.11/I35

Topic: C.10. Brain Injury and Trauma

Support: R01 NS069844

Title: The transmembrane protein Raw promotes Wallerian degeneration in Drosophila by restraining a protective JNK signaling pathway
Abstract: Mitogen Activated Protein Kinase (MAPK) signaling via the c-Jun N-terminal Kinase (JNK) is known to regulate multiple cellular responses to axonal injury. Inhibition of JNK impairs the ability of neurons to initiate new axonal regeneration in the peripheral nervous system, and also impairs the ability of distal axons that are removed from their cell body to undergo Wallerian degeneration. Recently, our lab identified the Drosophila transmembrane protein Raw as a pro-degenerative protein whose loss strongly delays the degeneration of severed neurites. The delay of degeneration in raw mutants is sensitive to dominant negative inhibition of JNK and downstream transcription factors Fos and c-Jun in motoneurons. These observations suggest the existence of a protective pathway mediated by JNK signaling that influences the resilience of axons to degeneration, which contrasts with the previously known roles for JNK in promoting axonal degeneration. In Drosophila the dual leucine zipper kinase DLK (known as Wallenda in Drosophila) regulates a MAPK signaling pathway that can inhibit degeneration following its activation in a preconditioning injury (Shin et al., 2012). However, our genetic data indicate that Raw and Wallenda signaling function independently. We propose that Raw represents a new inroad to disentangle multiple protective and degenerative pathways regulated by JNK. We will present current work to understand the mechanism of axonal protection in raw mutants, which appears resistant to reduced levels of the of the NAD biosynthetic protein NMNAT, an important protective factor in axons.

References

Disclosures: T. Waller: None. Y. Hao: None. C.A. Collins: None.
Abstract: Nerve damage and neuropathies can contribute to pain, loss of sensation, weakness, and systemic complications. Localized gene delivery to nerve tissue has the potential to enable many novel therapies for these conditions. Current delivery methods face many practical and translational hurdles, including heavy reliance on viral infection, stochasticity, lack of specificity, and cellular damage. Unique nerve anatomy presents challenges for targeted delivery to axons of motor and sensory neurons which can span both the central (CNS) and peripheral nervous system (PNS). Our novel non-viral tissue nano-transfection (TNT) chip platform can be used to deliver therapeutic cargo to nerve tissue at both levels (i.e., peripherally and centrally) via the use of solid state nanochannels, coupled with nano-electroporation and nano-electrophoresis. Such nanochannels were fabricated via a combination of cleanroom-based manufacturing techniques. This novel platform was then used to controllably deliver a variety of cargos to the CNS and PNS of mice, including plasmid DNA and CRISPR/Cas9 components. Nano-electroporation conditions were optimized by delivering labeled plasmids at different voltages and pulse lengths. Delivery efficacy and retrograde transport from PNS to CNS was evaluated via immunofluorescence microscopy and qRT-PCR at different levels, including peripheral nerve bundles, dorsal root ganglion (DRG), and spinal cord (SC). Laser speckle imaging and electrophysiology measurements were used to evaluate potential alterations in perfusion and functionality post-TNT. Tissue sections collected shortly after transfection revealed successful cargo delivery following a short-lived (<100 ms) pulsed electric field across the nanostructured platform. Fluorescence intensity demonstrated up to ~50,000 fold change with respect to controls when varying voltage alone, and differences across groups of up to 20x when varying the duration of nano-electrophoresis. Immunofluorescence analysis and qRT-PCR confirmed tissue transfection and strong plasmid DNA and CRISPR/Cas9 activity at the peripheral nerve level, with varying degrees of expression/activity at the DRG and SC levels depending on the nano-electroporation conditions. No significant behavioral changes (e.g., paw clenching, gait perturbations) were noted in treated mice. Our nanostructured platform, with the use of non-viral cargo, showed the ability to efficiently transfect peripheral and central nerve tissue in a targeted and controlled manner. Ongoing studies focus on modulating tissue repair via induced tissue plasticity following TNT-based delivery of reprogramming factors.


Poster

301. Peripheral Nerve Injury

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: 301.13/I37
Title: Rapid depletion of the ESCRT protein Vps4 underlies injury-induced autophagic impedance and Wallerian degeneration

Authors: H. WANG, K. ZHANG, Q. WANG, S. ZHANG, *Y. (. FANG;
Interdisciplinary Res. Ctr. on Biol. and Chem., SIOC, Chinese Acad. of Sci., Shanghai, China

Abstract: Axonal degeneration is a prominent feature of acute neural injury and chronic neurodegenerative diseases. In particular, the distal segment of injured axons undergoes an active and highly regulated self-destruction process, termed Wallerian degeneration. Recent work of Wallerian degeneration has been centered on the genes and pathways regulating NAD+ metabolism such as the NAD+ synthase Nmnat and the NAD+ hydrolase SARM1. However, apart from the NAD+ mechanism, other crucial signal transduction pathways involved in axonal injury and degeneration remain poorly understood.

In our previous work, we developed an in vivo model of nerve injury using the Drosophila wing (Fang et al., 2012; Fang et al., 2013; Fang and Bonini, 2015). In this study, we have combined multiple in vitro and in vivo neural injury models, including the Drosophila wing nerve, primary mouse DRG neurons, the mouse optic nerve, and the spinal cord of cynomolgus monkeys to investigate the role and regulation of autophagy in axonal degeneration. We found that the basal levels of axonal autophagy (evident by the formation of mCherry-Atg8a puncta) are low in general, even in aged flies. However, upon axotomy, there is a rapid and massive autophagy induction in the distal segment of the injured axons. The response can be seen as early as 3 hr after injury and is much earlier than when fly axons start to degenerate (12~24 hr).

Next, by performing a transgenic RNAi screen in flies, we identified the ESCRT component Vps4 as a novel essential gene for axonal integrity (Wang et al., 2019). We found that upregulation of Vps4 significantly delays degeneration of injured fly wing axons. We further revealed that Vps4 is required and sufficient to promote autophagic flux in axons and mammalian cells. Moreover, using both in vitro and in vivo models, we showed that the function of Vps4 in maintaining axonal autophagy and suppressing Wallerian degeneration is conserved in mammals. Finally, we uncover that the Vps4 protein is rapidly depleted in injured mouse DRG axons as well as the spinal cord of monkeys, which may underlie the injury-induced autophagic impedance and the subsequent axonal degeneration. Together, Vps4 and ESCRT may represent a novel signal transduction mechanism in axonal injury and Wallerian degeneration.

Key reference:

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Poster

301. Peripheral Nerve Injury

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 301.14/I38

Topic: C.10. Brain Injury and Trauma

Support: Innovation Award, Thompson Family Foundation Initiative at Columbia University

Title: Integrin protects nociceptive neurons in models of paclitaxel-mediated peripheral sensory neuropathy

Authors: *G. J.-E. SHIN1, M. PERO2,4, L. HAMMOND1, A. BURGOS1, A. KUMAR2, F. BARTOLINI2, W. B. GRUEBER1,3, 

Abstract: Chemotherapy induced peripheral neuropathy (CIPN) is a major side effect from cancer treatment with no known method for prevention or cure in clinics. CIPN primarily affects unmyelinated nociceptive sensory terminals. Despite the high prevalence of CIPN, molecular and cellular mechanisms that lead to CIPN are still poorly understood. Here, we used a genetically tractable Drosophila in vivo model and primary sensory neurons isolated from adult mouse to examine the mechanisms underlying CIPN and protective pathways. Using nociceptive neurons in Drosophila larvae we found that chronic treatment with paclitaxel resulted in neuronal degeneration and a change in branch distribution across the sensory terminal field. These larvae also showed a reduced thermal nociceptive response, mimicking the pathology of CIPN patients presenting loss of thermal nociception. We subsequently found that nociceptive neuron-specific overexpression of integrins, heteromeric receptors that link cell membranes to the extracellular matrix, protected Drosophila larvae from paclitaxel-mediated cellular and behavioral phenotypes. We found that this protection is specific to integrins, suggesting that facilitating connections between neuronal arbors and surrounding extracellular matrix is critical in maintaining the morphology and the function of nociceptive neurons against paclitaxel insult. Using primary dorsal root ganglia neuron cultures isolated from adult mouse, we further show that overexpression of human ITGB1, a common β subunit in integrin heterodimers expressed in vertebrate sensory neurons, also prevented degeneration following paclitaxel treatment. Our preliminary results indicate that endogenous integrin protein levels decrease following paclitaxel treatment in Drosophila in vivo and primary sensory neurons isolated from adult mouse, suggesting a paclitaxel-mediated induction of lysosomal integrin degradation. Using live
imaging and superresolution approaches, we provide supportive evidence that paclitaxel treatment changed the endosomal-lysosomal pathway in *Drosophila in vivo*. We found that lysosomes were often enlarged by paclitaxel treatment, whereas recycling endosomes co-localized less with integrins and showed reduced mobility. Altogether, our study suggests conserved mechanisms of paclitaxel-induced integrin degradation and the therapeutic potential of restoring integrin levels to antagonize paclitaxel-mediated toxicity in sensory neurons.


**Poster**

**302. Neural Injury and Treatment**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 302.01/I39

**Topic:** C.11. Spinal Cord Injury and Plasticity

**Title:** Antiallodynic effect of GPR55 and GPR119 receptors in processing neuropathic pain in rat

**Authors:** *A. ZUÑIGA*¹, H. ROCHA-GONZALEZ¹, F. FLORES-MURRIETA², J. REYES-GARCIA¹;

¹ESM-IPN, Mexico, Mexico; ²INER, Mexico, Mexico

**Abstract:** Orphan G-protein coupled receptors are proteins that represent a wide range of new therapeutic targets with biological functions to be discovered. GPR55 and GPR119 receptors have been reported on neurons of the nervous system, so they could play an important role in the modulation of neuropathic pain. The aim of this work was to evaluate the antiallodynic effect of GPR55 and GPR119 receptors in the treatment of neuropathic pain induced by L5-L6 spinal cord ligation in rat. Mechanical allodynia was measured using von Frey filaments by the Kim and Chung model. Male Wistar rats (180-200 g) were submitted to L5-L6 spinal nerves ligation to induce neuropathic pain. After two weeks of recovery, the rats were administered intrathecally with saline, or increasing doses of antagonist CID16020046 (GPR55 antagonist, 1-300 μg), O-1062 (GPR55 antagonist, 30-300 μg), AS1269574 (GPR119 agonist, 1-300μg) or G-protein antagonist peptide (non-selective GPR119 antagonist, 0.001-1ng). After administration, CID16020046 and AS1269574 had an efficacy of 52.1% ± 2.3 and 58.8% ± 1.9, respectively. In addition, intrathecal administration of O-1062 and the G-protein antagonist peptide decreased the antiallodynic effect induced by CID16020046 and AS1269574, respectively. Data suggest that GPR55 and GPR119 receptors participate in the modulation of neuropathic pain.

**Disclosures:** A. Zuñiga: None. H. Rocha-gonzalez: None. F. Flores-murrieta: None. J. Reyes-garcia: None.
**Poster**

**302. Neural Injury and Treatment**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 302.02/I40

**Topic:** C.11. Spinal Cord Injury and Plasticity

**Support:** MAFAT Grant 01021574

**Title:** Blood-glutamate scavenging: An effective neuroprotective treatment in spinal cord injury model

**Authors:** *A. MATUZANY-RUBAN¹, Y. GOLDSHMIT², E. BANIAS³;
²Steyer Sch. of Hlth. Professions, Fac. of Med., ³Fac. of Med., ¹Tel Aviv Univ., Tel Aviv, Israel

**Abstract:** Neurotrauma causes immediate elevation of extracellular glutamate levels, which creates excitotoxicity, scar formation and consequently neuronal death. We have implanted a novel strategy to reduce excitotoxicity by administration of a blood Glu scavenger (BGS) such as the blood resident enzyme and its co-substrate. In our recent study, we demonstrated that administration of BGS induces a remarkable protection of neurons and axonal regeneration, and as a result, a significant functional recovery in spinal cord injury model. The role of exercising in enhancing recovery following spinal lesions has also been studied extensively. Although exercising does not facilitate axonal regeneration, a combination of BGS with exercise has the potential to reduce scar formation and neuronal death, and to enable regeneration through the lesion site.

**Objective** To evaluate the effect of BGS treatment and/or exercises on survival of neurons at lesion sites, as well as on scar formation and axonal regeneration after spinal cord injury.

**Study design**

In this study, we induced a spinal cord injury (hemisection) in mice. Immediately after SCI mice were randomly divided into (1) untreated (n=7), (2) BGS treated (n=9), (3) exercise (n=7) and (4) BGS treatment combined with exercises groups (n=9), in accordance with power analysis. Mice were treated with BGSs for five consecutive days, followed by exercises for three months. Immunostaining was performed in order to characterize the neuroprotective effects of BGS. In addition, anterograde tracing was carried out fourteen weeks after lesion induction and the labeled axons were quantified.

**Results** So far, our results have demonstrated that the combined treatment has decreased the inflammatory cell's activation significantly more than each treatment by itself (% of decrease of Iba1 immunofluorescence vs. vehicle-control: BGS 25.6±9.5, exercise 21.6±12.3, combined treatment 43.6±12.3). Examination of lesion size and GFAP density around the lesion site, has revealed that only the BGS treatment has decreased lesion size and attenuated the astrocytic gliosis, while treadmill training alone had no effect on these parameters (% of decrease of GFAP...
immunofluorescence compared to vehicle-control: BGS 32.5±11.5, exercise 13.9±7.9, combine (31.9±6.3) p<0.05; one way ANOVA, Bonferroni's Multiple Comparison Test). Effect of the treatments on axonal regeneration currently under analysis

Summary
Our results strongly support the hypothesis that excess Glu plays a significant negative role in contributing to motor decline and early mortality in SCI. Thus, its removal is of particular therapeutic relevance.

Disclosures: A. Matuzany-Ruban: None. Y. Goldshmit: None. E. Banias: None.

Poster

302. Neural Injury and Treatment

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 302.03/I41


Support: NIH Grant HD097737

Title: Targeting HIF1a for axon regeneration

Authors: *P. J. WARD1, Z. HASSAN2, K. CAMPBELL2, C. WYNANS2, S. WARIYAR1, O. MISTRETTA1;
1Emory Univ. Sch. of Med., Atlanta, GA; 2Emory Univ. Col. of Arts and Sci., Atlanta, GA

Abstract: Compared to the central nervous system (CNS), peripheral nervous system (PNS) neurons mount a limited regenerative program after axonal injury that can be further enhanced by activity-dependent therapies, such as exercise. The mechanism by which neuronal activity enhances axon regeneration is incompletely understood, and defining how injured neurons respond to activity-dependent therapies may reveal new therapeutic targets to improve recovery following both CNS and PNS injuries. We hypothesized that exercise utilizes an oxygen-sensitive transcription factor, HIF1a, to enhance axon regeneration following injury. We found that running exercise caused nuclear translocation of HIF1a in axotomized sensory and motoneurons leading to enhanced axon regeneration and improved functional recovery following nerve injury. Additionally, we identified a pharmacologic inhibitor (FG4592) of prolyl hydroxylases that stabilizes HIF1a. FG4592 increased DRG neurite outgrowth in vitro and enhanced axon regeneration in vivo. Manipulation of HIF1a via activity-dependent therapies or pharmacologically may lead to significant improvements in axon regeneration and functional recovery.

Disclosures: P.J. Ward: None. Z. Hassan: None. K. Campbell: None. C. Wynans: None. S. Wariyar: None. O. Mistretta: None.
Poster

302. Neural Injury and Treatment

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 302.04/142


Support: FAPESP - 2016/25478-9

Title: Neuroprotection and immunomodulation by dimethyl fumarate after ventral root avulsion and reimplantation

Authors: *P. R. KEMPE¹, G. B. CHIAROTTO¹, B. BARRAVIERA², R. S. FERREIRA, Jr², A. L. OLIVEIRA¹;
¹Lab. of Nerve Regeneration, Univ. of Campinas, Campinas, Brazil; ²Ctr. for the Study of Venoms and Venomous Animals, São Paulo State Univ., Botucatu, Brazil

Abstract: Brachial plexus lesion within the spinal canal, including the avulsion of roots, is a recurrent outcome of high-energy trauma following impact of the forequarter, with dislocation of the shoulder girdle. Experimentally, ventral root avulsion (VRA) can be obtained in rats after an abrupt separation of the motor roots from the surface of the spinal cord, what results in ipsilateral paralysis of the affected limb. As a result, most of the axotomized motoneurons degenerate up to the second week post-injury, combined with a significant loss of synapses and increased glial reaction, triggering a chronic inflammatory state. It is believed that pharmacological treatment associated with roots reimplantation can overcome the degenerative effects of VRA. Therefore, our goal was to test if dimethyl-fumarate (DMF), an FDA approved drug with neuroprotective and immunomodulatory effects, associated with fibrin sealant (FS), a biological glue used for tissue repair, could improve regenerative response and lead to motor function recovery. Thus, adult female Lewis rats were subjected to unilateral VRA of L4-L6 roots followed by reimplantation and daily treatment with DMF (15 mg/Kg) for one or four weeks. Control group was VRA followed by vehicle treatment. The evaluated survival times post-surgery were 1 and 12 weeks. The analysis involved neuronal survival by Nissl staining, glial reactivity by immunofluorescence (anti-GFAP for astrocytes and anti-Iba-1 for microglia), synapse preservation (anti-VGLUT1 for glutamatergic inputs and anti-GAD65 for GABAergic inputs), gene expression (pro- and anti-inflammatory cytokines and interleukins) and motor function recovery (Catwalk system). Our results indicate that DMF treatment is neuroprotective and immunomodulatory since it preserves 36% of motoneurons and around 70% of GABAergic and glutamatergic synapses. DMF also decreased astrogliosis and microglial reaction combined with downregulation of pro-inflammatory gene transcripts. Importantly, the pharmacological treatment was further enhanced when associated with root reimplantation using FS, reaching 70% of motoneuron survival and more than 80% of synaptic inputs preservation. Also, glial
reaction decreased almost by a half. Conversely, such protective effects reflected in 50% motor function improvement, showing the efficacy of employing combined regenerative approaches following spinal cord root injury.


Poster

302. Neural Injury and Treatment

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 302.05/I43


Support: KAKENHI Grant 17H04318

Title: The efficacy of C5a receptor antagonist for human iPSC-derived neural stem/progenitor cell transplantation in the injured spinal cord of mice

Authors: *R. SHIBATA*¹², N. NAGOSHI¹, M. KHAZAEI³, M. G. FEHLINGS³, M. MATSUMOTO¹, H. OKANO², M. NAKAMURA¹;
¹Dept. of Orthopaedic Surgery, Keio Univ. Sch. of Med., Tokyo, Japan; ²Dept. of Physiology, Keio Univ. Sch. of Med., Tokyo, Japan; ³Dept. of Genet. and Develop., Krembl Res. Institute, Univ. Hlth. Netw, Toronto, ON, Canada

Abstract: [Introduction] We previously reported the efficacy of human-iPS derived neural stem/progenitor cell transplantation for spinal cord injury (SCI) in the subacute phase. However, this procedure is not effective in the acute phase due to the inflammatory response occurring immediately after SCI, which impacts transplanted cell survival and differentiation. C5a, which is one of the complement components, is a powerful chemoattractant and recruits inflammatory cells through binding C5a receptor. Therefore, the objective of this study is to suppress the inflammatory response immediately after SCI using C5a receptor antagonist (C5aRA) as an immunosuppressant, thus improving the efficacy of hiPSC-NS/PC transplantation for SCI in acute phase. [Methods] We used immuodeficient SCID-Beige mice that lack lymphocytes and NK cells. First, to evaluate the influence of C5aRA on the inflammatory response post-SCI, we induced a thoracic spinal contusion injury in mice. We quantified inflammatory cytokines and inflammatory cells in injured spinal cord tissues using qPCR and flow-cytometry. Next, we divided the SCI mice into 4 groups (PBS only, C5aRA only, PBS + transplantation (PBS+TP), C5aRA + transplantation (C5aRA+TP)). Immediately after SCI, C5aRA or PBS was administrated once a day for 4 consecutive days, and then, 5.0 × 10⁵ hiPSC-NS/PCs were transplanted into the lesion epicenter on day 4. We evaluated cell survival rate by Bioluminescent Imaging (BLI), hindlimb motor function by BMS score, and the
differentiation profile of the graft hiPSC-NS/PCs by immunohistochemistry. [Results] C5aRA administration significantly reduced IL-1b, IL-6 and TNFα at 12 hours and macrophages at 4 days after SCI (p<0.05). The C5aRA+TP group had better functional improvement as compared to the PBS only group (p<0.05). BLI revealed that the C5aRA+TP group had a significantly higher cell survival rate compared to the PBS +TP group (p<0.05). There was no significant difference in the differentiation profiles of the graft hiPSC-NS/PCs between C5aRA+TP group and PBS+TP group. [Conclusion] The present study demonstrated that administration of C5aRA could suppress the inflammatory response during the acute phase of SCI, and also improve the survival rate of transplanted hiPSC-NS/PCs and enhance motor functional restoration. hiPSC-NS/PCs transplanted with C5aRA are a promising treatment for acute phase SCI patients.


Poster

302. Neural Injury and Treatment

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 302.06/I44


Support: NRF-2015H1D3A1066543
       NRF-2017R1C1B2011772
       HI16C1559

Title: TRPV4 instigate endothelial disruption and secondary damage in spinal cord injury

Authors: *H. KUMAR¹, I. HAN²;
1CHA Univ., Seongnamsi, Korea, Republic of; 2CHA Univ., Seongnam, Korea, Republic of

Abstract: Transient receptor potential vanilloid type 4 (TRPV4), is a polymodal ionotropic receptor and plays a vital role in a multitude of physiological processes and upregulated in a variety of pathological conditions. TRPV4 expression and function is not known after spinal cord injury (SCI). We examined the TRPV4 role and its involvement in major biological cascades in the pathology of SCI. We studied in a clinically relevant model of moderate compression (35 g for 5 min at T10 level in rats) for SCI. Also, we checked the TRPV4 expression in injury dependent manner (compression using 20 g, 35 g and 50 g for 5 min) and transaction model of SCI. We quantitatively estimate Ca²⁺ at the same time points using two-photon microscopy and co-related the TRPV4 expression with Ca²⁺ after SCI. Interestingly, the TRPV4 expression was increased during the inflammatory phase after SCI and was linked with endothelial damage. Activation of TRPV4 was associated with endothelial damage and TRPV4 inhibition using specific antagonist (RN-1734 5 mg/kg, i.p.) attenuated the inflammatory cytokines, chemokines,
promotes vascular stabilization and prevented the tight junctions protein degradation and blood-spinal cord barrier (BSCB) break down after SCI. Likewise, TRPV4 KO mouse showed reduced inflammation and prevented the tight junctions protein degradation and BSCB breakdown after SCI (20 g for 1 min). Moreover, TRPV4 KO mouse abridged secondary damage as observed by a reduction in glial scar formation and protection of endothelial cell as compared with wild-type mouse. Thus, our result suggests that increased TRPV4 expression was associated with the early inflammatory phase of SCI, tissue damage, vascular destabilization, BSCB breakdown, and cell injury. TRPV4 inhibition serves as a promising therapeutic strategy to attenuate neuropathic pain, secondary damage and promoting vascular stabilization after SCI.

**Keywords:** TRPV4; Spinal Cord Injury; Vascular stabilization; Inflammation; Blood-spinal cord barrier, Neuropathic pain, Endothelial Cells

**Disclosures:** H. Kumar: None. I. Han: None.

**Poster**

**302. Neural Injury and Treatment**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 302.07/J1

**Topic:** C.11. Spinal Cord Injury and Plasticity

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Raquel Oliveira PhD fellowship from NORTE2020, ref. NORTE-08-5369-FSE-000026
POCI-01-0145-FEDER-007274

**Title:** The success of onabotulinumtoxina in treating neurogenic bladder after spinal cord injury: Mechanisms beyond SNAP-25 cleavage

**Authors:** *R. OLIVEIRA*¹³, S. CHAMBEL¹³, F. CRUZ²³⁴, C. D. CRUZ¹³;

**Abstract: Introduction & Aims** Injections of onabotulinumtoxinA (Onabot/A) into the bladder wall is the gold-standard treatment for neurogenic detrusor overactivity (NDO), leading to long-lasting improvements on urinary function. Onabot/A impairs vesicle-mediated neurotransmission on motor, autonomic and sensory bladder fibers by specifically cleaving the synaptic associated protein (SNAP-25). However, mechanisms underlying the enduring effects of the toxin on the lower urinary tract are still mostly unknown, but likely reflects neuronal stress. Here we investigated signs of neuronal injury and stress in bladder afferents after treatment with Onabot/A. **Material & Methods** Female rats were submitted to T8/T9 largely incomplete spinal
cord transection (SCT). Control animals underwent sham surgery. Awake cystometries were performed at baseline, 1 week and 4 weeks after SCT to evaluate the development of urinary dysfunction. At 4 weeks post-SCT, when NDO is typically present, rats received 10 bladder injections of Onabot/A 10U diluted in 50uL of saline. Control rats received saline. Three days later, awake cystometries were performed, after which rats were euthanized and bladders, dorsal root ganglia (DRG) L5-S1 and spinal cords (SC) collected. Bladders and SC were processed for immunohistochemistry and western blotting (WB) to evaluate the catalytic activity of Onabot/A and DRG were processed for WB to measure levels of cell stress markers and primary cell culture to evaluate neuronal growth and dendritic branching. **Results** Four weeks after SCT, rats presented increased bladder pressure and frequent bladder contractions associated with high voided volumes. Onabot/A treatment cleaved SNAP-25 in the bladder wall and improved urinary function. This was accompanied by upregulation of cellular stress markers, such as ATF3 and PERK. In vitro assays, used to evaluate intrinsic ability of terminal growth, suggested a tendency for reduced dendritic length in Onabot/A-treated SCT rats, but not in control animals. **Conclusions** Preliminary results suggest that improvements in NDO following bladder treatment with Onabot/A may reflect the occurrence of metabolic injury in affected bladder afferents, along SNAP25 cleavage. Induced neuronal stress could justify the prolonged duration of Onabot/A beneficial effects in SCI-induced bladder dysfunction.

**Disclosures:** R. Oliveira: None. S. Chambel: None. F. Cruz: None. C.D. Cruz: None.

**Poster**

**302. Neural Injury and Treatment**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Posterm #:** 302.08/12

**Topic:** C.11. Spinal Cord Injury and Plasticity

**Support:** NIH NINDS 5RO1NS092680-02

**Title:** Targeting intra-axonal RyR and IP$_3$R mediated Ca$^{2+}$reduces secondary axonal degeneration following a contusive-SCI *in vivo*

**Authors:** *B. C. OREM$^1$, A. RAJAEE$^2$, D. P. STIRLING$^3$; $^1$Anatom. Sci. and Neurobiology, Kentucky Spinal Cord Injury Res. Ctr., $^2$Kentucky Spinal Cord Injury Res. Center, Neurolog. Surgery, Univ. of Louisville, Louisville, KY; $^3$Neurolog. Surgery & Microbiology and Immunol., KSCIRC, Univ. of Louisville, Louisville, KY

**Abstract:** Preventing axon loss after traumatic spinal cord injury (SCI) is an important therapeutic goal. Axonal Ca$^{2+}$ overload is established as a key facilitator of axonal injury following SCI. Previously we showed that intracellular calcium release through both RyRs and IP$_3$Rs contributes to axonal dieback and axonal loss following an *ex vivo* laser-induced SCI.
Here, we use an intravital imaging approach to assess the role of RyR- and IP$_3$R-mediated Ca$^{2+}$ release on secondary axonal degeneration following a clinically-relevant in vivo contusion model in real-time. We hypothesize that blocking intracellular Ca$^{2+}$ release from RyR and IP$_3$R will diminish secondary axonal degeneration after SCI. Briefly, adult, 6-8 week old Advillin-Cre:Ai9 mice that express “floxed” tdTomato in ascending dorsal columns of the spinal cord were imaged using two-photon excitation microscopy. Mice were subjected to a mild, 30 kdyn contusion at T12 and received the RyR blocker Ryanodine (50 µM) or the IP$_3$R blocker 2-APB (100 µM) intrathecally 3 hr and 24 hr post-SCI. We found that Ryanodine treatment significantly increased axonal survival at 24 hr post-SCI (Ryanodine, 54.1 ± 12.9 %; mean percentage normalized to baseline ± SD; control, 34.6 ± 7.7 %, p<0.05; t-test, N = 5-7 mice per group) compared to vehicle controls. Similarly, inhibition of IP$_3$R with 2-APB significantly increased axonal survival (2-APB, 55.8 ± 16.3 %; control, 35.6 ± 10.7 %, p<0.05, Mann-Whitney Rank Sum test, N = 6 mice per group) compared to vehicle at 24 hr post injury. Together, these data reveal a role of RyRs and IP$_3$Rs on axonal degeneration in vivo after SCI and suggest that blocking intracellular Ca$^{2+}$ release is axoprotective in a clinically relevant contusion model.

**Disclosures:** B.C. Orem: None. A. Rajae: None. D.P. Stirling: None.

**Poster**

**302. Neural Injury and Treatment**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 302.09/J3

**Topic:** C.11. Spinal Cord Injury and Plasticity

**Support:** Shriners Hospitals for Children Research Grant (#85115)
Craig H. Neilsen Foundation Senior Research Grant (#546798)

**Title:** Chemogenetic modulation of afferents via DREADDs affects kinematics in a rat contusion model of SCI

**Authors:** *K. M. KEEFE*¹, J. T. EISDORFER¹, T. HALLOWELL³, O. HAJI-MAGHSOUDI⁴, K. M. RAUSCHER¹, G. M. SMITH², M. LEMAY¹, A. J. SPENCE¹;

**Abstract:** Spinal cord injury (SCI) causes life-long neurological impairment, significantly affecting quality of life. Previous studies demonstrate that epidural stimulation of sensory neurons can improve functional outcomes after SCI. However, this type of stimulation is not selective to neuronal subtypes and thus the contribution of specific fibers to recovery is difficult to discern. Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) are
chemogenetic tools used to selectively control neurons with administration of its ligand, clozapine-N-oxide (CNO). DREADDs could therefore be used to evaluate how subsets of sensory neurons (e.g. proprioceptors vs. cutaneous afferents) influence recovery after SCI. This study aimed to determine whether modulation of large diameter sensory afferents with DREADDs can affect locomotor functional outcomes after a contusive SCI. An adeno-associated viral vector (AAV2) encapsulating the excitatory DREADD (hM3Dq) was injected into L1-L3 dorsal root ganglia (DRG) prior to SCI. Excitatory DREADDs act to depolarize neurons and increase their baseline excitability. The lumbar DRG were chosen as their rostral segments are hypothesized to contain central pattern generating circuitry in rats. High-speed video kinematics were recorded at 3, 4, and 6 weeks after contusion with administration of CNO (4 mg/kg). A PCA of 28 kinematic variables was analyzed in no-injury, contused with CNO administration and sham DRG surgery, and contused with excitatory DREADDs afferent activation by CNO groups. The first 3 principal components (PCs) of the PCA explained 77% of the variance (n=11 rats). DREADDs activation caused an increase in PC2, which was most heavily loaded on maximum hip extension. These data are consistent with our targeting of L1-L3 DRGs, which supply muscles primarily about the hip, and simulation results by other groups suggesting that afferent stimulation extends joints. Subsequent examination of mean hip angle specifically showed a significant difference between SCI-DREADDs-CNO and SCI-Sham-CNO animals (t-test; p<0.05; n=11 rats). These results suggest that chemogenetic modulation of afferents can affect kinematics in a rat contusion model of spinal cord injury.


Poster

302. Neural Injury and Treatment

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 302.10/J4


Title: Neuroprotective effect of sodium propionate in neuroinflammation models

Authors: *I. PATERNITI, M. LANZA, G. CASILI, A. FILIPPONE, M. CAMPOLO, S. CUZZOCREA, E. ESPOSITO;
Univ. of Messina, Messina, Italy

Abstract: Sodium propionate (SP) is one of the main short chain fatty acids (SCFA) that can be produced naturally through host metabolic pathways. The physiological effects of SP have been documented and include the reduction of pro-inflammatory mediators in an vivo model of colitis. Thus, the aim of this study was to evaluate the neuroprotective effects of SP in reducing inflammatory process associated to neurological disorders. We performed both in vitro model of
Alzheimer’s disease, induced by oligomeric Aβ1-42 stimulation, and in in vivo model of spinal cord injury (SCI) in which neuroinflammation plays a crucial role. For in vitro model, the human neuroblastoma SHSY-5Y cell line was first differentiated with retinoic acid (100 μM) for 24 hours and then stimulated by oligomeric Aβ1-42 (1μg/ml) and treated with SP at three different concentrations (0.1- 1- 10 μM) for another 24 hours. Instead, the in vivo model of SCI was induced by extradural compression of the spinal cord at T6-T7 levels, using an aneurysm clip, and animals were treated with SP (10-30-100 mg/kg o.s) 1 and 6 h after SCI. Our results demonstrated that both in in vitro neuroinflammatory model and in vivo model of SCI the treatment with SP significantly reduced NF-κB nuclear translocation and IκBα degradation, as well as decreases COX-2 and iNOS expressions evaluated by Western blot analysis. Moreover, we showed that SP treatment significantly ameliorated histopathology changes and improved motor recovery in a dose-dependent manner. In conclusion, our results demonstrated that SP possesses neuroprotective effects, suggesting it could represent a target for therapeutic intervention in inflammatory disorders such as neurodegenerative disease and central nervous system injury.


Poster

302. Neural Injury and Treatment

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 302.11/J5


Support: European Research Council
Læge Sofus Carl Emil Friis og hustru Olga Doris Friis’ Legat
Novo Nordisk Foundation Laureate Program

Title: Nimodipine prevents the development of spasticity after spinal cord injury by blocking Cav 1.3 calcium channels

Authors: M. MARCANTONI1, P. LOW2, A. FUCHS2, O. KIEHN1, *C. BELLARDITA1;
1Neurosci. Dept., University of Copenhagen, Denmark; 2Neurosci. Dept., Karolinska Institute, Sweden

Abstract: Spasticity, one of the most frequent comorbidities of spinal cord injury (SCI), has been shown to disrupt motor recovery and quality of life. Despite major progress in neurorehabilitative and pharmacological approaches, no curative treatment for spasticity exists. Here, we investigated the possibility that early onset and long-term blockage of the Cav 1.3 calcium channels in a mouse model of chronic SCI prevent the development of spasticity.
Constitutive knockout of Ca\textsubscript{V}1.3 channels or conditional deletion in neuronal subtypes abolished increased muscle tone and spasms typical of chronic SCI. Furthermore, pharmacological blockade of Ca\textsubscript{V}1.3 channels with nimodipine starting in the acute phase of SCI completely prevented the development of increased muscle tone and spontaneous spasms. Notably, the aberrant muscle activities were permanently blocked even after termination of the treatment. This study has identified a specific target and a potentially curative treatment protocol for the prevention of spasticity after SCI.

**Disclosures:** M. Marcantoni: None. P. Low: None. A. Fuchs: None. O. Kiehn: None. C. Bellardita: None.

**Poster**

**302. Neural Injury and Treatment**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 302.12/J6

**Topic:** C.11. Spinal Cord Injury and Plasticity

**Title:** The neurokinin 2 receptor agonist \([\text{Lys}^5,\text{MeLEU}^9,\text{Nle}^{10}]\text{-NKA}_{(4-10)}\) produces urination and defecation in rats: Involvement of neurokinin 1 and neurokinin 2 receptors

**Authors:** *J. B. COOK, R. PIATT, L. MARSON; Dignify Therapeut., Research Triangle Park, NC

**Abstract:** Individuals with spinal cord injury or neurodegenerative disorders such as multiple sclerosis and Parkinson’s disease, often have bladder and/or gastrointestinal dysfunction that require the use of catheters and bowel programs to empty the bladder and bowel. Similarly, bladder and bowel dysfunction is prevalent in the aging population. Therefore, there is a need for drugs that promote emptying of the bladder and/or bowel. Our group is investigating neurokinin 2 receptor (NK2R) agonists for this indication. In the current study, we tested the ability of subcutaneous injection of \([\text{Lys}^5,\text{MeLEU}^9,\text{Nle}^{10}]\text{-NKA}_{(4-10)}\) (LMN-NKA) to induce urination and defecation in awake naïve rats. Since a potential side effect of LMN-NKA is flushing of the ears and paws, we examined the role of neurokinin 1 receptors (NK1R) and NK2R in the LMN-NKA induced flushing responses. Adult Sprague Dawley rats (n=23) were habituated to metabolism cages and handling for 3 days. Behavior was monitored before (10-20 min, pre-observation period) and after injections of LMN-NKA (3-100 µg/kg) and the time to urination and defecation, the voided volume and fecal weight, and flushing, was monitored for 30 min post LMN-NKA. In the antagonist study, vehicle (Veh), the NK1R antagonist CP-99,994 (1 mg/kg), or the NK2R antagonist GR159897 (1 mg/kg) were administered prior to the pre-observation period and then either saline (Sal) or 100 µg/kg LMN-NKA was injected, and behavior observed for 30 min. Injection of Sal, Veh, CP-99,994, or GR159897 alone resulted in minimal voiding or defecation and no flushing was observed. LMN-NKA induced a dose related increase in voiding,
defecation, and flushing at 10-100 μg/kg with a 100% responder rate at the highest dose. Urination, defecation and flushing occurred within 10 min of LMN-NKA dosing. The LMN-NKA induced urination was not reduced by the NK1R antagonist, however, pre-dosing with the NK2R antagonist reduced urination. LMN-NKA induced defecation was blocked by the NK2R antagonist, but the NK1R antagonist had no effect. Pretreatment with the NK1R antagonist significantly reduced the onset and duration of flushing produced by LMN-NKA, while flushing was still observed after the NK2R antagonist. Therefore, LMN-NKA induced voiding and defecation appears to be mediated via the NK2R and flushing by the NK1R. The results suggest that more selective NK2R agonists could be developed to promote voiding without concomitant side effects, such as flushing.


Poster

302. Neural Injury and Treatment

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 302.13/J7


Title: The fatty acide amide hydrolase inhibitor, PF-3845, attenuates inflammation and improves functional recovery in mice with spinal cord injury

Authors: *M. L. LANZA, G. CASILI, A. FILIPPONE, M. CAMPOLO, I. PATERNITI, S. CUZZOCREA, E. ESPOSITO; Univ. of Messina, Messina, Italy

Abstract: The endocannabinoid (eCB) system has emerged as a prominent lipid signalling network widely expressed in the body and involved in multiple adaptive responses. The eCB system comprises endogenous lipid transmitters such as N-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG) known as endocannabinoids, eCBs that are synthesized “on demand” are primarily involved in neuroprotective pathways producing neuro-modulatory, anti-inflammatory and anti-oxidative effects. It is noteworthy that increased AEA concentrations after SCI is thought to prevent neuroinflammation by regulating key-processes overall triggering anti-apoptotic, anti-oxidative and anti-inflammatory mechanisms. However, AEA is short-lived mediator and its levels are tightly regulated by a rapid enzymatic degradation mediated by fatty acid amide hydrolase (FAAH). The aim of the study is to considered this enzyme as potential drug targets and its inhibition could enable the pharmacological modulation of AEA levels to take advantage of their effects.
We performed an in vivo model of SCI, using a compression model, and we treated animals with PF3845, a selective and potent FAAH inhibitor, 1 hour and 6 hour after SCI. At 24 hours we scarified animals to evaluate the anti-inflammatory and neuroprotective effects of PF3845. The results obtained demonstrated that PF3845 significantly reduced the histological alterations induced by SCI; considerably decreased GFAP expression, NF-κB translocation and degradation of IxB-α, as well as diminished the expression of pro-inflammatory mediators COX-2, iNOS, TNF-α and IL-1β.

Thus, inhibition of FAAH can boost the endogenous levels of AEA enhancing its protective effect, suggesting that FAAH inhibitors could represent a novel therapy to control neuroinflammatory conditions of SCI.


**Poster**

302. Neural Injury and Treatment

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 302.14/J8

**Topic:** C.11. Spinal Cord Injury and Plasticity

**Title:** Pharmacological induction of oxidative metabolism enhances neural differentiation and recovery after spinal cord injury

**Authors:** *S. DOLCI*¹, G. MARTANO³, G. FIERABRACCI¹, S. ZORZIN¹, R. ZAMFIR¹, E. BOTTANI⁴, P. DELFINO¹, M. DI CHIO¹, M. MATTIOLI⁵, A. BACHI³, D. BRUNETTI⁶, M. RAGNI⁶, P. BOSSOLASCO⁷, D. BARDELLI⁷, V. CORBO¹, E. NISOLI⁶, A. VALERIO⁵, *G. F. FUMAGALLI², F. BIFARI², I. DECIMO¹;

¹Diagnostics & Publ. Hlth., ²Univ. of Verona, Verona, Italy; ³IFOM the FIRC Inst. of Mol. Oncology, Milan, Italy; ⁴Div. of Pharmacology, Univ. of Brescia, Brescia, Italy; ⁵Univ. of Milan, Milano, Italy; ⁶Univ. of Milan, Milan, Italy; ⁷IRCCS Inst. Auxologico Italiano, Milan, Italy; ⁸Univ. of Brescia, Brescia, Italy

**Abstract:** Spinal cord injury (SCI) results in irreversible sensory, motor, and autonomic impairments with high social and medical care costs. SCI pathophysiology involves several aspects including cell death and neural tissue loss, dysfunction of the cell metabolism and the inflammatory reaction of the immune system. Current SCI therapies aim at minimize the spinal cord damage and a therapeutic approach able to promote functional recovery is still lacking. Our hypothesis is that rewiring neural cell metabolism could improve SCI outcome. We designed a novel formulation made by essential amino acids, TCA cycle precursors and co-factors capable to increase oxidative metabolism and mitochondrial function and explored its effect in vitro on NSC neuronal differentiation and in vivo in a mouse model of severe SCI. We found that the
treatment greatly enhances oxidative phosphorylation, ATP production in differentiating neurons derived from both murine meningeal and SVZ NSCs and human iPSCs. The enhanced mitochondrial function was associated with an increase in the neuronal differentiation and spine maturation. Global transcriptomic analysis further indicates that treated neurons strongly induce Nrf2 mediated gene expression that greatly increases glutathione metabolism and improved ROS scavenging mechanism. Therefore, we evaluated the effect of treatment in vivo in a severe contusive SCI mouse model. Importantly, we start our treatment following the sub-acute phase (therapeutic setting). We found that the treatment significantly increased functional recovery and limited the instauration of a spastic condition of the ankle joint. In line with the behavioural data, we observed a reduction of the cyst and glial scar volumes and higher number of preserved/regenerated neurons in the treated injured spinal cord. Metabolomic analysis revealed that the treatment significantly increases energy metabolism and oxidative phosphorylation of the injured spinal cord tissue. In conclusion, our results indicate that nutrient induced metabolic switch toward oxidative metabolism, increases the ROS defences and enhances neuronal differentiation with potential therapeutic impact in severe SCI.


Poster

302. Neural Injury and Treatment

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 302.15/J9


Support: Craig H. Neilsen Foundation
NIH Grant R01NS091582
NIH Grant F32NS111241

Title: Mitochondrial uncoupling with 2,4-dinitrophenol exerts age dependent effects after spinal cord injury

Authors: *A. N. STEWART¹, K. E. MCFARLANE¹, H. J. VEKARIA², W. M. BAILEY¹, B. ZHANG¹, S. P. PATEL¹, P. G. SULLIVAN², J. C. GENSEL¹;
¹Spinal Cord and Brain Injury Res. Ctr. and the Dept. of Physiol., ²Spinal Cord and Brain Injury Res. Ctr. and the Dept. of Neurosci., Univ. of Kentucky, Lexington, KY
Abstract: The average age at time of spinal cord injury (SCI) is increasing. Aging exacerbates SCI in part by increasing reactive oxygen species (ROS) damage, however the contribution of mitochondria derived ROS to age-dependent recovery after SCI is not well understood. Here we tested the age-dependent effects of 2,4-dinitrophenol (DNP), a mitochondrial uncoupler that increases nutrient consumption and decreases ROS formation. Graded doses of DNP were delivered for 1-week after SCI in young- (4-month-old; 4-MO) and middle-aged (14-MO) mice. We detected a significant neuroprotective effect of DNP (1-mg/kg/day) at 7-days post-injury (DPI) for 14-MO SCI-mice but toxic effects of the same dose for 4-MO SCI-mice. Specifically, 14-MO SCI-mice treated with 1.0 mg/kg/day DNP had improved tissue sparing and lowered 3-nitrotyrosine (3-NT; protein nitration product) accumulation, with no apparent effects on neuron survival. In contrast, 4-MO SCI-mice treated with DNP had a significant loss of motor neurons surrounding the lesion and no reduction of 3-NT accumulation. Similar age-dependent effects of DNP were observed at 28-DPI in both aged and young mice. Specifically, treatment with DNP significantly improved locomotor function of 14-MO mice but worsened locomotor abilities of 4-MO mice (BMS scale). No DNP-mediated increase in tissue sparing was detected in 14-MO mice at 28-DPI, while 4-MO mice treated with DNP experienced a significant decrease in tissue sparing at the lesion epicenter. Collectively, these data demonstrate that mild mitochondrial uncoupling exerts age-dependent effects on SCI pathophysiology. Further, our data implicate metabolic processes in age-specific secondary injury events after SCI.


Poster

302. Neural Injury and Treatment

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 302.16/J10


Support: New York State Department of Health Adelson Medical Research Foundation

Title: Targeting alpha-tubulin acetylation to promote neurite outgrowth and functional recovery after injury

Authors: *V. WONG¹, C. PICCI³, M. SWIFT⁵, M. LEVINSON⁶, D. GOLDBERG¹, A. SAUVE⁷, B. LANGLEY⁴, D. WILLIS²;
Abstract: Damage to the central nervous system (CNS), such as spinal cord injury, results in neuronal and axonal degeneration and subsequent neurological dysfunction. Endogenous repair in the CNS is impeded by inhibitory cues, such as chondroitin sulfate proteoglycans (CSPGs) and myelin-associated glycoprotein (MAG), which prevent axon regeneration. Previously, it has been demonstrated that the inhibition of histone deacetylase-6 (HDAC6) can promote microtubule α-tubulin acetylation and restore the growth of CSPGs- and MAG-inhibited neurites. Since the acetylation of α-tubulin is regulated by two opposing enzymes, HDAC6 (deacetylation) and α-tubulin acetyltransferase-1 (αTAT1; acetylation), we investigated the regulation of these enzymes downstream of a growth inhibitory signal. We used dissociated mouse cortical neurons incubated with CSPGs or MAG for various indicated times. We examined changes in tubulin acetylation levels, HDAC6 and αTAT1 expression, and neurite growth using molecular and imaging techniques. Our findings show that exposure of neurons to soluble CSPGs and MAG substrates cause an acute and RhoA-kinase-dependent reduction in α-tubulin acetylation and αTAT1 protein levels, without changes to either HDAC6 levels or HDAC6 activity. The CSPGs- and MAG-induced reduction in αTAT1 occurs primarily in the distal and middle regions of neurites and reconstitution of αTAT1, either by Rho-associated kinase (ROCK) inhibition or lentiviral-mediated αTAT1 overexpression, can restore neurite growth. Lastly, we demonstrate that CSPGs and MAG signaling increases αTAT1 levels post-transcriptionally via a ROCK-dependent increase in αTAT1 protein turnover. Together, these findings define αTAT1 as a novel potential therapeutic target for ameliorating CNS injury characterized by growth inhibitory substrates that are prohibitive to axonal regeneration. This study represents a paradigm shift as αTAT1 provides a new target to promote axonal regrowth, in addition to HDAC6, which has received considerably more attention. Our immediate future plan is to complement these findings in vitro to in vivo setting using transgenic (namely HDAC6 and αTAT1) knockout mice with spinal cord injury. Moreover, we are currently conducting a high throughput drug screen to discover compounds that can promote α-tubulin acetylation in neurons in vitro. We will perform in vivo dosing studies of candidate compounds in spinal cord injured animals and examine their potential for functional recovery.


Poster

302. Neural Injury and Treatment

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 302.17/J11


Support: CIHR Grant MOP133460
Development of SOX9 antagonists for the treatment of neurological injuries

Authors: *T. HRYCIW¹, N. M. GEREMIA¹, M. DINUNZIO¹, B. URQUHART², A. BROWN¹;
¹Robarts Res. Inst., ²Physiol. and Pharmacol., Western Univ., London, ON, Canada

Abstract: The transcription factor SOX9 plays key roles during development, being required for male sex differentiation and chondrogenesis. In adults, SOX9 is found in astrocytes and in several stem cell niches where it serves to maintain “stemness”. After spinal cord injury (SCI) or stroke, SOX9 expression in reactive astrocytes promotes production of chondroitin sulfate proteoglycans (CSPGs) that inhibit axonal growth. Conditional Sox9 ablation reduces CSPGs in the injured spinal cord, increases reparative axonal sprouting and improves locomotor recovery. Similarly, in the middle cerebral artery occlusion model of stroke conditional Sox9 ablation improves recovery by promoting axonal sprouting from uninjured areas of the brain onto targets denervated by the injury. To inhibit SOX9 activity on extracellular matrix gene promoters, we designed a fusion peptide consisting of the TAT cell-penetrating sequence and a SOX9 interfering peptide. This peptide was able to decrease the expression of SOX9 target genes in primary astrocyte cultures and in the chondrogenic cell line ATDC5 as assayed by real time PCR. Intravenous injection of the SOX9-targeting peptide in SCI mice at 24 and 48 h post-injury resulted in uptake by glial cells in the injured cord as assessed by immunohistochemistry, and decreased SOX9 target gene expression assayed at 72h by real time PCR. A computational screen of ~12 million compounds predicted to interfere with SOX9 identified ten candidate SOX9 inhibitors. One candidate, designated as Z02, decreased SOX9 target gene expression in vitro. Mass spectrometry studies showed that intravenous administration resulted in accumulation of Z02 in the spinal cord of both uninjured and spinal cord injured rats indicating that Z02 is capable of crossing the blood-spinal cord barrier. Staining cord sections with Wisteria Floribunda lectin showed that treatment of SCI rats (T8 contusion) with Z02 resulted in a reduction of perineuronal nets surrounding neurons compared to vehicle-treated controls. Behavioural studies are underway to determine whether the reduction of CSPG levels in perineuronal nets by Z02 correlates with locomotor improvements following spinal cord injury.


Poster

302. Neural Injury and Treatment

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 302.18/J12

Enhancing KCC2 activity reduces development of spasticity after spinal cord injury

Authors: *J. N. BILCHAK, K. YEAKLE, G. CARON, M.-P. COTE; 
Neurobio. and Anat., Drexel Univ., Philadelphia, PA

Abstract: Within a year after a spinal cord injury (SCI), the majority of patients find themselves thwarted in the recovery of motor function by the development of spasticity. Among the multifaceted factors contributing to the hyperexcitability of spinal networks after chronic SCI is a shift in chloride homeostasis. SCI triggers a significant decrease in the expression of KCC2, a chloride transporter, which leads to a decrease in chloride extrusion capability and subsequently intracellular chloride accumulation in neurons. This shift in chloride homeostasis significantly contributes to reflex dis-inhibition and spasticity. Interestingly, exercise training can improve spastic symptoms via an increase in KCC2 expression in lumbar motoneurons. However, SCI is accompanied by a substantial number of co-morbidities that often delay the onset of exercise-based therapies or cause safety concerns for the patient during training sessions. Thus, patients are routinely medicated with anti-spastic medications. While these work efficiently to suppress spastic events, they induce significant side effects and depress the overall spinal excitability, making them incompatible with activity-based therapies. A promising alternative is to instead directly restore endogenous inhibition by restoring chloride homeostasis. We have previously shown that acute treatment with the KCC2 enhancer, CLP257, reduces signs of spasticity in chronic SCI rats. Here, we sought to increase KCC2 activity chronically after SCI, and assess spastic symptoms as they develop in the weeks following injury. In addition, we investigated the effects of combining this novel compound with an exercise-training regimen. Adult female Sprague-Dawley rats were implanted with chronic EMG electrodes, received a severe contusion injury at T9, and were treated daily with either CLP290 or vehicle starting one day after injury and continuing for 4 weeks. Exercised animals were step-trained on a treadmill daily. Once a week, EMG activity was recorded in awake, behaving animals to assess spontaneous spasms, responses to muscle stretch, and stepping ability on a treadmill. Our results suggest that chronic treatment with CLP290 reduces the development of spontaneous spasms, helps maintain normal responses to stretch, and improves stepping ability. Importantly, CLP290 treatment in exercised animals showed no visible detriment to the benefits of exercise on locomotor function. This work reveals the substantial potential for KCC2 enhancers as a novel spasticity treatment that can be used in tandem with activity-based therapies.

Disclosures: J.N. Bilchak: None. K. Yeakle: None. G. Caron: None. M. Cote: None.
**Poster**

**302. Neural Injury and Treatment**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 302.19/J13

**Topic:** C.11. Spinal Cord Injury and Plasticity

**Support:** Supported by the Rick Hansen Foundation through the ICORD-Rick Hansen Institute – Blusson Integrated Cure Partnership

**Title:** Testing combinatorial treatments of promising FDA approved neuro-protective drug candidates in a cervical hemi-contusion model of rats

**Authors:** *W. PLUNET, N. JANZEN, J. LIU, E. RAFFAELE, S. KAMAKARI, O. SEIRA, K. KOLE, Y. JIANG, L. MCPHAIL, W. TETZLAFF; ICORD, Univ. of British Columbia, Vancouver, BC, Canada

**Abstract:** A significant number of FDA approved drugs have demonstrated efficacy in preclinical spinal cord injury (SCI). However, when tested clinically they have failed to produce meaningful results. This lack of robustness is due to a number of factors in the pre-clinical testing including injury model, treatment timing, and the studies being underpowered, to name a few. We therefore wanted to test the most promising FDA approved drugs, alone and in combination, when these are administered 3 hours after a cervical spinal cord hemi-contusion injury using group sizes of n = 16-21. We initially tested 8 different FDA approved drugs (riluzole, valproic acid, fluoxetine, metformin, inosine, rosuvastatin, glibenclamide, tamoxifen) that had been reported to improve functional recovery in SCI models. In our experiments none of the 8 treatments improved recovery compared to control groups, and only glibenclamide improved the amount of spared spinal cord tissue. Having poor success with numerous individual treatments we decided to test combination treatments. We tested glibenclamide plus taxmoxifen (G + T), and in another group: glibenclamide, tamoxifen plus inosine (G + T + I). We found that both treatment groups showed a modest (p < 0.05) improvement in the staircase compared to the control group, while on the ladder test G + T + I resulted in a nearly 50% reduction of mistakes compared to controls. These results suggest a combinational approach may allow a recovery level that is not observed with single treatments, in particular when initiated somewhat delayed at 3 hours after injury. Supported by the Rick Hansen Foundation through the ICORD-Rick Hansen Institute - Blusson Integrated Cure Partnership.

**Poster**

**302. Neural Injury and Treatment**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 302.20/J14

**Topic:** C.11. Spinal Cord Injury and Plasticity

**Support:** Upstate Foundation

**Title:** Evaluation of cABC delivery methods on the response of oligodendroglia after spinal cord injury

**Authors:** *D. J. OSTERHOUT*, J. R. SIEBERT, A. CHENG, B. AHMAD;  

**Abstract:** The tissue surrounding a spinal cord lesion is well known as being inhibitory to regenerative and repair processes, due to the expression of chondroitin sulfate proteoglycans (CSPGs). The deposition of CSPGs at the lesion starts immediately after injury and forms a glial scar, which hinders both axonal regeneration and the migration of oligodendrocyte progenitor cells (OPCs). However, many studies have demonstrated that treating CSPGs with the enzyme chondroitinase (cABC), which removes the glycosaminoglycan (GAG) side chains from the protein core of the molecule, creates a permissive environment for both axonal sprouting and OPC migration. While cABC treatment of a spinal cord injury (SCI) in vivo has shown promise in stimulating axonal regeneration, the labile nature of the enzyme means biological activity is typically lost only a few days post-administration. This poses an interesting problem: how to protect the biological activity of cABC for long term treatment of the glial scar.

In our recent studies, we have been testing a biodegradable nanosphere delivery system designed to provide a slow continuous release of active cABC. We have previously demonstrated that this drug delivery method can significantly enhance the axonal regeneration response as compared with an injection of cABC. In this study, we examined the migration of OPCs to the lesion with two different cABC delivery methods: nanospheres and injection. The total number of OPCs surrounding the lesion site was significantly increased after cABC administration by either method, compared to controls. The enhanced OPC migration is evident at early times post-injury, and continues through one month. However, nanosphere delivery of cABC provided more continuous digestion of CSPGs, along with an increasing number of OPCs at 1, 2 and 4 weeks post injury when compared to a direct injection of cABC. While the number of OPCs tends to plateau at one month, the extended digestion of CSPGs allows for a persistent accumulation of OPCs in and around the lesion. Collectively, these data demonstrate that utilizing a biodegradable delivery system, allowing for a steady release of enzymatically active cABC, will maximize its effects of on both axonal sprouting and OPC migration after SCI.

Poster

302. Neural Injury and Treatment

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 302.21/J15


Support: California Institute for Regenerative Medicine (CIRM) (RT3-07616)
Dr Miriam and Sheldon G. Adelson Medical Research Foundation (AMRF)

Title: Survival of human stem cell-derived motor neurons in a non-human primate model of chronic conus medullaris/cauda equina spinal cord injury

Authors: *N. P. BISCOLA*¹, J. H. NIETO¹, R. DATTA², M. C. CONDRO², P. MEERA³, D. MOORE², N. ZHANG¹, M. OHLSSON⁶, K. A. REIMANN⁷, K. L. CHRISTE⁵, B. G. NOVITCH³,⁴, H. I. KORNBLUM²,⁵,⁴, L. A. HAVTON¹,³,⁴,


Abstract: Conus medullaris (CM) / cauda equina (CE) forms of spinal cord injury (SCI) represent about 20% of all SCI cases in the United States. Paralysis, sensory damage, and loss of bladder, bowel, and sexual functions are common after this injury. Neurodegenerative reactions, including neuronal death, contribute to the functional impairments and there are no treatments available to reverse these deficits. The present study investigated a new approach to replace degenerating motor neurons in a non-human primate model of CM/CE form of spinal cord injury at 24 hours after surgery (n=1), 2 months post-surgery (n=3), and 7 months post-surgery (n=4). A total of 8 female rhesus macaques were subjected to a new immunosuppression protocol that included anti-thymocyte globulin, tacrolimus, prednisone, and anti-CD40 treatment. The animals underwent an L6-S3 ventral root avulsion (VRA) injury, replantation of the avulsed L6 and L7 ventral roots into the lateral funiculus, and injection of approximately 250,000 human stem cell-derived motor neuron into the L5 spinal cord segment. Anatomical studies showed survival of human cells in the spinal cord of all animals. Formation of rosettes expressing neural progenitors SOX2, and NESTIN were identified at 2 months post-surgery. The human cells also showed labeling for motor neuron and oligodendrocyte progenitors, such as OLIG2, but no markers for astrocytes (GFAP) or microglial cells (IBA1) were identified. At 7 months post surgery no rosettes, astrocytes, or microglial cells were identified, although oligodendrocyte progenitors
were present. No tumor formation was detected and a human neuronal phenotype was confirmed by STEM 121 and βIII-tubulin markers. The integration of human cells in the primate spinal cord was confirmed using pre-embedding immuno-gold labeling for analysis of STEM121 in the electron microscope. Human cell processes formed parallel tracts in the grey and white matter, and human cells were able to form synapses with host motor neurons, demonstrated by synaptophysin and STEM 121 staining. Behavioral analysis showed a VRA-induced left leg weakness, but preserved ability to use the affected limb for climbing, balancing, and stepping. Functional analysis using cystometrogram and pelvic floor EMG recordings showed preserved micturation reflexes and absence of adverse functional effects. We conclude that grafted human cells supported by a new immunosuppression protocol, may show long-term survival and form neural circuits in the primate spinal cord after a CM/CE injury.


Poster

302. Neural Injury and Treatment

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 302.22/J16


Support: Line of research No. FIS/IMSS/PROT/G16/1605
Line of research No. FIS/IMSS/PROT/G17/1676
CONACYT CVU: 824097
IMSS Scholarship 99097193

Title: Evaluation of the immunomodulatory effect of peptides in rats with traumatic spinal cord injury

Authors: J. VILCHIS VILLA1, D. PARRA VILLAMAR1, A. IBARRA2, L. BLANCAS ESPINOZA1, J. TOSCANO ZAPIEN1, E. GARCÍA VENCES2, R. H. RODRIGUEZ BARRERA3, A. FLORES ROMERO2, *R. S. GARCÍA1;
1Inst. Mexicano Del Seguro Social, Hosp. de Pediatría, UIMI, Ciudad de Mexico, Mexico;
2Univ. Anahuac-Centro De Investigacion Camina, Huixquilucan Edo. De Mexico, Mexico;
3Univ. Autónoma Metropolitana Iztapalapa, Mexico City, Mexico

Abstract: Spinal cord injury (SCI) is a disabling neurological condition with a high impact on the quality of life of patients. After the initial mechanical damage, several biochemical mechanisms occur in the neuronal, vascular, and immune systems (secondary damage). One of the main goals for therapy is to achieve motor and sensory recovery. New therapies have been
explored, and have shown significant effects on motor recovery as well as on structural features. We present the proposal of a combinatorial therapy of immunomodulatory peptides that have already shown significant effects on motor recovery in rats with SCI, which resulted more effective than individual use. The objective was to evaluate combinations of the peptides MLIF (A), A91 (B), and GSH-MEE (C) and their effects in acute and chronic stages of SCI to know their specific responses. Female Sprague Dawley rats (230-250g) underwent a laminectomy at the T9 vertebra and a moderate contusion. They were divided into 6 groups (n=36): 1) Sham, 2) PBS, 3) A + B, 4) A + C, 5) B + C, and 6) A + B + C. The presence of lipid peroxidation metabolites (HNE, MDA and nitrites) and gene expression were evaluated 3 and 7 days post-injury and treatment (acute stage); motor recovery and histology of spinal cord were evaluated two months post-injury (chronic stage). In the acute stage, the concentration of nitrites along with IL-1β, IL-6 and IFN-γ expressions were significantly lower in the A + B + C group vs PBS at 3 and 7 days, respectively. No significant changes were observed in levels of HNE or MDA. In the chronic stage, there was a significant motor improvement in the A + B + C group vs all groups, in addition to greater preservation of parenchyma and motor neurons in the area of injury, greater white matter in the caudal area, and less presence of collagen. In conclusion, 3-peptide combinatorial therapy improves the motor recovery in rats, modulating inflammation in the acute stage, therefore preserving the integrity of the tissue in the chronic stage.


Poster

302. Neural Injury and Treatment

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 302.23/J17


Support: FAPESP grant 2018/06316-3

Title: Modulation of enhancer of zester homolog 2 (EZH2) activity promotes innate inflammatory response alteration and leads to tissue regeneration and better motor function recovery after rat spinal cord injury
Authors: *F. F. CORREIA*¹, B. C. MORENA¹, F. R. C. GONÇALVES¹, V. PASCHON², A. H. KIHARA¹;
¹UFABC, São Bernardo do Campo, Brazil; ²Univ. Federal Do ABC, São Bernardo Do Campo, Brazil

Abstract: Spinal cord injury (SCI) is a traumatic neurologic disorder with clinical relevance due to the disruption of neuronal communication between the peripheral and central nervous system (CNS). After the primary injury, often caused by mechanical trauma, the condition may be aggravated by local tissue inflammation. Nowadays, it is already known that epigenetic-related processes contribute to the spread of CNS damage. Epigenetic is usually related to alterations in the chromatin structure which are capable to change the genetic expression without altering the DNA and are mainly related to histones modifications through methyl and acetyl group addition. Within this context, global methylation and specially the methylation on lysine 27 of histone 3 produced by Enhancer of Zeste Homolog 2 (EZH2) was related to modulation of inflammatory response and cellular death in CNS infections. However, the role of EZH2 after neuronal injury is not well described. The aim of this work was to describe the role of EZH2 activity on inflammation progress and tissue regeneration through pharmacology inhibition (GSK343) one hour after rat SCI. To this end, we combined immunofluorescence (IF) analyses with motor function evaluation as provided by Basso, Beattie, and Bresnahan (BBB) test and grid scale. Results were compared by student test T or two-way repeated measures ANOVA followed by appropriate post-hoc test. All procedures were conducted in accordance with UFABC Animal Care Ethics Committee (#2066300916). IF results in sections of the spinal cord revealed that after acute SCI (24h after injury) the number of CD86 positive cells changes in the white matter. When compared to controls (only vehicle) the treatment with GSK343 increases the number of CD86-positive cells (588 ± 251 vs. 1298±150; N= 5; P<0.01), while the pixel intensity analysis demonstrated a reduction on individual brightness of this marker (0.01±0.002 vs. 0.007 ± 0.001; N= 5; P<0.01). Moreover, when compared to controls, GSK343 treatment after SCI increased the score in BBB test 6 weeks after injury (7.28±1.6 vs. 14.55±2.2; N= 9; P<0.05), grid scale (0.0% ±33.3 vs. 83.33% ±36.07 vs.; N= 6; P<0.05) and subBBB scale (4.28±1.04 vs. 7.88±1.50; N= 9; P<0.05). In addition, 6 weeks after injury, IF results evidenced that when compared to controls, growth associated protein 43 (GAP43) pixel intensity is higher in the rostral region of the spinal cord (8.64 ± 0.05 vs. 17.3±1.95; N=3; P>0.01). All these data suggest a participation of EZH2 in the immunological innate response, especially on CD86 expression. The presence of EZH2 may influence in the adaptive response, which is related to the axonal regeneration and motor function recovery.

Poster

303. Spinal Cord Injury and Plasticity: Neurophysiology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 303.01/J18


Support:  Shriners Hospital for Children's Research Grant (#85115)
         Craig H. Neilsen Foundation Senior Research Grant (#546798)

Title: Effect of clozapine-N-oxide (CNO) on H-reflex of naïve female Sprague Dawley rats

Authors: *J. T. EISDORFER¹, K. M. KEEFE¹, K. M. RAUSCHER¹, G. M. SMITH², M. A. LEMAY¹, A. J. SPENCE¹;
         ¹Bioengineering, ²Lewis Katz Sch. of Med., Temple Univ., Philadelphia, PA

Abstract: Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) are chemogenetic tools that can selectively control excitability of neurons. DREADDs are activated by clozapine-N-oxide (CNO), a ligand thought to otherwise be pharmacologically inert. However, recent studies report CNO is back-metabolized to clozapine and can cause confounding effects as clozapine blocks neurotransmitter release at several endogenous receptors. Our study evaluated whether CNO modulates the H-reflex, an assay of spinal cord circuit function. The H-reflex measures the fraction of motor units activated by a stretch reflex pathway upon electrical stimulation of a peripheral nerve. Electrical stimulation causes an orthodromic volley of spikes in large afferents that elicits a reflex EMG response (H-wave). It also activates efferents which directly produce a muscle response (M-wave). Changes in maximum H-to-M-wave (H-M) ratio reflect changes in reflex pathway excitability. Further, changes in the difference in current between H- and M-wave threshold (deltaHM) indicate changes in excitability of the afferents responsible for the reflex pathway. Our study recorded the H- and M-wave from the interosseous muscle in the hind paw via electrical stimulation of the tibial nerve. We performed H-reflex on naïve female Sprague Dawley rats in the absence of DREADDs with and without intraperitoneal injection of CNO (4 mg/kg). H-reflex experiments were conducted at ‘pre’, ‘active’ and ‘post’ phases that correspond to: immediately before CNO injection; during strongest CNO effects (30 min); and after CNO wash-out (2 h), respectively.

We found maximum H-M ratio was not significantly different across phases within the same group (pre 0.30 +/- 0.07; CNO active 0.25 +/- 0.13; mean +/- s.d., n=6; p=0.92 and p=0.83 for control (n=7) and CNO (n=6) groups, respectively; 2-way ANOVA on pre condition baselined data) and between groups for all phases (p=0.35). With our sample size and observed standard deviation, we would have detected a 0.13 difference in H-M ratio with 80% statistical power, corresponding to approximately half the change seen after spinal cord transection. We also found deltaHM was not significantly different within the same group (CNO active 0.23 +/- 0.16; post...
0.26 +/- 0.25; mean +/- s.d., n=6; p=0.87 and p=0.55 for control (n=7) and CNO (n=6) groups, respectively; 2-way ANOVA on baselined data) and between groups (p=0.55). These results indicate CNO or back-metabolized clozapine does not affect the H-reflex in naive rats to within our statistical power. This suggests at this dosage of CNO, the H-reflex may be used to assay spinal cord circuit function in conjunction with DREADDs.


Poster

303. Spinal Cord Injury and Plasticity: Neurophysiology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 303.02/J19


Support: Merit Review Funding from the Department of Veterans Affairs.
New York State Spinal Cord Injury Research Board (SCIRB)
Craig Neilsen Foundation

Title: Differential effects of low frequency (0.2 Hz) and high frequency (20Hz) spinal electromagnetic stimulation in modulating parameters of H-reflex responses in chronic spinal cord injured rats

Authors: *L. LIANG1,3, H. A. PETROSYAN1,3, S. A. SISTO4,2, V. L. ARVANIAN3,1;
1Neurobio. and Behavior., 2Dept. of Physical Therapy, Sch. of Hlth. Technol. and Mgmt., Stony Brook Univ., Stony Brook, NY; 3Northport Veterans Affairs Med. Ctr., Northport, NY; 4Univ. at Buffalo, Buffalo, NY

Abstract: Spinal cord injury (SCI) is a devastating condition that affects many important body functions of individuals. Significant deficits in transmission is one of the reasons behind functional shortfalls. In our previous animal studies, using intracellular recordings from individual motoneurons we have demonstrated that after mid-thoracic SCI synaptic transmission to lumbar spinal cord is significantly diminished. Various treatment interventions that enhance transmission in damaged spinal cord lead to recovery of motor and sensory function. Our recent results revealed that non-invasive spinal electromagnetic stimulation (SEMS) applied at low (0.2 Hz) frequency was able to induce facilitation of synaptic transmission in chronic SCI animals. In contrast to facilitatory effects of low-frequency SEMS, transcranial electromagnetic stimulation (TMS) applied at low frequencies has been reported to be inhibitory, while high frequency TMS is known to induce facilitation. In this study, we have examined and compared effects of repetitive SEMS applied at low (0.2 Hz) and high (20Hz) frequencies on H-reflex in non-injured and chronically injured rats. H-reflex is recognized as a reliable neurophysiological measure of
spinal circuitry that is distorted following SCI. Using animal models of SCI, we have also examined underlying mechanisms of SEMS induced plasticity. In particular, we have examined the role of NMDA receptors reported to be involved in modulation of transmission in damaged spinal cord. Our results revealed that high frequency (20Hz) spinal stimulation significantly reduced the amplitude of H-reflex and induced rightward shift of the H-reflex threshold current in chronic SCI rats. In contrast, low frequency (0.2Hz) spinal stimulation induced increase of H-reflex amplitude and the leftward shift of the threshold. These effects of both low and high frequency SEMS were long lasting and lasted for about 2 hours post SEMS. Most importantly SEMS induced significant improvements in recovery of frequency dependent depression (FDD) of H-reflex. Importantly, after SEMS administration there is a statistically significant difference in H-reflex amplitude at all frequencies tested i.e. 0.2, 0.5, 1, 2, 5, and 10Hz stimulations in chronic SCI rats. These results suggest that, depending on frequency of stimulation, SEMS may induce differential effects on spinal plasticity and could be used as a novel non-invasive technique for modulation of neuro-muscular circuits after chronic SCI or other neurological disorders. In fact, our current study examines effects of low (0.2) frequency and high (20 Hz) frequency SEMS in humans.


Poster

303. Spinal Cord Injury and Plasticity: Neurophysiology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 303.03/J20


Support: NIBIB-NIH R21EB020318
Travis Roy Foundation to Cure Spinal Cord injury

Title: Repetitive paired brain and spinal cord stimulation strengthen spared circuits and reduces hyperreflexia after cervical spinal cord injury

Authors: *A. PAL¹, A. RAMAMURTHY¹, H. PARK¹, T. BETHEA¹, A. GARCIA-SANDOVAL², S. RATNADURAI-GIRIDHARAN³, W. VOIT², J. B. CARMEL¹;
¹Neurol. and Orthopedic Surgery, Columbia Univ. Med. Ctr., New York, NY; ²Materials Sci. and Engin. and Bioengineering, The Univ. of Texas at Dallas, Richardson, TX; ³Burke Neurolog. Inst., White Plains, NY

Abstract: Pairs of stimuli engage associative learning mechanisms to change the nervous system. We developed a paired stimulation approach that relies on proper timing of motor cortex and spinal cord stimulation to alter spinal sensorimotor circuits. When pairing is performed repeatedly, there is a lasting and robust increase in cortical and spinal excitability in uninjured
rats. The present study tests the efficacy of paired stimulation in rats with spinal cord injury (SCI). We hypothesized that the tissue spared by SCI would enable paired stimulation to augment motor responses but decrease hyperreflexia. Cortical and biceps EMG electrodes were implanted in adult rats, and baseline testing was done a week before SCI. A moderate C4 contusion (200 k dyne) was performed, and thin and softening electrode arrays were inserted over C5-C6. Beginning 11 days after SCI, we tested the effects of repetitive paired stimulation over 10 days. The protocol uses intermittent bursts to deliver 3000 stimuli over 30 minutes every day for 10 days. After this protocol, rats with SCI had large (>150%) augmentation of both cortical and spinal motor evoked potentials (MEPs) that decreased over 2 hours to 50% increase. The magnitude and the duration of the effects were similar in rats with and without SCI, indicating that spared circuits mediate pairing effects. In the group of rats with repetitive stimulation, but not an injury only control group, MEPs increased more than 2-fold over the 10-day stimulation period. The difference between groups was maintained weeks after SCI, indicating a durable change in physiology. In both groups, SCI diminished the rate-dependent decrease in the H-reflex, consistent with hyperreflexia. After 10 days of paired stimulation, rats with stimulation had much less hyperreflexia than injury-only controls. Thus, paired stimulation produces stronger cortical and spinal MEPs, decreased hyperreflexia and the circuits spared after SCI were sufficient to enable long-term plasticity.


Poster

303. Spinal Cord Injury and Plasticity: Neurophysiology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 303.04/J21


Support: New York State Spinal Cord Injury Research Board (SCIRB)

Merit Review Funding from the Department of Veterans Affairs

Craig H. Neilsen Foundation

Title: Delayed implantation of neural stem cells (NSCs) combined with spinal electro-magnetic stimulation (SEMS) and exercise training in chronic spinal cord injured rats

Authors: *V. L. ARVANIAN1,2, P. J. HORNER4, R. C. KRENCIK4, L. LIANG1,2, N. BALLAS3, K. LASEK2, S. GUMUDAVEILL1, V. ALESSI1,2, H. PETROSYAN1,2;

1Norhtport VAMC, Northport, NY; 2Neurobio. and Behavior, 3Stony Brook Univ., Stony Brook, NY; 4Ctr. for Neuroregeneration, Dept. Neurosurg., Houston Methodist Res. Inst., Houston, TX
Abstract: One of the major devastating consequences following severe SCI is the destruction of local cells that are required for proper function and neuromodulation of spino-muscular motor circuitry. We examined the advantage of a transplantable source of neurons and oligodendrocytes into damaged spinal cord in combination with SEMS and exercise. We have recently demonstrated that SEMS could strengthen transmission and facilitate NMDA receptor function in damaged spinal cord. Importantly, exercise training, applied after SEMS, induced sustained improvements of transmission and most importantly function following contusion SCI. In the current study, adult rats received T10 contusion SCI and human induced pluripotent stem cell (hiPSC)-derived neural stem cells (NSC) were implanted 2 weeks post injury; one week after implantation the rats received SEMS followed by treadmill training, every other day for 6 weeks. Three groups of animals were examined. Gr.1 received control PBS injections and no other treatment or NSCs; Gr.2 received implantation of NSCs only; Gr.3 received implantation of NSCs followed by SEMS and treadmill exercise. All rats received injections of cyclosporine daily during survival period to protect human derived NSCs from immune-mediated rejection in rat spinal cord. Locomotor function was assessed using BBB scoring and automated Catwalk. Bladder function was assessed with metabolic chambers weekly. Electrophysiological recordings were performed at the end of survival period to examine effects of treatment on transmission at spino-muscular circuitry. After completion of behavioral and electrophysiology evaluations, the rats were perfused, and spinal cords removed and prepared for immunochemistry evaluation. Results revealed no adverse effects from implantation of NSCs. Animals that received NSCs combined with chronic SEMS/exercise treatment exhibited better recovery of locomotor function, bladder control and transmission compared with rats that received NSCs only. However, no significant difference was observed between NSCs only group and control group with no NSCs. Immunohistochemistry results revealed that survival rate of NSCs was very low, thus significant improvements of motor and bladder function in combination group could potentially be attributed to the beneficial effects of SEMS, secreted factors by implanted NSCs and exercise. Currently, the use of neural progenitor cells driven to a more mature phenotype and development of new implantation techniques are being tested in our lab that will increase the survival rate of implanted stem cells and to induce functional changes in chronic stage of severe spinal cord injury.


Poster

303. Spinal Cord Injury and Plasticity: Neurophysiology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 303.05/J22

Support: NIH Grant 1R01NS102871

Title: Hyperexcitation in mouse sympathetic neurons after spinal cord injury

Authors: K. TIAN\(^1\), Y. LI\(^2\), M. L. MCKINNON\(^2\), *S. HOCHMAN\(^3\), A. A. PRINZ\(^1\);
\(^1\)Dept. of Biol., \(^2\)Dept. of Physiol., Emory Univ., Atlanta, GA; \(^3\)Emory Univ. Sch. Med., Atlanta, GA

Abstract: Thoracic sympathetic postganglionic neurons (tSPNs), innervated by preganglionic neurons in the spinal cord, integrate synaptic inputs from the spinal cord to regulate downstream effector targets including vasculature. Vasomotor disorders such as autonomic dysreflexia after spinal cord injury (SCI) have been know for decades, yet little is known about the cellular and synaptic plasticity of tSPNs after SCI. We have recently characterized the cellular properties of mouse tSPNs using whole-cell recording and computational modeling [1]. Preliminary studies comparing tSPN cellular properties after high thoracic cord transection showed recovery of firing properties (similar f-I curves) measured 3-6 weeks after injury but with increased frequency in spontaneous synaptic events [2]. Furthermore, a previous RNA-Seq study identified two types of tSPNs, NA2 and NA3, that innervate the vasculature [3], but whether and how the mechanisms regulating their excitability differ is unknown.

To investigate the questions above, we employed an ensemble modeling approach to build a database of physiologically-realistic tSPN models that encompass a wide range of cellular dynamics and cell sizes [4]. From the database we identified NA3-like neurons and found that compared to all the other neurons in the database, NA3-like neurons have (i) higher densities of currents modulating spike rate adaptation with lower densities of leak current, and (ii) greater ability to integrate modeled synaptic inputs, which may lead to hyperexcitation after SCI.

Acknowledgements

Ensemble modeling was performed on the Neuroscience Gateway Portal [5]. This work is supported by the CMBC Interdisciplinary Neuroscience Pilot Research Fund at Emory University and NIH grant 1R01NS102871.

References
1. McKinnon et al., (2019) eNeuro eN-TNWR-0433-18R2
2. Li et al., (2018) Society of Neuroscience 387.07

Disclosures: K. Tian: None. Y. Li: None. M.L. McKinnon: None. S. Hochman: None. A.A. Prinz: None.
Title: Electrophysiological mechanisms underlying cervical epidural stimulation in adult rats

Authors: *P. D. SHARMA, P. TENDOLKAR, P. SHAH;
Sch. of Hlth. Technol. and Mgmt., Stony Brook Univ., Stony Brook, NY

Abstract: Regaining upper limb function remains the top priority of population that have sustained a cervical spinal cord injury (cSCI). There is growing indication that electrical epidural stimulation (ES) of the spinal cord can restore volitional leg movements and promote functional upper limb motor recovery post-cSCI. Collective evidence from rodent, human and computational studies suggest that the physiological recovery following lumbar ES is mediated via direct activation of sensorimotor spinal neural networks. However, the mechanisms underlying cervical ES have not been directly studied. Identifying neural pathways that are affected with cervical ES is a critical step prior to establishing the use of cervical ES as a potential neuromodulatory therapy after a cSCI.

In this work, we electrophysiologically identified spinal structures that are activated with cervical ES using in-vivo spinal motor evoked potentials (sMEPs). Three stimulation protocols were implemented for this purpose: single pulse, paired-pulse with different inter-stimuli intervals (ISI) and tonic stimulation at multiple frequencies. For each protocol, bipolar stimulation at C6 and C8 spinal segments (pulse duration = 0.2ms) was utilized. Simultaneous recordings were obtained from four chronically implanted forelimb muscles in 11 non-injured awake rats.

Single pulse stimulation revealed that sMEPs are comprised of three distinct waveforms. Based on the latency, amplitude and spike morphology, we termed these waveforms as afferent (AfR), efferent (EfR) and polysynaptic (PsR) responses. The AfR appeared at lower intensities at 4-6ms, while the EfR generally appeared at higher intensities at 1.5-3ms. The PsR was present at all intensities, had a long latency window of 15-25ms, and consisted of unsynchronized spiking activity. Paired-pulse and different frequency stimulation revealed a significant reduction in AfR and PsR activity at lower ISIs and higher frequencies (10-100ms, 1-30Hz) respectively. The EfR remain unchanged in both protocols.

The EfR and AfR evoked by cervical ES share similarities to the early and middle responses elicited by lumbar ES, which has been shown to directly interact with efferent and afferent
fibers. The long latency of the PsR is indicative of polysynaptic activity with likely contribution from supraspinal pathways. The suppression of AfR activity during paired-pulse and different frequency cervical ES suggests that this modulation is most likely mediated via afferent fibers. There is a high chance that similar to lumbar ES, cervical ES mediated recovery following cSCI is dependent on this path.

Disclosures: P.D. Sharma: None. P. Tendolkar: None. P. Shah: None.

Poster

303. Spinal Cord Injury and Plasticity: Neurophysiology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 303.07/J24


Support: NIH Grant K01HD084672-04

Title: Serotonin supersensitivity and injury severity contribution to spasm intensity in chronic spinal cord injury

Authors: *O. A. SHELTON1, V. M. TYSELING2, M. JIANG3, D. BIRCH1;
2Dept. of Physical Therapy and Human Movement Sci. and Physiol., 3Dept Physiol, Feinberg Sch. Me, 1Northwestern Univ., Chicago, IL

Abstract: Spinal cord injuries (SCI) disrupt messages between the brain and the body distal to the injury. This results in paralysis below the injury level and severely impairs function. In addition, most people with SCI develop hyperreflexia and involuntary muscle contractions, or spasms, that cause even more movement dysfunction as well as pain, contractures, and safety issues. One contribution to the generation of spasms is the interruption of 5-HTergic descending projections from the raphe nuclei. These projections modulate neuronal excitability through 5-HT signaling onto spinal neurons. In the intact cord, 5-HT signaling increases the net excitability of the spinal cord and enhances the motor output of the spinal cord. Following chronic SCI, diminished 5-HT signaling alters the excitability of the spinal cord and its sensitivity to 5-HT caudal to the injury and these changes contribute to the development of spasms. While changes in 5-HT sensitivity have been shown to contribute to development, it is unclear how much of the variability in the intensity of spasms is dependent upon the degree of 5-HT sensitivity. In this study we examine whether the difference in intensity of spasms can be explained by the degree of increase of 5-HT sensitivity of the spinal cord. We used a chronic impact injury mouse model of SCI. We assessed the amount of functional locomotor recovery at 10wks and compared this recovery to the electrical outputs from muscle and nerve tissue in the SCI mice. To compare how muscle contractions were impacted by our drug interventions we tested spasms in vivo by recording the flexor withdrawal EMG response to an electrical stimulus three months post injury.
To directly measure the spinal reflexes, we removed the sacral section of the spinal cord, stimulated the dorsal roots, and recorded the ENG response through the ventral roots. Changes in 5-HT response in multiple spinal neuron types has been shown to contribute to spasm generation. The current study investigates how much the variability in spasm intensity correlates with the degree of 5-HT sensitivity.

**Disclosures:** O.A. Shelton: None. V.M. Tysseling: None. M. Jiang: None. D. Birch: None.

**Poster**

**303. Spinal Cord Injury and Plasticity: Neurophysiology**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 303.08/J25

**Topic:** C.11. Spinal Cord Injury and Plasticity

**Support:** CSCR15FEL002

**Title:** Mapping cerebrovascular reactivity in response to breath-hold task after spinal cord injury: A functional near-infrared spectroscopy study

**Authors:** *K. KARUNAKARAN*1,2,3, N. D. CHIARAVALLOTTI4, B. B. BISWAL5;  

**Abstract:** Impairment of the sympathetic control after spinal cord injury results in cardiovascular deficits such as supine hypotension, orthostatic hypotension and cardiac arrhythmia. Injuries above T6 level further experience an impairment of cerebrovascular reactivity and neurovascular coupling properties of cerebrovascular control. It is unclear if cerebrovascular control is also affected in lower-level injuries. Functional near-infrared spectroscopy (fNIRS) is a promising technique to non-invasively and reliably measure the hemodynamic response of the brain in an upright position at a high temporal resolution. Using a breath-hold paradigm with fNIRS, the cerebrovascular reactivity can be quantified in response to global hypercapnia. Thirteen individuals with paraplegia resulting from SCI and 13 HC were recruited and scanned during a 15 second breath holding task for a total duration of 5 minutes. Hemoglobin concentration changes were quantified from the sensorimotor cortex, supplementary motor area and prefrontal cortex. Results indicate that SCI group (including 7 subjects with injuries below T6) exhibit a greater delay and larger pre-stimulus undershoot in oxy-hemoglobin concentration change to breath holding in bilateral lateral sensorimotor areas. The overall increase in oxy-hemoglobin change was similar in both healthy and SCI groups. Current findings suggest an intact static autoregulation but impaired dynamic autoregulation of cerebral blood
flow after paraplegia. Further, the application of fNIRS to assess cortical reorganization following SCI is unique and expands our understanding of the neurophysiology after SCI.

**Disclosures:** K. Karunakaran: None. N.D. Chiaravalloti: None. B.B. Biswal: None.

**Poster**

**303. Spinal Cord Injury and Plasticity: Neurophysiology**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 303.09/J26

**Topic:** C.11. Spinal Cord Injury and Plasticity

**Support:** K12HD073945 1R01 NS11234

**Title:** Intraspinal neural synchrony: Oscillating between interpretations

**Authors:** *J. G. MCPHERSON*1,2,3,4, M. F. BANDRES3,4, V. MELEDO4, M. BUCHANAN3,4; 1Physical Therapy, 2Anesthesiol., 3Biomed. Engin., Washington Univ. in St. Louis, St. Louis, MO; 4Biomed. Engin., Florida Intl. Univ., Miami, FL

**Abstract:** In supraspinal neural structures, band-specific local field potential (LFP) power and oscillatory neural activity are associated with particular behavioral states and underlying neural processes. Supraspinal LFPs can also be controlled volitionally. As such, supraspinal LFPs and neural oscillations are thought to convey meaningful information. Little is known about LFPs and oscillatory activity in the spinal cord, however. Indeed, many questions remain open. For example, does spontaneous intraspinal oscillatory activity even exist? Can intraspinal LFPs be compartmentalized into discrete, physiologically relevant frequency bands? If so, does sensorimotor integration predictably modulate band-specific power in intraspinal LFPs? In this exploratory study, we characterize lamina-specific spontaneous intraspinal LFPs and oscillatory activity. We then characterize intraspinal LFPs and oscillatory activity during periods of increased neural transmission in spinal pain pathways, non-pain-related sensory pathways, and motor pathways (respectively). Finally, we characterize changes in intraspinal LFPs and oscillatory activity associated with altered spinal GABA-ergic signaling. All experiments were approved by the FIU IACUC and conducted in adult male Sprague-Dawley rats (n=20). After T13-L2 laminectomy, microelectrode arrays were implanted at the L5 dorsal root entry zone. Electrodes were distributed from the superficial dorsal horn to the ventral horn. Sensory transmission was modulated by mechanical stimulation of the L5 dermatome, motor transmission was modulated via intraspinal microstimulation, and GABA-ergic signaling was modulated through use of different anesthetic agents. Outcome measures included: time-frequency analysis of LFP power during sensorimotor integration; presence, location, and frequency of spontaneous oscillations; coherence between multi-unit neural activity across laminae; and temporal measures
of synchrony amongst co-active units across laminae. Our primary finding is that intraspinal LFP power is modified in a frequency- and lamina-specific manner by changes in pain, non-pain-related sensory, and motor transmission. Analyses of firing synchrony between co-active units suggests that these changes may reflect information processing and functional connectivity across laminae. We also find evidence of spontaneous, lamina-specific bursts of oscillatory activity that can likewise be modified by sensorimotor integration. Future work is required to investigate intraspinal LFP and oscillatory activity in awake, behaving animals and to determine if it can be volitionally controlled.


Poster

303. Spinal Cord Injury and Plasticity: Neurophysiology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 303.10/J27


Support: JSPS KAKENHI Grant Number 18K08811

Title: Changes in spinal dorsal horn neuron characteristics from the early stage of peripheral nerve injury to chronic pain transition

Authors: *M. KURABE, M. SASAKI, H. BABA; Anesthesiol., Niigata Univ, Sch. Med., Niigata, Japan

Abstract: Background: Chronic pain is a major health issue and its treatment presents a challenge. The plasticity of neuronal transmission in the spinal dorsal horn (SDH) is thought to be a key mechanism in developing chronic pain. The functional and temporal changes of SDH neurons that cause the transition to chronic pain remain unknown. Clarifying long-term remodeling processes in the SDH after nerve injury will aid in developing treatments for chronic pain. In this study, we aimed to clarify remodeling processes in the SDH after nerve injury.

Methods: We divided adult male Wistar rats into 2 groups: control and chronic constriction injury (CCI) model rats. In vivo patch-clamp recordings were prepared from the SDH neurons. We compared spontaneous excitatory postsynaptic currents (sEPSCs), neuronal response properties, and time-dependent changes of receptive field (RF) after nerve injury. We next identified excitatory neurons indirectly through the absence of staining for Pax2, and positive labeling with the pan-neuronal marker NeuN. The number of excitatory neurons was analyzed at various times after nerve injury.

Results: We observed evoked EPSCs induced by noxious or innoxious stimuli and categorized neurons into 4 types: response to only noxious stimuli; response to only innoxious stimuli;
response to both noxious and innoxious stimuli; and nonresponding neurons. The relative proportion of the 4 types of neurons differed significantly between naïve and CCI rats. In addition, the RF in CCI rats was significantly increased on days 14 and 28 after nerve injury. Then, we recorded sEPSCs and evoked EPSCs. Both the frequency and amplitude of sEPSCs from CCI rats were significantly increased until 14 days after injury. However, they all gradually decreased thereafter and reached almost control levels on the 28th day. In accordance with this result, the number of Pax- and NeuN+ neurons was decreased at day 28.

Conclusions: This study is the first to demonstrate long-term remodeling processes in the SDH after nerve injury. The changes of properties and excitatory inputs in SDH neurons may have contributed to the RF expansion in CCI rats. Hence, the inhibition of early remodeling caused by nerve injury in the SDH may be a strategy for reducing the transition to chronic pain.

Disclosures: M. Kurabe: None. M. Sasaki: None. H. Baba: None.

Poster

303. Spinal Cord Injury and Plasticity: Neurophysiology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 303.11/J28


Support: National Institutes of Health/National Institute of Neurological Disorders and Stroke Grant 1RO1NS100810-01A1

US Department of Veterans Affairs

Title: Spasticity and spasms in knee extensor muscles in people with incomplete spinal cord injury

Authors: *B. A. DEFOREST*1,3, S. SANGARI1,3, J. BOHORQUEZ2, M. PEREZ1,3,4;
1Dept. of Neurolog. Surgery, The Miami Project to Cure Paralysis, Univ. of Miami, Miami, FL;
2Dept. of Biomed. Engin., Univ. of Miami, Coral Gables, FL; 3Shirley Ryan Ability Lab., Chicago, IL; 4Bruce W. Carter Dept. of Veterans Affairs Med. Ctr., Miami, FL

Abstract: Spasticity (i.e. velocity-dependent resistance to stretch) and spasms (i.e. involuntary and uncontrolled muscle contractions) are common symptoms present in humans with spinal cord injury (SCI). These two complications are commonly thought to be related, but there is no conclusive evidence that support this view. Clinically, spasticity is evaluated in individual muscles with the Modified Ashworth Scale (MAS), while spasms are assessed with the Penn Spasm Frequency scale (PSFS). To examine relationships between spasticity and spasms, we examined the MAS, PSFS, and the cutaneous reflex in the knee extensor muscles in people with motor incomplete SCI. Spinal reflexes are thought to contribute to the short-latency component
of the cutaneous reflex while persistent inward currents are thought to contribute to the long-latency component of the cutaneous reflex. We found that the area of the short-latency component of the cutaneous reflex was correlated with the MAS but not the PSFS (onset to 500 ms post stimuli; Spearman rho=0.884; p=0.001). The long-latency component of the cutaneous reflex (>500 ms post stimuli) was not correlated with the MAS (rho=0.477; p=0.164) and the PSFS (rho≤0.467; p≥0.173). Notably, the area of the short- and long-latency components of the reflex were correlated (r=0.961; p<0.001). These results suggest that while there is no relationship between clinical assessments of spasticity and spasms, the cutaneous reflex reveals a physiological relationship between these two complications. We argue that the cutaneous reflex might be a useful tool for making inferences about spasticity and spasms following SCI.


Poster

303. Spinal Cord Injury and Plasticity: Neurophysiology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: 303.12/J29


Support: National Institutes of Health/National Institute of Neurological Disorders and Stroke
US Department of Veterans Affairs

Title: Bilateral asymmetries in assessments of sensory function after spinal cord injury

Authors: *B. CHEN1,2, M. A. PEREZ1,3,2;
1Dept. of Neurolog. Surgery, The Miami Project to Cure Paralysis, Univ. of Miami, Miami, FL;
2Shirley Ryan Ability Lab., Chicago, IL; 3Bruce W. Carter Dept. of Veterans Affairs Med. Ctr., Miami, FL

Abstract: Sensory function is typically altered following spinal cord injury (SCI). Therefore, using sensitive assessments to detect changes in spared sensory pathways is critical for rehabilitation procedures and plasticity outcomes. Previous studies showed discrepancies in the level of spared sensory function detected by the international standards for neurological classification of spinal cord injury (ISNCSCI) and the electrical perceptual threshold (EPT). The goal of the present study was to compare spared sensory function as revealed by light touch and pinprick tests of the SNCSCI and the EPT exams in 16 individuals with chronic incomplete cervical SCI bilaterally. In the evaluation per dermatome, we found that the light touch component of the ISNCSCI exam detected asymmetries in 17 to 29 % of the subjects tested and the pinprick component of the ISNCSCI exam detected asymmetries in 17 to 47 % of the subjects tested. Notably, the EPT exam detected asymmetries in 82 to 94 % of the subjects.
tested. Our results demonstrate that EPT is a more sensitive tool to detect bilateral asymmetries in spared sensory function after SCI.

**Disclosures:** B. Chen: None. M.A. Perez: None.

**Poster**

**303. Spinal Cord Injury and Plasticity: Neurophysiology**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 303.13/J30

**Topic:** C.11. Spinal Cord Injury and Plasticity

**Support:** NIH Grants NS092961 and NS078680
DoD Grant W81XWH-17-1-0304

**Title:** Altered neural activity and functional connectivity in injured cervical spinal cord in monkeys


**Abstract:** Our previous high-resolution fMRI study of monkey spinal cord revealed that spinal grey matter horns demonstrate strong resting state functional connectivity (rsFC) within and cross spinal segments. Traumatic spinal cord injury (SCI) reduced the strengths of these rsFC below injury segments, but these later returned to their pre-injury strengths. This dynamic process correlated strongly with the recovery of hand use behavior in squirrel monkeys after a unilateral section of the dorsal column (DCL) [Chen et al. 2015 PNAS]. Inter-horn rsFC thus appears to reflect the functional integrity of intraspinal circuits, suggesting that rsFC metrics may serve as a biomarker of SCI severity and for predicting recovery. An improved understanding of the dynamic processes involved in the functional plasticity of intraspinal circuits, and their relationships to functional and behavioral impairments, would provide critical mechanistic information for identifying targets and time-windows for therapeutic interventions. The current study aims to (1) validate rsFC fMRI findings by comparing directly rsFC with FC measures of local field potential (LFP) coherence at 2 or 6 months after SCI, and (2) relate changes in LFP coherence to the recovery of impaired digit use in performing food reaching-grasping-retrieving tasks. To date, we have studied 4 monkeys with repeated fMRI acquisitions at high field (9.4T) followed by microelectrode electrophysiology recordings at 2 or 6 months after a DCL. All MRI images were acquired under light isoflurane anesthesia using a surface transmit-receive coil. High-resolution structural and BOLD fMRI images were acquired with the same geometry. Four 16-channel microelectrode arrays were placed bilaterally at two segments. Both fMRI and LFP signals were acquired in response to 8 Hz vibrotactile stimulation and at rest. Standard fMRI data preprocessing and LFP denoising steps were applied. We found that broad-band LFP coherence
between dorsal horns of the segment below injury was stronger than that of the above-injury segment. Across LFP bands, the differences between above and below-lesion horns varied. Coherence of gamma band signals was stronger, and coherence of theta and delta bands were weaker, below the segment of the injury compared to their corresponding measures above the segment at 6 months post-injury, a time when each monkeys’ hand use returned to pre-injury levels. Preliminary analyses also revealed reduced fMRI responses to tactile stimulation in below-injury segments compared to the normal cord. These results confirm the interpretation of BOLD rsFC as markers of changes in spinal cord circuits following injury.

**Disclosures:**  
**P.F. Yang:** None.  
**T.L. Wu:** None.  
**N. Byun:** None.  
**J.C. Gore:** None.  
**L.M. Chen:** None.

### Poster

**303. Spinal Cord Injury and Plasticity: Neurophysiology**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 303.14/J31

**Topic:** C.11. Spinal Cord Injury and Plasticity

**Support:** NIH R01 NS078680  
Dana Foundation

**Title:** Loss of tactile input differentially alters the responsiveness and functional connectivity of primate somatosensory cortices

**Authors:** L. CHEN, A. MISHRA, F. WANG, *P.-F. YANG, J. C. GORE;  
Radiology and Inst. of Imaging Sci., Vanderbilt Univ., Nashville, TN

**Abstract:** The somatosensory system undergoes drastic reorganization after the loss of its sensory inputs following spinal cord injury (SCI). This plastic process is believed to play a critical role in mediating recovery of some lost functions through mostly unknown mechanisms. Despite its high functional importance, the dynamic process of plasticity has not been systematically characterized in primates. The primary goal of the present study is to fill in this critical knowledge gap by (1) using stimulus-driven and resting state fMRI (rsfMRI) to characterize and monitor longitudinally the plasticity that occurs within the somatosensory cortices following loss of tactile input by a targeted unilateral dorsal column transection of the cervical spinal cord, and (2) relating changes in fMRI responses and resting-state functional connectivity (rsFC) to impairments in skilled hand use in squirrel monkeys over a period of two months. An improved understanding of the dynamic processes of mesoscale functional circuits and their relationship to the degree of recovery of impaired hand use would provide critical mechanistic information for identifying targets and time window for therapeutic interventions. The fMRI study was conducted in six monkeys under light isoflurane anesthesia at ultra-high
field (9.4T), which offers high contrast- and signal-to-noise ratios. A 3-cm surface transmit-receive coil was positioned over the primary (S1), secondary (S2) somatosensory, and insular cortices. High-resolution T2*-weighted anatomic (68x68µmx2000 µm³) and fMRI (547x547x2000 µm³) images were acquired. Vibrotactile stimuli (8Hz in 30s off/on cycles) were used to map digit locations, quantify cortical responsiveness, and locate digit regions before (baseline) and at different time points after a unilateral dorsal column lesion (DCL). Standard fMRI data preprocessing steps were applied. We measured the voxel-wise temporal fMRI signal magnitude at the task frequency using FFT and identified those voxels that showed statistically significant differences in BOLD signals between stimulus on and off periods. We found that the signals in deeper cortical layers dropped drastically compared to superficial regions in S1 after an injury, whereas they were more uniform beforehand. Pair-wise rsFC analyses of areas 3a, 3b, 1, and 2 revealed weakened FC across areas. In summary, a near complete loss of ascending tactile inputs following spinal cord injury leads to differential effects in cortical responsiveness to 8 Hz vibrotactile stimulation in S1 versus S2 and insular areas as well as functional connectivity strengths between S1 subareas.

Disclosures:  
P. Yang: None.  
L. Chen: None.  
A. Mishra: None.  
F. Wang: None.  
J.C. Gore: None.

Poster

304. Peripheral Auditory System

Location:  
Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 304.01/J32

Topic:  
D.06. Auditory & Vestibular Systems

Title:  
Discovery of PIPE-505, a small molecule therapeutic for the treatment of sensorineural hearing loss (SNHL) associated with cochlear synaptopathy

Authors: *D. LORRAIN, M. POON, K. J. STEBBINS, G. EDU, A. BROADHEAD, J. SEIDERS, J. ROPPE;
Pipeline Therapeut., San Diego, CA

Abstract: PIPE-505 is a gamma secretase inhibitor (GSI) in development for the treatment of SNHL associated with cochlear synaptopathy. A series of in vitro and in vivo studies in animal models of auditory loss have demonstrated two distinct mechanisms of action (MOA) that may restore hearing function in patients with SNHL. Specifically, treatment with PIPE-505 lead to 1) regeneration of synapses between Type I (spiral ganglion neuron) SGN and (inner hair cell) IHC via the Netrin/DCC path and 2) increased outer hair cells via the Notch pathway. Mouse dissociated cochlea cultures were used to demonstrate that inhibition of GS induces neurite outgrowth of Type I SGNs. Function blocking antibodies against the axon guidance pathway (e.g., deleted in colorectal cancer [DCC] and netrin) prevented PIPE-505 induced neurite outgrowth.
outgrowth, indicating that neurite stimulation is generated by PIPE-505 via the netrin-DCC pathway. Importantly, inhibition of GS prevented the cleavage of DCC, resulting in persistent expression of select receptors and consequent increased axonal length. This observation with PIPE-505 has been extended to in vivo models of hearing loss. In a noise-induced cochlear synaptopathy model local administration of PIPE-505 regenerated IHC sensory synapses and improved ABR Wave I function. In a guinea pig kanamycin ototoxicity model, intratympanic PIPE-505 restored IHC synapse number and this effect was long lasting. To our knowledge this is the first time this effect has been observed and described. From a more established perspective, PIPE-505 treatment showed evidence of generating outer hair cells (OHC). This effect has been well described and involves notch-mediated Atoh1 expression. In summary, nonclinical studies have demonstrated the potential for PIPE-505 to restore synaptic function at the IHC, as well as regenerate OHCs through a separate mechanism. The lead therapeutic MOA, regeneration of the cochlear synapse, should augment signal-to-noise processing and manifest as improved speech-in-noise comprehension, a chief auditory complaint and unmet need of patients with SNHL.

**Disclosures:**

- **D. Lorrain:** A. Employment/Salary (full or part-time); Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **M. Poon:** A. Employment/Salary (full or part-time); Full, Pipeline Therapeutics. **K.J. Stebbins:** A. Employment/Salary (full or part-time); Pipeline Therapeutics. **G. Edu:** A. Employment/Salary (full or part-time); Pipeline Therapeutics. **A. Broadhead:** A. Employment/Salary (full or part-time); Pipeline Therapeutics. **J. Seiders:** A. Employment/Salary (full or part-time); Pipeline Therapeutics. **J. Roppe:** A. Employment/Salary (full or part-time); Pipeline Therapeutics.

**Poster**

**304. Peripheral Auditory System**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 304.02/J33

**Topic:** D.06. Auditory & Vestibular Systems

**Support:** NIDCD (1R01 DC016099) to ENY.

**Title:** Electrophysiological characterization of type II spiral ganglion neurons

**Authors:** *M. C. PEREZ-FLORES, J. HAN LEE, S. PARK, M. KANG, E. N. YAMOAH; Physiol. and Cell Biol., Univ. of Nevada Reno, Reno, NV

**Abstract:** In mammals, spiral ganglion afferent neurons (SGNs) that innervate cochlear hair cells, comprise two distinct population. Myelinated type I SGNs represent ~95% of the afferent neuron population and extend one peripheral process which form a single synapse with one inner
hair cell (IHC). The type II SGNs are unmyelinated and constitute only 5% of the population, they receive synaptic inputs from ~9 outer hair cells (OHCs). Type I neurons have been extensively characterized because they overpass the number of type II neurons in a relation of 20:1. In contrast, type II neurons have small size and thin axons, making them difficult for in vivo recording. Biochemically, type I and II SGNs comprise molecularly distinct cell populations. Type I neurons are characterized as consisting of three molecular subtypes with additional variations existing across the tonotopic axis. In type II neurons an average of 6,320 genes/cell were detected. Even if such molecular profile can be used for subtype classification, further functional characterization remains unknown. Recent studies in type II fibers suggest that they may mediate the sensation of auditory pain. However, their very extensive neurite projection from the soma is not amenable for adequate current and voltage clamp characterization. Thus, the best strategy to study type II neurons is cell isolation for their subsequent recording. In this work we have identified strategies to record from the bonafide type II neurons, we have benefited from the fact that a) the neurons are unmyelinated which is an advantage to use patch electrodes; b) as type II are peripherin positive, we could potentially use EGFP-positive transgenic mouse model to isolate type II SGNs and identify them accurately. We took advantage of the transgenic mouse model to provide evidence that 1) all peripheral neurons express peripherin at early stages, and this expression is time-dependent. 2) In three weeks old mice, peripherin expression remains specifically in type II neurons. Myelination of type I neurons suppresses their peripherin expression. 3) In culture, after 24 hr the number of peripherin-positive neurons increased in a time-dependent manner, as myelin detached from type I neurons. Thus, removal of myelin may trigger EGFP expression of the transgenic mouse model. For this reason, we only recorded from peripherin positive neurons maintained in culture for less than 24 hr. 4) type II neurons expressed heterogeneous firing properties, from slow to fast adapting. This work was supported by the funding the NIDCD (1R01 DC016099) to ENY.

Disclosures: M.C. Perez-Flores: None. J. Han Lee: None. S. Park: None. M. Kang: None. E.N. Yamoah: None.

Poster

304. Peripheral Auditory System

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 304.03/J34

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC016732
Purdue University

Title: Spatiotemporal susceptibility of the cochlea and the statoacoustic ganglion to Zika virus infection
**Abstract:** Congenital Zika Syndrome caused by vertical transmission of Zika virus (ZIKV) to the gestating fetus often results in microcephaly, ventriculomegaly, and sensorineural hearing loss. It has been reported that ~6% of ZIKV-associated microcephalic newborns show diminished otoacoustic emissions and auditory brainstem responses, suggesting at least some pathogenesis may originate within the cochlea and/or the statoacoustic ganglion (SAG). Both the sensory cochlea and the SAG derive from a common neurosensory domain of the otocyst that also gives rise to vestibular sensory organs. Previous research has demonstrated that ZIKV preferentially infects neural progenitor cells of the central nervous system and causes increased cell death and reduced proliferation of infected cells. Thus, we hypothesized that the neurosensory primordium would be more susceptible to ZIKV infection than other inner ear tissues. To investigate this hypothesis, we mapped the spatio-temporal susceptibility of the embryonic cochlea and SAG to ZIKV during development in two vertebrate models – chicken embryos and mouse cochlear cultures.

For chicken embryos, ZIKV was delivered into the fluid cavity of the otocyst at embryonic day (E)2 to 5 when it is most accessible for injection, followed by histological evaluations of the inner ear 2 to 8 days-post-infection (dpi). Cells harbouring replicating virus were detected by immunostaining of double-stranded RNA. We observed infection of both sensory and nonsensory otic epithelia, the SAG, and the periotic mesenchyme. In some cases, we also noticed smaller than normal SAGs about a week after E2 and E3 ear injections; this pathology may result from increased cell death observed within the ganglion at 2 dpi.

Mouse cochlear cultures were used to deliver ZIKV at different developmental stages from proliferative prosensory cells to post-mitotic differentiated cells. Cochleas were explanted at E12.5, E15.5 or postnatal day 2, exposed to ZIKV for 1 to 2 days, and cultured for 1 to 5 dpi. Infection was blocked when cochlear cultures were preincubated with the ZIKV-117 antibody. Like the chicken data, we detected ZIKV in hair cells (HCs), supporting cells and periotic mesenchyme. At all ages, inner HCs were somewhat more susceptible to infection than outer HCs.

Apart from the SAG of chick embryos, there was limited evidence that ZIKV increased cell death in the inner epithelium in either model system, although various post-infection time points remain to be studied. Our findings suggest the possibility that direct ZIKV infection of the peripheral auditory system may be a contributing factor to hearing loss associated with congenital Zika syndrome.

**Disclosures:** D.M. Fekete: None. N.H. Sammudin: None. A. Thawani: None. V. Munnamalai: None. H. Reygaerts: None. A. Wozniak: None. R.J. Kuhn: None.
Title: Non-invasive induction of mild therapeutic hypothermia mitigates noise-induced hearing loss

Authors: *S. RINCON SABATINO*¹, R. SANGALETTI², M. HOFFER², C. KING⁵, S. RAJGURU³;
¹Biomed. Engin., Univ. of Miami, Coral Gables, FL; ²Otolaryngology, Univ. of Miami, Miami, FL; ³Biomed. Engin. and Otolaryngology, Univ. of Miami, Coral Gables, FL; ⁴Physiol. and Biophysics, Univ. of Miami, Miller Sch. of Med., Miami, FL; ⁵Lucent Med. Systems, Kirkland, WA

Abstract: Motivation: Noise-induced hearing loss (NIHL) is a leading sensorineural conditions, with an estimated 1.1 billion people at risk of NIHL due to occupational and recreational exposure to hazardous sounds (>85 dB SPL). In the present study, we assessed the use of localized mild therapeutic hypothermia (*mTH*) in the preservation of neural structures post-exposure to acute noise trauma. Methods: Male and female juvenile Brown Norway rats (15-20 weeks old) were randomly separated into four groups: Normothermic NIHL, *mTH*-treated NIHL, *mTH* Control, and Non-exposed Control. Auditory brainstem responses were performed to quantify changes in hearing threshold in anesthetized rats prior to NIHL and up to 3 months post-exposure. NIHL animals were subjected, under isoflurane anesthesia, to two hours of continuous 4-8 kHz noise at 105 dB SPL. Mild hypothermia (31-33 ºC) was induced locally in cochleae of *mTH*-treated rats at 15 minutes post-exposure for a two-hour period. Following completion of experiments, cochleae were harvested for immunohistological quantification of inner and outer hair cell survival, ribbon synapse density and strial edema. To study neuroprotective mechanisms of *mTH* for NIHL, transcriptomic changes in apoptosis-related genes were performed by RNA-sequencing of cochlear sensory epithelium and further pathway analysis was performed by qRT-PCR. Results: At 1-day post-exposure, Normothermic animals had significantly higher threshold shifts than *mTH*-treated animals across all frequencies tested. Mild hypothermia treatment showed greater recovery in ABR thresholds and wave I and IV amplitudes in the days following noise exposure with nearly full recovery to control values, whereas the normothermic group elicited significantly higher thresholds at early timepoints and only a partial recovery at 28 days.
post-exposure. Significant synaptic ribbon loss was observed in representative regions corresponding to 8 and 16 kHz only in normothermic cochleae at 1- and 3-days post-exposure. Results of RT-PCR show that mTH prevents additional damages by inhibiting the activation of caspase 3 and 8, thus interrupting the extrinsic apoptotic pathway. **Conclusions:** Delivery of non-invasive, controlled and localized mild therapeutic hypothermia to the inner ear is feasible and safe. Mild therapeutic hypothermia post-NIHL is efficacious and significantly lowers hearing threshold shifts and preserves residual hearing. Therapeutic hypothermia provides significant protection from neuropathy and synaptopathy likely via downregulation of apoptotic pathways and inflammation.

**Disclosures:** S. Rincon Sabatino: None. R. Sangaletti: None. M. Hoffer: None. C. King: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds): Restor-Ear Devices LLC, Lucent Medical Systems. S. Rajguru: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Restor-Ear Devices LLC.

**Poster**

**304. Peripheral Auditory System**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 304.05/J36

**Topic:** D.06. Auditory & Vestibular Systems

**Support:** NIH Grant R01DC016066 (L.S.)
NIH Grant R01DC06283 (M.E.W.)
Amelia Peabody Charitable Fund

**Title:** Noise damage and repair in zebrafish: Modeling acoustic overstimulation in lateral-line organs

**Authors:** M. HOLMGREN\(^1\), M. E. RAVICZ\(^2,3\), K. E. HANCOCK\(^2,3\), M. E. WARCHOL\(^1\), *L. SHEETS\(^1;\)
\(^1\)Dept. of Otolaryngology–Head and Neck Surgery, Washington Univ. Sch. of Med. In St. Lo, St. Louis, MO; \(^2\)Dept. of Otolaryngology–Head and Neck Surgery, Harvard Med. Sch., Boston, MA; \(^3\)Eaton-Peabody Lab., Massachusetts Eye and Ear, Boston, MA

**Abstract:** Sensory hair cells are specialized receptors for hearing and balance. Loud or extended noise exposure damages hair cells, often resulting in either loss of synaptic connections with auditory nerve fibers in the cochlea or hair-cell death. Currently, the cellular mechanisms underlying hair-cell damage following noise exposures are poorly defined. Determining these mechanisms is a critical step toward understanding the underlying causes of sensorineural hearing loss.
Zebrafish lateral-line neuromast hair cells are similar to mammalian hair cells at the molecular and cellular level, but are superficially localized on the skin, making them pharmacologically and optically accessible within intact larvae. We therefore exposed 7-day-old free-swimming zebrafish larvae to strong water current (60 Hz; 1.04 +/- 0.01 m/s²) in a multi-well dish attached to an electrodynamic shaker. Fish exposed to either a brief pulse followed by 2 hours of uninterrupted stimulation or short, repeated stimulation spanning a total of 2 hours showed a subset of neuromasts with disrupted morphology and significantly reduced hair-cell innervation. Additionally, fish exposed to the uninterrupted stimulus showed a significant loss of hair cells accompanied by recruitment of macrophages, indicating noise-induced injury. Hair-cell function, as measured by uptake of the cationic dye FM1-43, was also significantly reduced in noise-exposed neuromasts. Notably, noise-induced damage appears to repair within hours; hair-cell morphology and number returned to normal 4-8 hours following noise and corresponded to clearance of damaged hair cells by macrophages. Moreover, exposed hair cells with recovered morphology showed normal afferent innervation, suggesting nerve contacts also repair. Functionally, FM1-43 uptake completely recovered 48 hours following noise exposure. These observations support zebrafish as a useful model for defining the cellular pathways that contribute to hair-cell damage resulting from noise and for determining how hair-cell organs may repair following noise damage.

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**Poster**

**304. Peripheral Auditory System**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 304.06/J37

**Topic:** D.06. Auditory & Vestibular Systems

**Support:** NIBIB grant EB018297
R01DC004820
NIH R01-04084

**Title:** Contrasting mechanisms for hidden hearing loss: Synaptopathy vs myelin defects

**Authors:** *M. BUDAK¹, K. GROSH², G. CORFAS⁵, M. R. ZOCHOWSKI³, V. BOOTH⁴;
¹Biophysics, ²Mechanical Engin. & Biomed. Engin., ³Physics, ⁴Mathematics & Anesthesiol., Univ. of Michigan, Ann Arbor, MI; ⁵Kresge Hearing Res. Inst., The Univ. of Michigan, Ann Arbor, MI

**Abstract:** Hidden hearing loss (HHL) is an auditory neuropathy characterized by normal hearing thresholds but reduced amplitude of the sound-evoked auditory nerve compound action potential
It has been proposed that in humans HHL leads to speech discrimination and intelligibility deficits, particularly in noisy environments. Animal models originally indicated that HHL can be caused by moderate noise exposures or aging, and that loss of inner hair cell (IHC) synapses could be its cause. Recently, Wan and Corfas (2017) provided evidence that transient loss of cochlear Schwann cells also causes permanent auditory deficits in mice, which have characteristics of HHL, showing that the only histological finding in the mice after Schwann cell regeneration is a permanent disruption of the myelination patterns at the heminode of auditory nerve (AN) peripheral terminals. To shed light on mechanisms of HHL and to test their impact on AN activity, we constructed a reduced biophysical model for a population of AN fibers. We found that the amplitude of simulated sound-evoked AN CAPs is lower and has a greater latency when the heminodes are disorganized across the population, i.e. they are placed at uneven distances from the hair cell synapse rather than at the stereotypic distance as seen in the normal cochlea. Model analysis confirms that disruption of the position of the heminode causes loss and desynchronization of AN spikes, thus leading to a disruption of temporal resolution and reduction of the sound-evoked AN CAP. We also simulated synaptopathy by removing high threshold IHC-AN synapses and found that the amplitude of simulated sound-evoked AN CAPs decreases while latency remains unchanged, corresponding with observations in noise exposed animals. Thus, model results explain the differential effects caused by demyelination and synaptopathy underlying auditory deficits in HHL.


Poster

304. Peripheral Auditory System

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 304.07/J38

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant R01 DC016595

Title: Pou3f4 expressing otic mesenchyme cells promote spiral ganglion neuron survival

Authors: *P. M. BROOKS, M. L. MACRAE, K. M. RANGOUSSIS, T. M. COATE; Biol., Georgetown Univ., Washington, DC

Abstract: Current therapies to improve hearing, including hearing aids and cochlear implants, require intact and functional auditory neurons. Within the inner ear, the primary auditory neurons, spiral ganglion neurons (SGNs), are surrounded by mesenchyme cells which express the transcription factor Pou3f4 (also known as Brn4). Mutations in Pou3f4 have been associated with DFNX2, the most common form of X-linked deafness and typically include developmental
malformations of the middle and inner ear, and profound lifelong hearing loss. Presently, it is known that interactions between Pou3f4-expressing mesenchyme cells and SGNs are important for proper axon bundling during development. However, although Pou3f4 remains expressed into adulthood, the functional significance of Pou3f4 for postnatal SGNs remains elusive. To address this question, we first carefully documented Pou3f4 protein expression in early postnatal mouse cochlea followed by comparisons of SGNs in Pou3f4 knockout mice (Pou3f4<sup>y/−</sup>) and littermate controls (Pou3f4<sup>y/+</sup>). In Pou3f4<sup>y/−</sup> mice, SGN density begins to decline by the end of the first postnatal week, with approximately 25% of SGNs ultimately lost. Interestingly, this period of neuronal loss coincides with a transient population of Pou3f4-expressing cells particularly around and within the spiral ganglion. Given the developmental interaction between Pou3f4 and SGNs, we used a sparse labeling model to confirm that there is no difference in the percentage of neurons making contact with the sensory domain which could account for the observed neuronal loss. Instead, these data suggest that Pou3f4-expressing mesenchyme cells play a role in maintaining SGN populations during the early postnatal period. We are currently determining the extent to which Pou3f4 is necessary to maintain all, or only select, SGN subtypes.


Poster

304. Peripheral Auditory System

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 304.08/J39

Topic: D.06. Auditory & Vestibular Systems

Support: University of Miami Provost Research Awards
        University of Miami College of Arts and Sciences
        University of Miami Gabelli Fellowship

Title: Molecular mechanisms of alcohol-induced developmental defects in the zebrafish inner ear

Authors: *L. Y. ZAMORA<sup>1</sup>, I. SKROMNE<sup>2</sup>, Z. LU<sup>1</sup>;
        <sup>1</sup>Biol., Univ. of Miami, Miami, FL; <sup>2</sup>Biol., Univ. of Richmond, Richmond, VA

Abstract: Alcohol exposure is known to cause fetal alcohol spectrum disorders (FASD) that may manifest as lifelong physical, cognitive, and behavioral anomalies of human development. In our previous studies, we uncovered an alcohol-sensitive period, 12 to 17 hours post-fertilization (hpf) when the developing inner ear and lateral line were most vulnerable to alcohol insult. However, the mechanisms by which early alcohol exposure affects the developing
auditory system is still largely unknown. In this study, we determined if biological processes necessary for inner ear development are adversely affected by alcohol using transgenic, \textit{in situ} hybridization, and immunocytochemical techniques. To model fetal alcohol exposure, zebrafish (\textit{Danio rerio}) were treated in 2\% alcohol from 12 to 17 hpf. Vital biological processes that contribute to inner ear development were examined to uncover the mechanisms that underlie alcohol-induced inner ear defects. We focused on the Fibroblast Growth Factor (FGF) pathway, which is known as the major signaling required for inner ear development. First, we found \textit{Tg(HSP70:XFD)} embryos with heat-inducible FGF-inhibition had similar defective inner ear phenotypes compared to alcohol treatment. Next, we examined the effects of embryonic alcohol exposure on FGF signaling with an antibody targeting a FGF signaling marker, phosphoeryrized extracellular signal-related kinase (pERK). Results showed a reduction of inner ear pERK expression after alcohol exposure. In addition, we observed that alcohol exposure decreases gene expression of \textit{fgf3}, a critical ligand for initiation of FGF signaling, and an FGF target gene, \textit{sprouty4}. Furthermore, we administered SU5402, a pharmacological drug to inhibit FGF signaling at the intracellular receptor level. Treatment with SU5402 alone and SU5402 with alcohol resulted in similar inner ear defects, indicating that alcohol, like SU5402, may also affect FGF receptors. Our results provide evidence of molecular effects from alcohol exposure during inner ear development and serve to initiate the unraveling of complex and intricate mechanisms which lead to defective inner ear organogenesis. Overall, this is the first study to reveal that embryonic alcohol exposure suppresses FGF signaling, leading to decreased hearing capacity.

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Poster

304. Peripheral Auditory System

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 304.09/J40

Topic: D.06. Auditory & Vestibular Systems

Title: A backward encoding approach to recover subcortical auditory activity

Authors: *F. SCHMIDT*\textsuperscript{1}, G. DEMARCHI\textsuperscript{1}, F. GEYER\textsuperscript{2}, N. WEISZ\textsuperscript{1};
\textsuperscript{1}Ctr. for Cognitive Neurosci., \textsuperscript{2}Univ. of Salzburg, Salzburg, Austria

Abstract: Several subcortical nuclei along the auditory pathway are involved in the processing of sounds. One of the most commonly used methods of measuring the activity of these nuclei is the auditory brainstem response (ABR). Due to its low signal-to-noise ratio, ABRs have to be derived by averaging activity evoked by a high number (several thousand) of repetitions of e.g. clicks or tone bursts. To date no approach exists that can be used to non-invasively investigate both auditory brainstem activity following natural sounds (e.g. speech, music) and silent periods, for example, within selective attention tasks. For several cognitive neuroscientific questions this
is a severe limitation. We propose that by training a backward encoding model to reconstruct evoked ABRs from electrophysiological data, spatial filters (channel weights) can be obtained that are tuned to auditory brainstem activity. Since these filters can be applied to any other dataset (i.e. generalized) using the same spatial coverage, this could allow for the estimation of auditory brainstem activity from any continuous sensor level data. In this study, we established a proof-of-concept that by employing a backward encoding model generated using a click stimulation rate of 30 Hz we could predict the expected ABR activity recorded via electroencephalography (EEG) from an independent measurement, using a stimulation rate of 9 Hz. By showing that the individually predicted and measured ABRs are highly correlated ($r \sim 0.67$), we laid the necessary foundation to use this paradigm in more naturalistic listening situations.

**Disclosures:**  F. Schmidt: None. G. Demarchi: None. F. Geyer: None. N. Weisz: None.

**Poster**

**304. Peripheral Auditory System**

**Location:** Hall A

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**Topic:** D.06. Auditory & Vestibular Systems

**Support:** ERC Grant No. 670759
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DFG - DFG Research Center and Cluster of Excellence, Center for Nanoscale Microscopy and Molecular Physiology of the Brain
Leibniz program
BMBF - Optical Cochlear Implant (No. 13N13728)

**Title:** Towards the optical cochlear implant: Optogenetic stimulation of the auditory pathway

**Authors:** *D. KEPPELER*¹, A. DIETER¹, E. KLEIN², M. SCHWAERZLE³, P. RUTHER⁴, T. MOSER⁵;
¹Inst. for Auditory Neurosci., Goettingen, Germany; ²IMTEK, Univ. of Freiburg, Freiburg Im Breisgau, Germany; ³IMTEK, ⁴Univ. of Freiburg, Freiburg im Breisgau, Germany; ⁵Inst. for Auditory Neurosci., Univ. Med. Ctr. Goettingen, Goettingen, Germany

**Abstract:** With 500,000 patients worldwide, the cochlear implant (CI) is the most successful neuroprosthesis. In the majority of patients, direct electrical stimulation of the auditory nerve restores open speech perception bypassing the defective sensory organ. However, the major bottleneck of current CIs is the poor coding of spectral information, which results from broad current spread from each electrode contact within the saline solution filled cochlea. As light can
be more conveniently confined, optical stimulation of the auditory nerve presents a promising perspective for a fundamental advance of CIs. In this study, we established reliable expression of the ultra-fast channelrhodopsin (ChR) Chronos in mouse spiral ganglion neurons (SGNs) and demonstrated its support of high temporal fidelity in the auditory nerve. Further, we developed multichannel optical CIs based on micro-fabricated light-emitting diode (LED) arrays. We characterized them for optical stimulation of channelrhodopsin-2 (ChR2)-expressing SGNs in transgenic rats or gerbils using optical auditory brainstem responses (oABR) stimulated by individual or multiple LEDs. Positioning of the probes and atraumatic implantation was validated with micro-CT imaging. Multielectrode array recordings along the tonotopic axis of the inferior colliculus (IC) indicate spectral selectivity of optical stimulation closer to acoustic than electrical stimulation. This contribution will summarize the current state of optogenetic stimulation of the auditory pathway and report on recent breakthroughs on achieving high temporal fidelity, frequency resolution and establishing multichannel optical CIs.


**Poster**

**304. Peripheral Auditory System**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 304.11/J42

**Topic:** D.06. Auditory & Vestibular Systems

**Support:** Swedish Research Council  
Torsten Söderberg foundation  
County Council of Östergötland

**Title:** Radixin modulates outer hair cell stereocilia function

**Authors:** *S. PRASAD, A. FRIDBERGER;*  
Linköping Univ., Linköping, Sweden

**Abstract:** The RDX gene encodes the actin-binding protein radixin. In humans, RDX mutations cause congenital hearing loss, but radixin is expressed at high levels also in the mature inner ear, suggesting an important but yet undefined functional role. Based on its localization within sensory cell stereocilia, its actin-binding properties, and abundance, we hypothesized that radixin could affect the amount of force required to deflect stereocilia during sound stimulation. Previous work suggested that stereocilia stiffness may be actively regulated, but the underlying molecules were not determined. We applied quantitative time-resolved phase locked confocal imaging in combination with electrophysiology in semi-intact preparations of guinea pig
temporal bones to simultaneously visualize sound-evoked motion of stereocilia and record electrical potentials. The extracellular potentials are tuned to a particular sound stimulus of 84 dB SPL frequency near 220 Hz to get a maximum response. The acquired image sequences were low-pass filtered and motion quantified through optical flow analysis using Matlab. We show that blockage of radixin leads to increased stereocilia deflections during sound stimulation and increased electrically evoked motion of sensory cells. Both findings are consistent with reduced stiffness of stereocilia. However, blockade of radixin also decreased the electrical potentials produced by the sensory cells and showed mild loss in the hearing sensitivity measured from in vivo compound action potentials during sound stimulation, causing an acutely developing hearing loss. Thus, the present data shows that radixin is required for the mechanical stability of stereocilia and their ability to transduce sound into electrical potentials, thereby contributing strongly to the maintenance of hearing sensitivity in the adult inner ear. To conclude we have investigated the physiological relevance of radixin protein by looking into the importance of the regulation of radixin-mediated signaling processes as a novel mechanism for the regulation of stereocilia.

Disclosures: S. Prasad: None. A. Fridberger: None.

Poster

304. Peripheral Auditory System

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 304.12/J43

Topic: D.06. Auditory & Vestibular Systems

Title: Cochlear activity during silent periods shows a theta rhythmic pattern and is correlated to classical intermodal attention alpha effects
Authors: *M. H. A. KOEHLER, G. DEMARCHI, N. WEISZ;
Ctr. for Cognitive Neurosci., Univ. of Salzburg, Salzburg, Austria

Abstract: It is well established that the auditory efferent network can influence cochlear processes via direct and mediated projections from the auditory cortex to the superior olivary complex (SOC). Though, there is an ongoing controversy where in the processing hierarchy of this network attention processes take effect. So far all studies showing attentional modulations of cochlear responses have been limited to sound evoked responses (e. g. Wittekindt et al. 2014). The present study uses a trial-wise cueing paradigm to investigate audiovisual selective attention in humans simultaneously at the cortical and cochlear level during a stimulus-free cue-target period.

First, it was found that cochlear activity in silent cue-target periods was for both auditory and visual selective attention intrinsically modulated by a theta-rhythmic pattern (~4 Hz). Second, during periods of auditory selective attention slow modulations of cochlear activity were enhanced. Functional brain data revealed that posterior alpha and beta activity was enhanced during auditory selective attention. Interestingly, the found cochlear and ‘classical’ cortical posterior alpha attention effects showed a significant negative correlation.

It is hinted that the attentional sampling of the cochlea is putatively driven by a theta rhythm. Eventually, the correlation between cochlear and cortical attention effects suggests that participants showing the ‘classical’ posterior alpha modulations to a lesser extent appear to engage more strongly the efferent auditory system.

Disclosures: M.H.A. Koehler: None. G. Demarchi: None. N. Weisz: None.

Poster

304. Peripheral Auditory System

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 304.13/J44

Topic: D.06. Auditory & Vestibular Systems

Support: NSFC 31771618
NSFC 31571496

Title: Low Notch and high Wnt signaling determines hair cell fate in neuromast regeneration

Authors: Y. WU, F. ZHAO, P. WANG, F. RAO, X.-J. SONG, *D. LIU;
SUSTech Ctr. for Pain Medicine, Dept. of Biol., Southern Univ. of Sci. and Technol., Shenzhen, China

Abstract: The neuromast is a peripheral sensory organ of the lateral line system composed of the hair cells (HCs) and supporting cells (SCs). Numerous present on the body surface, teleost
neuromasts are ideal for study of mechanisms underlying damage-induced HC regeneration. We illustrate here that cisplatin-damaged zebrafish neuromasts generate new HCs within 12 hours post cisplatin treatment (12hpt), followed by proliferative HC regeneration, i.e., two new HCs are produced in pair. If the mitotic SCs of neuromasts are arrested at the S phase of the cell cycle before cisplatin treatment, some of them become new HCs within 12hpt, whereas if mitotic SCs are arrested at the M phase prior to cisplatin treatment, only proliferative HC regeneration happens. We find that a combined low Notch activity and high Wnt signaling state favors HC fate determination in some mitotic SCs, which are likely around the G1 phase, during and after HC damage. To support this view, in a mutant that lacks proliferative HC regeneration, unusual high Wnt activity is evident shortly after the cisplatin treatment and very few mitotic SCs enters the S phase. Therefore, a state of low Notch and high Wnt signaling somehow specifies the HC fate in some HC progenitor cells of ototoxin-damaged neuromast.

Disclosures:  Y. Wu: None. F. Zhao: None. P. Wang: None. F. Rao: None. X. Song: None. D. Liu: None.

Poster

304. Peripheral Auditory System

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 304.14/J45

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC017147
         NIH Grant DC015294
         Showalter Young Investigator Award
         Indiana University School of Medicine startup funding

Title: Functions of stereocilia base in auditory perception

Authors: *B. ZHAO;
Dept. of Otolaryngology Head & Neck Surgery, Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: The integrity of stereocilia, the mechanosensing organelles of inner-ear hair cells, is critical for auditory perception. The base of stereocilia shows a striking structural organization including the formation of a taper as well as cytoskeletal specializations including rootlet filaments. This region is critical for the morphogenesis of stereocilia and hair cell function since stereocilia pivot at their base during mechanical stimulation. However, molecular machineries that shape the stereocilia base are just beginning to be elucidated. By performing a screening, we have found several proteins localized at the basal region of stereocilia, including GRXCR2. Mutations in GRXCR2, which encodes a protein of undetermined function, cause hearing loss in humans and mice. We found that GRXCR2 forms a complex at the base of stereocilia with
taperin, another protein of unknown function required for human hearing. Stereocilia lacking GRXCR2 are longer than normal and disorganized due to the mislocalization of taperin, which could modulate the actin cytoskeleton in stereocilia. Remarkably, reducing taperin expression levels could rescue the morphological defects of stereocilia and restore the hearing of Grxcr2-deficient mice. Thus, our findings suggest that GRXCR2 is critical for the morphogenesis of stereocilia and auditory perception by restricting taperin to the stereocilia base.

Disclosures: B. Zhao: None.

Poster

304. Peripheral Auditory System

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: /Poster #: 304.15/J46

Topic: D.06. Auditory & Vestibular Systems

Support: Garnet Passe and Rodney Williams Memorial Foundation Research scholarship

Title: Apoptosis signal regulating kinase 1 (MAP3K5) inhibition attenuates aminoglycoside ototoxicity

Authors: *J. M. OGIER¹, B. NAYAGAM², R. BURT¹, E. DUNNE², P. J. LOCKHART¹; ¹Neurogenetics, ²Infection and Immunity, Murdoch Childrens Res. Inst., Parkville, Australia; ³Genet., The Univ. of Melbourne, Parkville, Australia

Abstract: More than one hundred medicines are ototoxic, meaning toxic to the ear. Of these, two drug classes are particularly problematic. The platinum based chemotherapeutics used in most anti cancer treatments, and, the aminoglycoside antibiotics used to manage chronic or multi drug resistant infection. These drugs save lives, but also destroy sensory hair cells within the ear. As a result, patients can experience devastating side effects, including permanent hearing loss, tinnitus, ataxia, nausea and vertigo. Ototoxic medicines have disabled millions of people worldwide, including approximately 1,280,000 children. We must find a way to prevent drug induced hair cell death, however, the process by which hair cells die remains uncertain. The c-Jun N-terminal kinase (JNK) pathway has been identified as a mediator of hair cell death. However, the role of upstream apoptotic regulator, Apoptosis signal-regulating kinase 1 (MAP3K5) has not been investigated in hair cells. Importantly, MAP3K5 is ubiquitously expressed, redox sensitive and drives the prolonged, apoptotic activation of JNK. However, MAP3K5 does not impact short term, homeostatic JNK activity. Therefore, we considered MAP3K5 as a target for preventing JNK mediated hair cell death. We hypothesised that MAP3K5 deficiency would prevent sensory cell death and protect against ototoxic drug-induced hearing loss. We used in vitro cochlear explants to (1) investigate the effect of MAP3K5 knockout on resistance to aminoglycoside-induced sensory hair cell death and (2) investigate the
efficacy of a MAP3K5 small molecule inhibitor in promoting sensory hair cell survival. We also investigated whether the MAP3K5 inhibitor changed aminoglycoside antibiotic efficacy. MCRI Animal Ethics approval A844. In vivo, MAP3K5 knockout has no effect on the development of the ear and hearing, as assessed by histology and the Auditory Brainstem Response. In vitro, MAP3K5 deficiency attenuates neomycin induced hair cell death. MAP3K5 inhibition significantly enhances the survival of aminoglycoside treated hair cells and the MAP3K5 inhibitor does not impair aminoglycoside activity against *pseudomonas aeruginosa*. Overall, MAP3K5 is a promising target for the prevention of drug induced hearing loss.


Poster

305. Auditory Cortex: Temporal and Frequency Factors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 305.01/K1

Topic:  D.06. Auditory & Vestibular Systems

Support:  UK Medical Research Council G0901321, MC_U135097128 and MC_UU_00010
American University of Beirut Research Board

Title:  Neuronal frequency tuning in human auditory cortex: A 7T fMRI adaptation and modelling approach

Authors:  *J. BESLE*¹, R.-M. SANCHEZ-PANCHUELO², S. FRANCIS², K. KRUMBHOLZ³;
¹Dept. of Psychology, American Univ. of Beirut, Beirut, Lebanon; ²Sir Peter Mansfield Imaging Centre, Sch. of Physics and Astronomy, ³Inst. of Hearing Research, Div. of Clin. Neuroscience, Sch. of Med., Univ. of Nottingham, Nottingham, United Kingdom

Abstract:  Frequency selectivity is a ubiquitous property of auditory neurons. Measuring it in human auditory cortex may be crucial for understanding common auditory deficits, but current non-invasive neuroimaging techniques can only measure the aggregate response of large populations of cells, thereby overestimating tuning width. Here we attempted to estimate neuronal frequency tuning in human auditory cortex using an fMRI-adaptation paradigm. We measured the BOLD response (sparse 2D GE-EPI sequence, 1.5mm resolution, TR=7.5s) in 12 participants to a high frequency (3.8 kHz) probe presented alone or preceded by adaptors at different frequencies (0.5 to 3.8 kHz). Adaptation tuning curves (the degree to which adapters at different frequencies decrease the response to the probe, assumed to reflect neuronal tuning) were constructed by subtracting the BOLD response to adaptors presented alone. Responses to adaptors alone were also used to estimate population response tuning curves. In addition, we delineated primary and secondary cortex in each subject using a combination of tonotopic and in-
vivo myelin mapping (phase-sensitive inversion recovery sequence, 0.6 mm resolution). Both adaptation and response tuning curves were narrower in primary than secondary auditory cortex but, contrary to expectation, adaptation tuning curves were as wide as population response tuning curves in both regions. To further examine this result, we implemented a simplified model of neuronal (frequency-specific) and BOLD (non-specific) adaptation effects in a tonotopically-organized neuron population and we showed that apparent adaptation tuning can result from non-specific BOLD adaptation effects combined with tonotopic organization. We then fitted the neuronal tuning width and other parameters of the model to our fMRI adaptation and population response tuning curves and found neuronal tuning width parameter values that were consistent with extrapolations from the primate electrophysiological literature. Our results show that it may be possible to estimate neuronal population tuning in auditory cortex using an fMRI frequency adaptation paradigm, provided that BOLD adaptation effects and the properties of tonotopic organization are adequately taken into account.

**Disclosures:** J. Besle: None. R. Sanchez-Panchuelo: None. S. Francis: None. K. Krumbholz: None.

**Poster**

**305. Auditory Cortex: Temporal and Frequency Factors**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 305.02/K2

**Topic:** D.06. Auditory & Vestibular Systems

**Title:** Targeted cortical manipulation of auditory perception

**Authors:** *S. CEBALLO*¹, Z. PIWKOWSKA², J. BOURG¹, A. DARET¹, B. BATHELLIER¹; ¹Paris-Saclay Inst. of Neurosci. (Neuropsi), Gif-Sur-Yvette, France; ²Dynamic Neuronal Imaging Unit, Inst. Pasteur, Paris, France

**Abstract:** Driving perception by direct activation of neural ensembles in cortex is a necessary step for achieving a causal understanding of the perceptual code and developing central sensory rehabilitation methods. Here, using optogenetic manipulations during an auditory discrimination task in mice, we show that auditory cortex can be short-circuited by coarser pathways for simple sound identification. Yet, when the sensory decision becomes more complex, involving temporal integration of information, auditory cortex activity is required for sound discrimination and targeted activation of specific cortical ensembles changes perceptual decisions as predicted by our readout of the cortical code. Hence, auditory cortex representations contribute to sound discriminations by refining decisions from parallel routes.

**Disclosures:** S. Ceballo: A. Employment/Salary (full or part-time); Centre National de la Recherche Scientifique (CNRS). Z. Piwkowska: A. Employment/Salary (full or part-time);
Poster

305. Auditory Cortex: Temporal and Frequency Factors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 305.03/K3

Topic: D.06. Auditory & Vestibular Systems

Support: DARPA NESD (N666001-17-C-4013)
Private gift

Title: Decoding complex sounds using broadband population recordings from secondary auditory cortex of macaques

Authors: *J. LEE1, C. D. HEELAN1,4, L. LYNCH1, R. O'SHEA1, D. BRANDMAN5, W. TRUCCOLO2,3, A. V. NURMIKKO1,3;
1Sch. of Engin., 2Neurosci., 3Carney Inst. for Brain Sci., Brown Univ., Providence, RI;
4Connexon Systems, Providence, RI; 5Dept. of Surgery (Neurosurgery), Dalhousie Univ., Halifax, NS, Canada

Abstract: Direct electronic communication with auditory and speech areas of the neocortex is a challenging addition for brain-computer interfaces. We report the first successful neural decoding of many English words with high intelligibility from intracortical spike-based neural population activity recorded from the secondary auditory cortex of macaques. Whereas the core of the secondary auditory cortex lies in the lateral sulcus, significant portions of the adjacent belt and parabelt lie on the superior temporal gyrus (STG). The STG is accessible to chronic implantation of microelectrode arrays (MEAs) for broadband population recording of spikes and field potentials. Our goal was to access auditory cortical circuits at a much higher spatial and temporal resolution than provided by ECoG or other epicortical electro-physiological techniques. We implanted two 96-channel intracortical arrays in the parabelt areas of the secondary auditory cortex in the rhesus macaque model and successfully decoded multiunit spiking activity to reconstruct intelligible English words and macaque calls. Using a novel neural processing toolkit (NPT), we performed an end-to-end neural decoding grid-search to explore the effects of signal properties, decoding algorithms, neural preprocessing, audio presentation, channel count and hyperparameters on reconstructing audio from full-broadband neural data recorded in STG. This computational experiment resulted in neural decoding models that successfully decoded neural activity into intelligible audio on the training, validation, and test data sets. We found that the LSTM RNN decoder outperformed other common neural decoding
algorithms. This is consistent with the findings of other studies in decoding neural activity recorded from the human motor cortex, and recent work from our group has demonstrated the feasibility of using LSTM RNN decoders for real-time neural decoding. We also showed that optimizing the neural frequency content via a bandpass filter prior to multiunit spike extraction provided a marginal decoding performance improvement. In sum, the results illuminated a view of the auditory cortex as a spatially distributed network and a general purpose processor of complex sounds. While we will continue to improve the performance of our neural decoding models, the presented results provide one starting point for future neural encoding work to “write in” neural information by patterned microstimulation to elicit naturalistic audio sensations towards the potential development of an auditory cortical prosthesis. Such future work can guide steps towards the potential development of an auditory cortical prosthesis.


Poster

305. Auditory Cortex: Temporal and Frequency Factors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 305.04/K4

Topic: D.06. Auditory & Vestibular Systems

Support: BBSRC New Investigator Award BB/M010929/1
Wellcome Principal Research Fellowship WT108369/Z/2015/Z

Title: Temporal and resolved harmonic pitch encoding in auditory cortical neurons

Authors: Q. GAUCHER1, A. Z. IVANOV3, M. PANNIELLO2, A. J. KING2, *K. M. M. WALKER2;  
1Dept. of Anatomy, Physiol. and Genet., 2Physiology, Anat. & Genet., Univ. of Oxford, Oxford, United Kingdom; 3The Univ. of Oxford, Oxford, United Kingdom

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 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”, primarily using temporal periodicity cues (Walker et al., 2019). Neurons that are sensitive to pitch cues are distributed widely across the auditory cortex (Bizley et al., 2010; Walker et al., 2011), and correlate with ferrets’ trial-to-trial pitch judgments (Bizley et al., 2013). However, these studies did not examine whether the “pitch-sensitive” 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
Here, we investigated the resolved harmonic and temporal periodicity cues that underlie pitch responses in ferret auditory cortex, and whether a subset of “pitch selective” neurons may integrate these cues across sounds with different spectra. We recorded the responses of large populations of individual neurons to a variety of sounds that varied in F0 (17 F0 values; 250 - 4000 Hz). The stimuli included click trains with varied temporal periodicity, pure tones, and harmonic tone complexes with filtering and phase manipulations to alter resolved harmonic or temporal envelope cues. Neural responses were measured from ketamine and medetomidine anesthetized adult ferrets using (1) high-channel-count multielectrodes (Neuropixels), and (2) 2-photon imaging of calcium dynamics in neurons expressing GCaMP6. We found that auditory cortical neurons often show greater sensitivity to temporal cues than resolved harmonic cues, in keeping with our recent behavioural findings (Walker et al., 2019). We also identified a subset of neurons with pitch-selective responses, similar to those previously reported in marmoset (Bendor & Wang, 2005), suggesting that specialized pitch processing also exists in non-primates.


Poster

305. Auditory Cortex: Temporal and Frequency Factors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 305.05/K5

Topic: D.06. Auditory & Vestibular Systems

Title: MEG correlates of periodicity relevant to pitch perception in human auditory cortex

Authors: *S.-G. KIM1, T. OVERATH1,2,3,4,5, W. SEDLEY6, S. KUMAR5,6, S. TEKI5, T. D. GRIFFITHS5,6,

Abstract: Pitch is a property of many sounds for which the cortical substrate is still debated. In this study we used MEG to estimate neural ensemble activity in sensor and source space using three types of pitch-evoking periodic stimuli at rates below and above the lower limit of pitch. The stimuli were harmonic complex tones (HC), click trains (CT) and iterated rippled noise (IRN). Trials consisted of noise-periodic-noise (NPN) or periodic-noise-periodic (PNP) segments in which the periodic segment was either above (250 Hz) or below (20 Hz) the lower limit of pitch. For ten healthy volunteers (age = 26 ± 7, 5 females), neuromagnetic responses while listening to
NPN and PNP trials were recorded using a 274-channel MEG system (CTF systems, Canada), and T1-weighted anatomical MR images were acquired. Using the minimum norm estimation (MNE) Python package, source activity of evoked responses was estimated via noise-normalized MNE. The pitch-associated periodic stimuli (at 250 Hz) were all associated with marked evoked responses at ~130 ms at both sensor and source levels after the NP transition (Figure 1). Evoked responses for PN transitions were much weaker (HC, CT) or absent (IRN). The non-pitch associated stimuli (at 20 Hz) were associated with weak or absent evoked responses at ~130 ms after the NP transition. On the supratemporal plane, evoked responses in Heschl’s gyrus showed the greatest peaks, particularly to NP transitions in 250-Hz trials involving HC and CT. Responses to pitch-associated periodicity also occurred in the more anterior temporal lobe and on the temporal convexity (Figure 1b). In an interval of 100 to 160 ms around the NP peak response, a repeated-measures ANOVA of sensor-level data revealed significant main effects for all factors (Periodicity-type, Fundamental-frequency, Transition-type, all p < 0.02), as well as for an RM ANOVA at source level with the additional factor region-of-interest (all p < 0.003). The data demonstrate cortical sensitivity to periodicity associated with pitch that is consistently present across different pitch-associated stimuli in the region of HG.

Figure 1. MEG evoked responses to onset and offset of pitch-eliciting periodicity. (A) Root mean squared (RMS) of all MEG sensors with respect to noise-to-periodic (NP) or periodic-to-noise (PN) transitions for various conditions (HC, harmonic complex; CT, click train; IRN, iterated rippled noise) with F0 of 250 Hz or 20 Hz. A time interval of [100, 160] ms for statistical inference is marked by shades. (B) Inter-subject average source activity estimated on cortical surface sources for NP and PN transitions, averaged over all types of conditions. (C) Region-of-interest (ROI)-wise source time series (maximal values within an ROI at each timepoint) over NP or PN transitions for auditory cortical ROIs (HG, Heschl’s gyrus; PT, planum temporale; STG, superior temporal gyrus; ROI contours are marked in (B)). A time interval of [100, 160] ms is also marked.


305. Auditory Cortex: Temporal and Frequency Factors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 305.06/K6

Topic: D.06. Auditory & Vestibular Systems

Support: RGC HK GRF 11100518
Title: Cortical mapping of prediction error responses to multiple sensory features

Authors: *H. AN¹, R. AUKSZTULEWICZ³, J. W. SCHNUPP²;
¹Biomed. Sci., ²City Univ. of Hong Kong, Hong Kong, Hong Kong; ³Dept. of Psychiatry, Univ. of Oxford, Oxford, United Kingdom

Abstract: Predictive coding is widely accepted as a comprehensive theory of neural processing underlying perceptual inference. However, it is unknown to what extent prediction violations of different sensory features are mediated in regions in auditory cortex, with different dynamics and by different mechanisms. This study investigated the neural responses to synthesized acoustic syllables which could be expected or unexpected along several features. By using electrocorticography (ECoG) in rat auditory cortex, we mapped regional differences in mismatch responses to different stimulus features. The subjects were 8 adults female Wistar rats with normal hearing and no prior exposure to the stimuli. Morphed syllables formed roving oddball sequences in which each stimulus was repeated 2-43 times (thereby forming a standard) with the inter-stimulus interval fixed at 300ms, and subsequently replaced with a deviant stimulus, differing from the standard along one of several acoustic features (duration, pitch, interaural level differences (ILD) and consonant identity). Each of these features could assume one of 9 different levels (durations: 55-95ms in 5ms steps; pitch mean F0: 0.7-1.4kHz logarithmically spaced; ILD : 60-68 dB SPL difference in 2 dB steps; consonant: /da/-/ba/ in equidistant steps), and the resulting deviance magnitude ranges between +4 and -4 steps. The deviant stimuli were then repeated to form new standards. We analyzed responses to the last repetition of a given stimulus (standard) and the first repetitions of a new stimulus (deviant). For the ECoG recording, we implanted urethane-anaesthetised rats with 8x8 electrode arrays covering a 3x3mm cortical patch encompassing primary and higher-order auditory cortex. In each rat, we presented a total 3800 stimuli, amounting to ~40 deviant stimuli (and their preceding standards) for each feature and level of deviance magnitude. We identified different the topographies and latencies of population activity in the rat auditory regions sensitive to expectation violation along different acoustic features. Responses to deviant stimuli increased in amplitude with increasing deviance magnitude. Mismatch response to both duration and ILD violations were observed in anterior auditory regions but at slightly earlier latencies for duration vs. ILD violations. In contrast, mismatch response to pitch and consonant identity were observed at posterior and dorsal electrodes, with consonant violations evoking earlier mismatch responses than pitch violations. This suggests differences in cortical regions and populations dynamics subserving prediction error signaling along different stimulus features.

Poster

305. Auditory Cortex: Temporal and Frequency Factors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 305.07/K7

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant T32-DC000023
NIH Grant R01-DC003180

Title: Variation in temporal coding of vocalizations along the anterior-posterior extent of marmoset auditory cortex

Authors: *X.-P. LIU*¹, X. WANG²;
¹Johns Hopkins Sch. of Med., Baltimore, MD; ²Dept Biomed Engin, Johns Hopkins Univ. Sch. Med., Baltimore, MD

Abstract: To gain insight into functional properties of the multiple fields in primate auditory cortex, we examined the responses of neurons to vocalizations across the anterior-posterior extent along the lateral sulcus in the marmoset (Callithrix jacchus), a highly vocal monkey species. Studies that have ventured anterior to the primary auditory cortex (AI) have seen longer response latencies and temporal integration windows and a scarcity of neurons with well synchronized responses to click trains and sinusoidal amplitude modulation, suggesting that anterior field neurons transform periodic auditory patterns into a rate code (Bendor and Wang, 2008; Camalier et al., 2012; Scott et al., 2010; Wang, 2007). In this study, we examined time domain processing of natural stimuli in both anterior and posterior fields by recording single unit responses to a set of 20 marmoset call types as well as sets of exemplars of each call type. To quantify temporal precision of evoked spike trains, we calculated the correlation index (CI) of the responses to multiple presentations (Joris et al., 2006), which indicates the degree of temporal variability in spike timing between repetitions. A subset of low spontaneous rate neurons displayed high CI values indicating timing variability less than a millisecond in response to some vocalizations. These neurons tended to have onset responses and be more prevalent in the posterior region. While the anterior region generally lacked such temporal precision, it featured other specializations, such as an area enriched in neurons with narrow frequency tuning, selectivity for slowly modulated vocalizations like phee calls, and robustness to broadband background noise. These specializations may form parallel forms of processing, for example, integrating across spectrum to enhance temporal resolution for detection of temporal edges or integrating across time to enhance selectivity for slower features. Neurons with higher and lower CI values responded in diverse ways to different exemplars of the same call types and may respectively use temporal or rate codes to encode these calls. These observations are reminiscent of recent studies in humans showing anterior-posterior differences in temporal responses along
Heschl's gyrus or superior temporal gyrus (Hamilton et al., 2018; Hullett et al., 2016; Jasmin et al., 2019; Norman-Haigene et al., 2015; Santoro et al., 2014), suggesting this may be a conserved organizational feature of the primate auditory cortex. These parallel processing strategies may help identify temporal and spectral features that are key to speech or music processing as well as animal communication sounds.

**Disclosures:** X. Liu: None. X. Wang: None.

**Poster**

**305. Auditory Cortex: Temporal and Frequency Factors**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 305.08/K8

**Topic:** D.06. Auditory & Vestibular Systems

**Support:** NIH T32 DC0046

**Title:** Structural changes in primary auditory cortex neuronal networks impact encoding fidelity in noisy environments

**Authors:** *K. V. SHILLING-SCRIVO*¹, P. O. KANOLD², B. BABADI³, A. SHEIKHATTAR⁴;

¹Univ. of Maryland Sch. of Med., Baltimore, MD; ²Biol., ⁴Dept. of Electrical and Computer Engin., ³Univ. of Maryland, College Park, MD

**Abstract:** Navigating complex auditory environments requires the ability to identify behaviorally meaningful sounds. In mouse auditory cortex, sound features in a quiet environment are encoded by small populations of neurons (Francis et al 2018; Liu et al 2019). However, most sounds exist in the presence of other background sounds that overlap it spectrally and temporally. To date it is unknown how populations of neurons encode sounds in the presence of a noisy background. We thus investigated how populations of neurons simultaneously encode both tonal foreground and noisy background sounds. We used *in vivo* two photon calcium imaging to record from 100s of neurons in primary auditory cortex (A1) in transgenic Thy1-GCaMP6s X CBA mice passively listening to tones in a broadband noise background. For each mouse, before imaging, A1 location was determined by widefield imaging. While imaging in A1, we presented 8 SAM tones (4-48 kHz ½ octave spacing, 5Hz carrier frequency, 10 repeats) at three levels (60,50,40dB) in the presence of 40dB broadband noise or in quiet. We imaged ~900 neurons and for each neuron, calculated the relative change in fluorescence for each trial (dF/F). Based on the average response to all stimuli, neurons clustered into three classes: (1) Neurons maximally responsive during the noise background (Noise-On); (2) Neurons maximally
responsive during tone onset (Tone-On); and (3) Neurons maximally responsive during tone offset (Tone-Off). Initial results showed that as the signal to noise ratio (SNR) decreases, Noise-On neurons remain more responsive than Tone-On or Tone-Off neurons. Noise correlation analysis showed that as SNR decreases, there was a corresponding decrease in noise correlation in the two tone-responsive classes of neurons (Tone-On and Tone-Off). Since neurons can be embedded in functional subnetworks, we utilized Granger causality (GC) analysis to identify neurons’ functional connectivity among these three classes (Francis et al 2018; Liu et al 2019). GC analysis showed that for the two tone-responsive classes of neurons decreasing SNR increased the number of GC linked neurons. In contrast Noise-On neurons showed no change in the number of GC linked neurons across SNR. Finally, we trained a naïve Bayes classifier on our data and found that as SNR decreases, more neurons are required to correctly identify the tone presented. Together, our results support a model in which different features of a stimulus are encoded by different populations of neurons in A1 and that as SNR decreases, more neurons are needed to differentially encode those stimulus features. Thus, the size of the active population in A1 seems to relate to coding fidelity.

Disclosures: K.V. Shilling-Scrivo: None. P.O. Kanold: None. B. Babadi: None. A. Sheikhattar: None.

Poster

305. Auditory Cortex: Temporal and Frequency Factors

Location: Hall A

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Support: NIH grant R01DC015232; NSERC PGS-D (Natural Sciences and Engineering Research Council);
FRQNT Doctoral Scholarship (Fonds de Recherche du Québec - Nature et Technologies)
College of Biological Sciences, U.C. Davis

Title: Responses to changes in amplitude modulation rate and spatial location in neurons in the auditory cortex in an awake macaque monkey

Authors: *B. M. BORMANN¹, D. A. BEAUREGARD CAZABON¹, K. E. NEVERKOVEC¹, K. B. CHEUNG¹, *G. H. RECANZONE²;
¹Ctr. for Neurosci., ²Ctr. for Neuroscience, Dept. of Neurobiology, Physiol. and Behavior, Univ. Of California, Davis, Davis, CA

Abstract: Acoustic signals are commonly thought to be processed in the primate auditory cortex in two main pathways, a caudal spatial pathway and a rostral non-spatial pathway. This
hypothesis predicts that particular spatial or non-spatial features of an acoustic stimulus are extracted from core auditory areas by belt and parabelt auditory areas, which are then further processed in the parietal and frontal lobes. It may also be that this segregation begins as early as primary auditory cortex (A1). We are currently testing this hypothesis by comparing the responses of the same auditory cortical neurons to complex stimuli that vary only in their temporal properties (amplitude modulation rate, AM) or spatial location. We presented two sequential 500 ms duration AM noise stimuli from free-field speakers to an alert male macaque monkey (20 years old) while extracellularly recording single neurons or small clusters of neurons in different auditory cortical fields. The first stimulus was a broadband noise with an AM rate of 25 or 33 Hz. The second stimulus could either vary in AM rate, from 13 to 50 Hz or 16 to 65 Hz, compared to the 25 or 33 Hz stimulus, respectively, or to the left or right by up to 12 degrees. The overall results showed that the AM frequency of the first stimulus had no effect on the firing rates of either presumptive core or belt neurons. In the core, however, neurons did tend to have higher firing rates to the second stimulus when it changed in AM rate as opposed to location. This was not seen in presumptive belt regions that were located medial or caudal to the core. Further, the normalized difference in firing rate across tested AM rates or locations to the second stimulus, a simple metric of AM and location tuning, was greater in the core than in the belt areas, with larger differences in the firing rate for changes in location compared to changes in AM rate. Again, such differences were not seen in presumptive belt areas. These results indicate that differences in the firing rate as a function of the temporal (AM rate) and spatial (location) attributes are present in primary auditory cortical areas, and that the extraction of these attributes in belt areas is likely to be a non-linear process.


Poster

305. Auditory Cortex: Temporal and Frequency Factors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 305.10/DP06/K10

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster

Topic: D.06. Auditory & Vestibular Systems

Support: NYUAD Institute G1001

Title: Tracking the building blocks of pitch perception in auditory cortex

Authors: *L. GWILLIAMS, E. ABRAMS, A. MARANTZ;
New York Univ., New York, NY
Abstract: While there is a general consensus that fundamental frequency, spectral content, and musical context contribute to pitch perception, it is currently unclear how and when these aspects of perceived musical pitch are neurally encoded during early auditory processing. To investigate this, we recorded brain responses to two types of tones: i) pure tones of fundamental frequency only (F0); ii) complex tones of five partials (integer multiples of, but not including, F0). Participants listened to musical tone sequences, ranging from 220-624Hz (the notes of the A, C, and Eb major scales), while magnetoencephalography (MEG) was recorded. Although the two tone-types have non-overlapping spectral content, they are perceived as the same pitch, thus creating an orthogonal relationship between the sensory input and perceptual output. Multivariate analyses were used to decode frequency and tone-type from the activity across MEG sensors. High decoding accuracy across time would suggest that these features are in fact encoded in the spatial pattern of neural responses to musical pitch. We found that a classifier trained at ~50 ms after the onset of the tone could accurately decode whether a listener was presented with a pure or complex tone based on the spatial pattern of activity. At 100ms, we could decode F0 from both tone-types, even for complex tones for which F0 was absent. Further, we were able to use a classifier trained on pure tones to accurately predict the frequency of the complex tones, suggesting that the missing fundamental is restored at this latency. From 200-300ms, tone-type decoding accuracy increased, and the F0 spatial pattern no longer generalised from one tone-type to the other. In sum, we confirm that central aspects of musical pitch perception are indeed encoded in early auditory neural responses. Overall, this suggests that independent neural mechanisms track the spectral content of musical tones and the present, or restored, fundamental frequency.

Disclosures: L. Gwilliams: None. E. Abrams: None. A. Marantz: None.

Poster

305. Auditory Cortex: Temporal and Frequency Factors

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Topic: D.06. Auditory & Vestibular Systems

Support: R01DC009607
U19NS107464

Title: Complex frequency interactions revealed by two-tone stimuli in awake mouse A1

Authors: *J. LIU, P. O. KANOLD;
Biol., Univ. of Maryland, College Park, MD

Abstract: Environmental sounds have rich spectral content and are characterized by specific spectral shape, e.g. harmonic structures. The auditory system has been traditionally probed with
pure tones but these spectrally simple stimuli might not be sufficient to capture how the auditory system integrates information over the frequency band. For example, the two-tone paradigm could reveal the inhibitory sideband flanking the characteristic frequency (CF), while pure tones typically fail to do so. In cat auditory cortex, two-tone paradigm has revealed diverse inhibitory sideband structures, with most cells having multi-peaked sidebands[1]. Thus, we investigated the response to both pure tones and two-tone stimuli in L2/3 of the primary auditory cortex of awake CBA/CaJ x Thy1-GCaMP6s mice. We found a diverse response pattern to two-tone stimuli. Although a large portion of neurons showed wide flanking inhibitory sidebands (~2-3 octaves), some neurons showed a mixture of two-tone suppression and two-tone facilitation, suggesting that the interactions of the simultaneously presented frequencies can be highly nonlinear and frequency specific. We also found that with the addition of a second tone, the signal correlations between neurons decreased, suggesting a decorrelation of neural responses by more complex stimuli. Furthermore, we separated onset and offset responses and found that offset responses can have distinct degrees of nonlinear interactions from onset responses. The complexity of these interactions suggests that the responses of A1 neurons can be spectral context dependent and likely encodes spectrally rich sound with high selectivity.


**Disclosures:**  
**J. Liu:** None. **P.O. Kanold:** None.

**Poster**

**305. Auditory Cortex: Temporal and Frequency Factors**

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**Topic:** D.06. Auditory & Vestibular Systems

**Support:** NIH Grant R01-DC014085  
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**Title:** High frequency time-locking in human auditory cortex to continuous speech

**Authors:** *J. P. KULASINGHAM*¹, C. BRODBECK¹, A. PRESACCO¹, S. E. KUCHINSKY², S. ANDERSON¹, J. Z. SIMON¹;  
¹Univ. of Maryland, College Park, MD; ²Audiol. and Speech Pathology Ctr., Walter Reed Natl. Military Med. Ctr., Bethesda, MD

**Abstract:** The neural processing of natural sounds, such as speech, changes along the ascending auditory pathway, and is often characterized by a progressive reduction in representative frequencies. For instance, the well-known frequency-following response (FFR) of the auditory
midbrain, measured with electroencephalography (EEG), is dominated by frequencies from ~
100 Hz to several hundred Hz, and time-locks to acoustic features (waveform and envelope) at
those rates. In contrast, cortical responses, whether measured by EEG or
magnetoencephalography (MEG), are thought to be characterized by frequencies of a few Hz to
a few tens of Hz, time locking to acoustic envelope features at those rates. In this study we show
that this separation by frequency is overly simplistic, even for non-invasive electrophysiological
recordings in humans. Using MEG we investigate high-frequency responses (80-300 Hz) to
continuous speech using neural source-localized reverse correlation, whose kernels are called
temporal response functions (TRFs). Continuous speech stimuli were presented to 40 subjects
(17 younger, 23 older) with clinically normal hearing and their MEG responses were analyzed in
the 80-300 Hz band. Consistent with the insensitivity of MEG to many subcortical structures, the
spatiotemporal profile of these response components indicate a purely cortical origin with ~40
ms peak latency and a right hemisphere bias. TRF analysis was performed using two separate
aspects of the speech stimuli: a) the 80-300 Hz band of the speech waveform itself, and b) the
80-300 Hz envelope of the high frequency (300-4000 Hz) band of the speech stimulus. Both of
these aspects contributed to the TRF, with the envelope dominating the response. Age-related
differences were also analyzed to investigate a reversal previously seen along the ascending
auditory pathway, whereby older listeners have weaker midbrain FFR responses than younger
listeners, but, paradoxically, have stronger low frequency cortical responses. In contrast to these
earlier results, this study did not find clear age-related magnitude differences in high frequency
cortical responses. Together, these results suggest that the traditional EEG-measured FFR has
distinct and separate contributions from both subcortical and cortical sources. The cortical
responses at FFR-like frequencies share properties with both midbrain responses at the same
frequencies and cortical responses at much lower frequencies.

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Kuchinsky: None. S. Anderson: None. J.Z. Simon: None.

Poster

305. Auditory Cortex: Temporal and Frequency Factors

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC003180

Title: Functional architecture of auditory cortex in awake marmosets revealed by multi-scale
multi-modal optical imaging
Authors: *X. SONG*, Y. GUO, C. CHEN, X. WANG;  
\(^1\)Dept. of Biomed. Engin., \(^2\)Johns Hopkins Univ., Baltimore, MD; \(^3\)Dept Biomed Engin, Johns Hopkins Univ. Sch. Med., Baltimore, MD

Abstract: The common marmoset (Callithrix jacchus), a highly vocal New World monkey species with a largely flat brain surface, has emerged in recent years as a promising non-human primate model for neuroscience research. Unlike most of the Old World primates, a significant proportion of auditory cortex in marmosets is directly accessible under the skull with optical imaging methods. Here, we applied wide-field enhanced intrinsic imaging, wide-field calcium imaging, and nearly silent two-photon calcium imaging methods in awake marmosets to study the functional architecture of auditory cortex. Previous studies have shown that primate auditory cortex consists of three layers of hierarchy, the core, the belt, and the parabelt. Responses to pure tone stimuli were largely confined to the putative core region through wide-field imaging. The low-frequency tonotopic reversal between A1 and R was clearly seen in all subjects. By increasing the bandwidth of sound stimuli, the responsive areas grew more laterally towards the putative belt region, suggesting the belt region preferentially responds to a wider spectral content and may carry out spectral integration over a broad frequency range. Variety of natural sounds can elicit responses that collectively cover the entire superior temporal gyrus (STG). However, data from wide-field calcium imaging revealed that the area close to superior temporal sulcus (STS), putatively the parabelt, carried more spontaneous or endogenic rhythm that appeared largely independent of sound stimulation. This form of rhythm may reflect the behavioral state of the subject. Zooming into the fine details, the general response patterns within each two-photon field-of-view were consistent with wide-field imaging results. However, individual neurons’ responses can be heterogeneous even for nearby neurons. The multi-scale multi-modal optical imaging approach reported here thus provides a new experimental paradigm for mapping the functional architecture of auditory cortex in awake condition in a high throughput way over conventionally electrophysiology methods. Research support: NIH DC003180

Disclosures: X. Song: None. Y. Guo: None. C. Chen: None. X. Wang: None.

Poster

305. Auditory Cortex: Temporal and Frequency Factors

Location: Hall A

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Program #/Poster #: 305.14/K14

Topic: D.06. Auditory & Vestibular Systems

Title: Sound representation in the mouse temporal association cortex (TeA)

Authors: *L. FEIGIN*, G.-I. TASAKA, I. MAOR, A. MIZRAHI;  
\(^1\)ELSC, \(^2\)Hebrew Univ. of Jerusalem, Jerusalem, Israel; \(^3\)Dept. of Neurobiology, ELSC, The
Abstract: Background: Temporal association cortex (TeA) is ventral to the secondary auditory cortex and dorsal to the Rhinal fissure. Only few studies reported the activity from TeA in response to sounds. TeA was reported to be tonotopically organized and suggested to be invariant to frequency and temporal modulations of natural sounds. But otherwise, TeA remains largely unstudied.

Results: To define the inputs to TeA, we first performed monosynaptic retrograde rabies tracing from starter cells in TeA. We found a surprisingly large number (>100) of brain regions projecting into TeA including cortical and subcortical regions. Quantitative analysis of the connectivity into TeA showed that the primary auditory cortex (A1) is its most prominent source of input (~45% of TeA’s total inputs). We, thus, recorded spiking activity from TeA with reference to A1.

To define the physiological responses of TeA to sounds, we used the newly developed high density multielectrode array “Neuropixels”. We recorded spiking activity from A1 and TeA simultaneously in ketamine anesthetized as well as in head restrained awake mice. An initial analysis shows that representations of pure tones are surprisingly similar in A1 and TeA. TeA spans a similar frequency range and response bandwidth to that of neurons in A1. Interestingly, neurons in TeA exhibit higher response latencies. For example, units in layer 5/6 have mean latencies that follow A1 by ~50 msec. Signal correlations, calculated for pure tone stimuli as well as complex sounds, and noise correlations are slightly elevated within units in TeA compared to units in A1. We are currently analyzing population responses within and across regions to uncover the transformation of sound representation from A1 to TeA, under normal condition and following experience dependent plasticity.

Conclusions: Our work shows that TeA is a higher order auditory field directly downstream of A1. The diverse connectivity into TeA, and its physiological signature suggests that this brain region sub-serves higher order auditory computations.

Disclosures: L. Feigin: None. G. Tasaka: None. I. Maor: None. A. Mizrahi: None.

Poster

305. Auditory Cortex: Temporal and Frequency Factors

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Support: Ramalingaswami fellowship (BT/RLF/Reentry/07/2014) to D.R. from the Department of Biotechnology, Ministry of Science and Technology, Government of India.
Title: How do structural constraints guide asymmetry in cortical organization of auditory steady state responses (ASSR)?

Authors: *N. KUMAR, S. KUMAR, D. ROY, A. BANERJEE;
Natl. Brain Res. Ctr., Gurugram, India

Abstract: Characterizing hemispheric dominance in the functional specialization of sensory processing is a fundamental question in cognitive neuroscience. Several studies have shown that structural and functional brain networks involved in auditory stimulus processing are distributed in the brain. One way to characterize the functional networks of auditory processing is to investigate the brain networks engaged in generating steady-state auditory responses (ASSR) - a neurophysiological marker of complex auditory processing.

Our earlier EEG studies with ASSR have revealed that entrainment of 40 Hz rhythms occurs in large-scale neural networks visible from scalp and source connectivity analysis. Interestingly, our results indicate there is a right hemisphere bias of leadership role in a community interaction during the binaural hearing of 40Hz rhythms. During the monaural stimulations, ipsilateral hemisphere was more dominant than contralateral in terms of information processing metrics, e.g., power spectra, global coherence, imaginary coherence, and Granger causality.

In this study, we have asked whether constraints in whole-brain structural connectivity with auditory brain regions as seed, is the cause of the asymmetry in brain functional organization. Diffusion imaging data was collected in normal healthy volunteers from which the structural connectome was computed. DTI data was collected from a total of 49 participants (25 young and 24 old). Obtained structural connectivity matrix was parcellated in 68 regions according to the Desikan-Killiany brain atlas. Structural connectivity matrix was used to estimate the length of fibers between any pair of parcels. Using a value of velocity 42 m/s as communication speed, we estimated the time delays that are involved in transmitting information between any pair of nodes. These time delays were used to couple the Kuramoto oscillators at the 68 parcels. The auditory parcels were triggered with 40 Hz intrinsic frequency while other parcels were set at an intrinsic frequency of 1 to 100 Hz.

The dynamical states of the network were investigated to interpret the underlying mechanisms of hemispherical asymmetry that we observed in empirical EEG data. Secondly, the computational model reveals that whole brain 40 Hz can be obtained by locally exciting the auditory brain parcels when the intrinsic frequency of neural oscillators reaches at least 20 Hz for the dorsal attention network and more than 30 Hz for the visual network.

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Poster

305. Auditory Cortex: Temporal and Frequency Factors

Location: Hall A

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH R01 DC002260

Title: Stimulus dependent transformations between synaptic and spiking receptive fields in auditory cortex

Authors: *K. X. KIM, C. A. ATENCIO, C. E. SCHREINER; Dept. of Otolaryngology–Head and Neck Surgery, Univ. of California San Francisco, San Francisco, CA

Abstract: Sound signals are transmitted via the ascending auditory pathway and arrive at the auditory cortex. The primary auditory cortex (A1) neurons, which are tonotopically arranged, integrate many synaptic inputs across frequencies, and the subthreshold activity is subsequently transformed into a spiking output. Although tonal receptive fields (TRFs) for responses to pure tones have been studied at the synaptic level, little is known about synaptic and spiking spectrotemporal receptive fields (STRFs) regarding responses to complex sounds. We examined the degree of synaptic integration generated from two different stimuli, pure tones and complex sounds (the dynamic moving ripple sound), and their output by characterizing receptive fields. In vivo whole-cell recordings were made from A1 neurons of anesthetized mice using the blind patch-clamp approach. Spectral tuning in STRFs from both spiking and subthreshold responses was more selective than that in TRFs. Specifically, the TRF bandwidths were approximately 1 octave wider than the STRF bandwidths. Furthermore, complex sounds carrying modulation information allowed us to determine filter modulation properties and nonlinearities. We found that STRFs derived from subthreshold activity illustrated more diverse frequencies and temporal modulation preferences than did spiking STRFs. Finally, STRF nonlinearities revealed the transformation from the synaptic input to the spiking output, with reduced event-generating noise and an increased feature selectivity. Our results suggest that stimulus-specific convergence and cortical dynamic processes determine the input-output transformation in A1 neurons.

Disclosures: K.X. Kim: None. C.A. Atencio: None. C.E. Schreiner: None.
Nicotine enhances temporal responsiveness in mouse A1

Authors: *I. INTSKIRVELI¹, E. PROVENZANO², R. METHERATE¹;
¹Dept. Neurobio. and Behavior, Univ. of California Irvine, Irvine, CA; ²Dept. of Cognitive Sci., Univ. of California San Diego, La Jolla, CA

Abstract: Neurons in primary auditory cortex (A1) adapt to repetitive sounds, i.e., they exhibit progressively decreased response magnitude to repetitive sounds. Since studies have demonstrated that systemic nicotine enhances sensory-evoked cortical responses, here we address how nicotine modulates response adaptation. We placed a 16-channel silicon multiprobe in A1 of adult, urethane anesthetized FVB mice and derived current-source density (CSD) profiles in response to 1 s trains of characteristic frequency (CF) tones presented at 2 Hz, 5 Hz, 10 Hz, 20 Hz and 40 Hz (30 dB above threshold, trains repeated 25 times). We analyzed two main current sinks in middle (approx. layer 4, L4) and deep (L5/6) layers. Stimulation produced adaptation of the L4 response that was weak at 2 Hz, stronger at 5-10 Hz and complete at 20-40 Hz. In contrast, the L5/6 sink exhibited no adaptation at 2-10 Hz, and simultaneously recorded brainstem responses (ABR) showed no adaptation even at 40 Hz. Systemic nicotine (2.1 mg/kg, s.c.) enhanced the L4 response to the first tone in each stimulus train and reduced its peak latency, consistent with prior studies. Additionally, nicotine enhanced adapted responses for stimulus trains at 2-10 Hz, but did not prevent complete adaptation of responses at 20-40 Hz. Nicotine also reduced response (peak latency) variability across 25 trials. In contrast to the effects in L4, nicotine had no effect on responses in L5/6. Although nicotinic enhancement in L4 was seen on both initial and later responses in each train (2-10 Hz), initial effects were stronger, leading to a greater degree of adaptation in nicotine. We suggest that nicotine modulates response properties in A1 to improve temporal consistency and to emphasize initial responses to repeated stimuli.

Disclosures: I. Intskirveli: None. R. Metherate: None. E. Provenzano: None.
305. Auditory Cortex: Temporal and Frequency Factors

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Title: Cooling of the auditory cortex modulates temporal processing of auditory thalamic neurons in awake marmosets

Authors: *Y. ZHANG, S. BAI, X. WANG, R. LI, H. SUN, L. GAO;
Interdisciplinary Inst. of Neurosci. and Technology, Key Lab. for Biomed. Engin. of Ministry of Education, Col. of Biomed. Engin. and Instrument Science, Zhejiang Univ., Hangzhou, China

Abstract: Temporal information is essential for perception and discrimination of communication sounds, such as human speech and animal vocalizations. So far, it remains largely unknown the thalamocortical circuit mechanisms underlying sound temporal processing in awake animals. In the present study, single unit recordings were performed in medial geniculate body (MGB) when the primary auditory cortex (A1) of awake marmosets were reversibly inactivated by cooling technique. We found that cooling of A1 has great influences on the temporal processing of MGB neurons. Firstly, A1 modulated differently on the onset and sustained sound driven responses of MGB neurons: sustained and late phase auditory responses diminished when A1 was cooled and retrieved when the temperature recovered to normal whereas onset responses were less affected. The result suggests that onset responses of MGB neurons largely inherited from the ascending auditory inputs whereas their sustained responses were due to the cortical feedback. Our results also support the hypothesis that sustained responses of A1 neurons were generated by intracortical inputs instead of thalamocortical inputs. Regarding the temporal responses of MGB neurons to time-varying stimuli, we found that cortical cooling either enhanced the synchronized responses of MGB neurons to slow repetition rates or weakened the non-synchronized responses of other MGB neurons to fast repetition rates, which suggests that cortical feedback control changed the temporal coding schemes of MGB neurons to time-varying stimuli: cortical feedback weakened the ability of MGB neurons to use temporal coding instead strengthen their ability to use rate coding strategy.

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Poster

305. Auditory Cortex: Temporal and Frequency Factors

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Topic: D.06. Auditory & Vestibular Systems

Support: WT102561/Z/13/Z

Title: Species differences in the cortical analysis of auditory time windows in primates

Authors: *P. DHEEREENDRA1, S. BAUMANN2, O. JOLY3, F. BALEZEAU1, A. THIELE1, T. D. GRIFFITHS1;
1Inst. of Neurosci., Newcastle Univ., Newcastle upon Tyne, United Kingdom; 2Dept of Exptl. Psychology, Oxford Univ., Oxford, United Kingdom; 3Brainomix, Oxford, United Kingdom

Abstract: We examined the brain basis for the processing of auditory time windows in stimuli with similar spectro-temporal complexity to human speech or macaque vocalisations. We created a synthetic stimulus by manipulating spectral flux, a timbral dimension, to systematically vary the time window duration required to analyse it. We conducted functional magnetic resonance imaging in awake macaques using this stimuli to test how the anatomy of their response patterns of time window processing compares to humans. Window of temporal integration was characterised in terms of the Pearson correlation (r) between amplitude spectra of adjacent timeframes [PMID 19052218] or in terms of time window within which any two frames show a minimum level of correlation thus shorter windows have lower r while longer windows have higher r. Individual core and belt areas were defined on 3 animals using tonotopic mapping and myelin mapping [PMID 25100930]. Sparse EPI images were acquired on a 4.7T vertical scanner whilst they carried out visual fixation. A general linear model analysis [SPM12] allowed single-subject inference to determine the relationship between BOLD and r in individual core and belt areas. Despite a similar overall pattern of changing preference, from shorter time windows in postero-medial areas to longer time windows in antero-lateral areas, monkeys exhibited a reduced sensitivity to longer time windows as compared to humans [PMID 19052218]. This difference in sensitivity is in line with recent behavioural results and might be explained by a specialization of the human brain for speech processing which requires sensitivity to longer time windows. We highlight specifically human response patterns that suggest an adaptation of a general primate brain mechanism, possibly an outcome of divergent evolution alongside the development of speech.

**Title:** Neural circuits underlying spectro-temporal integration of sounds

**Authors:** *A. M. KLINE*, D. A. APONTE, H. K. KATO;
Dept. of Psychiatry and Neurosci. Ctr., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

**Abstract:** The brain’s capacity to integrate the features of sensory stimuli is essential to understand and respond to our environment. In the auditory system, even in the face of multiple sound sources, our brain integrates acoustic components originating from the same source to reconstitute individual sound objects. In particular, integration of harmonic components is critical to accurately perceive physiologically relevant sounds such as language. In humans, one of the factors influencing the binding of harmonic components is timing of the spectral components’ onset within a 30-ms window. However, the neural circuits by which the auditory system integrates sounds in a timing-dependent manner remain unknown. In this study, we investigated the temporal integration of harmonic sounds in mouse auditory cortex. Using macroscopic imaging of all auditory cortical areas, we determined that higher-order area, A2, preferentially responds to harmonic vocalizations over pure tones. Furthermore, using two-photon calcium imaging in awake mice, we found that A2 neurons display preferential response to coincident harmonics compared to harmonic tones whose onsets are shifted in time. This timing-dependence was not observed in primary area A1, suggesting that A2 plays a unique computational role in processing harmonics. Finally, we developed a Go/No-go task in which mice were trained to discriminate between coincident harmonics and time-shifted distractors. Interestingly, we found that mice have a similar 30-ms window for integrating harmonic components, suggesting conserved neural circuitry across species. In the future, we will manipulate A2 activity during this task to investigate its causal role in the perceptual integration of harmonics. Determining the mechanism by which the auditory cortex integrates harmonic sounds will contribute to our knowledge of how we process language, as well as inform us about how speech perception could be affected in neurodevelopmental disorders.

**Disclosures:** A.M. Kline: None. D.A. Aponte: None. H.K. Kato: None.
Title: Imbalanced excitatory synaptic charge shapes direction selectivity in primary auditory cortex

Abstract: Frequency Modulation (FM) is a prevalent sound feature found in both human speech and animal vocalization. The brain's ability to detect the direction of frequency modulation is critical for vocal communication. Neurons in the primary auditory cortex (A1) have been reported to show selective firing to their preferred FM direction. However, it remains debated how direction selectivity maps spatially onto A1, and what circuit mechanisms underlie this selectivity. Here, we determined the FM tuning properties of a large population of A1 neurons using in vivo two-photon Ca\(^{2+}\) imaging in awake mice. We found that more than 70% of A1 neurons show direction-selective firing, and that the selectivity was topographically ordered along the A1 tonotopic axis. Interestingly, A1 neurons also showed robust direction selectivity to ethologically relevant slow-rate FM sweeps, which previous studies did not observe in anesthetized animals. To investigate the synaptic mechanism underlying the direction selectivity to slow FM sweeps, we measured synaptic currents using in vivo whole cell recordings. In contrast to previous models that explained direction selectivity based on different onset timings of EPSCs and IPSCs, we found that direction selectivity is rather driven by a difference in the total excitatory charge triggered by preferred and null directions. Null-direction FM sweeps evoked both significantly attenuated EPSCs and IPSCs, which is explained by a suppression of recurrent circuits in the cortex - a phenomenon we recently reported as “network suppression”. Taken together, our findings revealed a non-classical circuit mechanism that ensures direction selectivity of A1 neurons to slow FM sweeps which play important roles in our vocal communication.

Poster

305. Auditory Cortex: Temporal and Frequency Factors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 305.22/K22

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC003180

Title: Functional segregation of the marmoset auditory cortex by data-driven decomposition of responses to naturalistic sounds with wide-field optical imaging

Abstract: The common marmoset (Callithrix jacchus) is a highly vocal New World monkey species and has garnered considerable interest in recent years as a promising non-human primate model in neuroscience. To study the functional organization of the auditory cortex using this animal model, we developed techniques to perform multi-scale and multi-modal chronic optical imaging in awake marmosets. The imaging interface is a removable artificial dura (AD) based window with a diameter of 1/4 inches that covers the region between the lateral sulcus (LS) and superior temporal sulcus (STS). While previous studies have extensively evaluated neural activities in the core region of auditory cortex driven by pure tone stimuli, how the auditory cortex is spatially organized to process naturalistic sounds, especially in the secondary auditory areas, is still poorly understood. In this study, we used a hypothesis-free data-driven method to probe the functional organizations of the marmoset auditory cortex. This method was first developed by Norman-Haignere et al. (2015) for human fMRI studies. We played the same set of 165 naturalistic sounds to marmosets and measured activities in the auditory cortex with wide-field optical imaging. In contrast to the pure tone responsive area (mostly limited to the core region), this set of sound stimuli activated more lateral areas in marmoset auditory cortex (presumably the belt and parabelt regions), suggesting that the secondary auditory areas are sensitive to higher-order features in naturalistic sounds. With the matrix decomposition method used in Norman-Haignere et al. (2015) study, we found independent components located in both primary and secondary auditory areas that were sensitive to different sound features and sound categories. This approach provides a potential way to reveal new functional areas in the auditory cortex of non-human primate. In addition, we analyzed trial-to-trial variance of the responses to the natural sound stimuli. Some lateral areas showed neural activities that were more variable than areas closer to the lateral sulcus (putative core region) under the same stimulus context, suggesting that the activities in these areas may be influenced more by internal processing or the state of the animal, rather than solely dependent on stimulus driven responses.

Disclosures: Y. Guo: None. X. Song: None. C. Chen: None. X. Wang: None.
Title: Spike decoding of signals from noise stimuli in the auditory cortex of an awake macaque monkey

Authors: *D. BEAUREGARD CAZABON¹, B. BORMANN¹, K. NEVERKOVEC¹, K. CHEUNG¹, G. H. RECANZONE³², B. J. MALONE⁴;
¹Ctr. for Neurosci., ²Dept. of Neurobiology, Physiol. and Behavior, Univ. of California, Davis, Davis, CA; ³Ctr. for Neurosci., Univ. Of California, Davis, Davis, CA; ⁴Otolaryngology & Head & Neck Surgery, UCSF Sch. of Med., San Francisco, CA

Abstract: Most natural listening conditions consist of a multitude of different sounds from a variety of different locations. We currently have little understanding of how these different signal streams are decoded by core and belt auditory cortex into perceptually distinct events or objects. We therefore used spike train decoding techniques to determine the effects of various background distractors on the responses of neurons to amplitude-modulated sounds with complex spectra. We presented free-field stimuli to a passively listening, alert male macaque monkey (20 years old) while extracellularly recording single neurons or small clusters of neurons in different auditory cortical fields. Spike trains were analyzed in terms of their firing rates and their temporal patterns at timescales of milliseconds, using decoding algorithms based on simple linear classifiers. Target stimuli consisted of a 100% amplitude-modulated (AM) carrier composed of interleaved 1/3 octave bands of pink noise from 0 - 18 kHz, modulated at either 25 Hz or 33 Hz. Three classes of distractors were tested: 1) carriers with similarly constructed bands having the frequency ranges of the bands interleaved with that of the target ranges, but modulated at the other AM rate (i.e. 33 Hz target and 25 Hz distractor); 2) pink noise modulated at 5 Hz; and 3) unmodulated pink noise. All distractors were presented at three different intensities (+10, 0 and -10 dB relative to the target) and at locations either 45 degrees from the target in the ipsilateral or the contralateral hemifield relative to the target. Targets and distractors were presented either independently or in combination of one target and one distractor. Preliminary analysis suggested that cortical firing patterns were sensitive to multiple acoustic
features of the targets and distractors, including intensity, location, and modulation frequency. Although the spike trains of neurons in the core fields carried more information in their timing, on average, than did neurons in belt areas, prominent response modulation was not limited to the core fields. These findings suggest that both rate and temporal information in the firing rate patterns of auditory cortical neurons can accurately encode complex, concurrent acoustic stimuli.


Poster

305. Auditory Cortex: Temporal and Frequency Factors

Location: Hall A

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Topic: D.06. Auditory & Vestibular Systems

Support: NSERC PGS-D (Natural Sciences and Engineering Research Council)
FRQNT Doctoral Scholarship (Fonds de Recherche du Québec - Nature et Technologies)

Title: Effects of amplitude-modulated signal-in-noise auditory stimuli on the firing rates of auditory cortical neurons in an awake macaque monkey

Authors: *A. N. SANCHEZ¹, D. B. CAZABON¹, B. BORMANN¹, K. NEVERKOVEC¹, K. B. CHEUNG¹, G. H. RECANZONE¹,²;
¹Ctr. for Neurosci., ²Neurobiology, Physiol. and Behavior, UC Davis, Davis, CA

Abstract: The natural acoustic environment is normally very complex, with different types of sounds simultaneously emitted from various source locations. It remains unclear how the auditory system is able to disambiguate the different acoustic signatures into different sound sources. This raises the interesting questions of how neurons encode multiple sounds; if this encoding is a summation of firing rates, or if cells are performing more nuanced computations of the stimuli. Given that auditory cortex is necessary for the perception of complex stimuli, and the auditory cortex is known to be composed of a number of distinct cortical fields, another question is whether there are differences in how multiple stimuli are processed between auditory fields. We hypothesize that the addition of a second, or ‘distracter’ stimulus will predictably change firing rates of auditory neurons to a primary, or ‘target’ stimulus. We presented one or two stimuli to a passively listening, alert male macaque monkey (20 years old) while extracellularly recording single neurons or small clusters of neurons in different auditory cortical fields. Free-field stimuli consisted of an amplitude-modulated (AM) noise with broadband carriers (the target) presented independently or concurrently with an AM noise distracter which had 1) a spectrally different broadband carrier, 2) one of three different intensities (+10, 0 and -10 dB
relative to the target) and 3) a location 45 degrees from the target in the ipsilateral or contralateral hemifield. Generally, we found an effect of both distracter intensity and location for most neurons. Overall these effects were greater for presumptive core compared to presumptive belt cells. In most cases the responses for ‘distracter + target’ conditions were higher than the sum of the ‘target’ and ‘distracter’ conditions alone, yet were well correlated with measured responses. Regression analysis of the predicted and measured values showed that the regression coefficients were greater, and the slopes were closer to one, in presumptive core neuron responses compared to neuron responses in rostral and caudal regions. These findings suggest that the processing of concurrent sounds involves both linear and non-linear properties in the auditory cortex.


Poster

306. Auditory Processing: From Midbrain to Cortex

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 306.01/K25

Topic: D.06. Auditory & Vestibular Systems

Support: American Hearing Research Foundation
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Title: The roles of NPY neurons and NPY signaling in the inferior colliculus

Authors: *M. A. SILVEIRA¹, J. D. ANAIR¹, N. L. BEEBE³, L. L. BURGER², B. R. SCHOFIELD⁴, M. T. ROBERTS¹;
¹Dept. of Otolaryngology-Head and Neck Surgery, ²Mol. & Integrative Physiol., Univ. of Michigan, Ann Arbor, MI; ³Anat. & Nuerobiology, ⁴Anat. & Neurobio., Northeast Ohio Med. Univ., Rootstown, OH

Abstract: Located in the midbrain, the inferior colliculus (IC) integrates information from numerous auditory nuclei and is an important hub for sound processing. Despite its importance, little is known about the function and molecular identity of neurons in the IC. Using a multi-faceted approach, we have identified Neuropeptide Y (NPY) as a marker for a distinct neuron class in the IC. In the NPY-hrGFP mouse line, hrGFP-positive neurons are distributed throughout the IC, and hrGFP selectively-labels IC neurons that express NPY. Immunostaining showed that NPY neurons in the IC are GABAergic (98.5% of hrGFP-positive neurons co-label with an antibody against GAD67; total NPY neurons counted: 2673). Using design-based stereology, we found that NPY neurons represent ~25% of the GABAergic neurons in the IC. To examine the physiology of NPY neurons, we targeted whole cell patch clamp
recordings to hrGFP-expressing neurons in acute brain slices. These recordings demonstrated that NPY neurons exclusively exhibit a sustained firing pattern (spike frequency adaptation ratio < 2), express little Ih (sag ratio at -80mV = 0.88±0.09) and have a propensity to spontaneously fire, suggesting they might tonically release NPY in the IC. In post hoc reconstructions of recorded neurons, we found that NPY neurons have a stellate morphology and extend their dendrites across the laminar plane of the IC (n=24). Because we found that the NPY Y1 receptor is widely expressed in the IC, we next investigated the functional role of NPY signaling in the IC. Previous studies demonstrated that activity in the IC can induce a variety of unconditioned fear responses, and, in other brain areas, NPY is involved in fear and stress responses. To investigate if NPY signaling is involved in fear responses in the IC, we exposed mice to a novel environment for one hour, and we collected IC tissue 4 hours later to evaluate changes in expression of the Npy and Y1R genes. Our preliminary results show an increase in Npy expression in mice that were acutely exposed to a novel environment compared to mice that were habituated to the novel environment (n=8 - 9 mice per group, one-way two-tailed ANOVA followed by Tukey’s post hoc, p=0.001). No significant difference was observed between the control and habituated groups (p>0.05). Interestingly, for Y1R we observed a decrease in relative expression in the habituated group when compared to the control group (n=8 - 9 mice per group, one-way two-tailed ANOVA followed by Tukey’s post hoc, p=0.01). Thus, we propose that the release of NPY from NPY neurons influences unconditioned fear responses in the IC.

**Disclosures:** M.A. Silveira: None. J.D. Anair: None. N.L. Beebe: None. L.L. Burger: None. B.R. Schofield: None. M.T. Roberts: None.

**Poster**

**306. Auditory Processing: From Midbrain to Cortex**

**Location:** Hall A

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**Topic:** D.06. Auditory & Vestibular Systems

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**Title:** Dopamine heterogeneously alters processing in the inferior colliculus independent of stimulus type

**Authors:** *J. M. HOYT*, D. J. PERKEL, C. V. PORTFORS;  
1Washington State Univ. Vancouver, Vancouver, WA; 2Depts. Biol. & Otolaryngology, Univ. of Washington, Seattle, WA

**Abstract:** Background: The ability to understand speech relies on accurate auditory processing of complex sounds. However, communication disorders can arise from disruptions in the way
individual neurons process sounds or in the ways neurons interact with one another. For example, individuals with Parkinson’s disease suffer from speech perception deficits, suggesting that dopamine is involved in the encoding of complex sounds. The primary auditory nucleus in the midbrain, the inferior colliculus (IC), is rich in both dopaminergic terminals and dopamine receptors. Recent studies from our lab demonstrated that dopamine can increase or decrease responses of many neurons in the IC of mice, although the stimuli used were pure tones or white noise. Thus, it is currently unknown whether dopamine alters processing of complex, salient sounds, and whether the observed heterogeneous effects might differ between types of stimuli. In this study, we tested whether dopamine alters responses of IC neurons to vocalizations. We also tested whether the type of stimulus affects the directional (i.e., increased or decreased) effects of dopamine in the IC. **Methods:** We recorded extracellular responses of single neurons in the IC of awake mice. We compared evoked responses to tones, white noise, and mouse vocalizations, as well as spontaneous activity before and after iontophoretic application of dopamine. **Results:** The effects of dopamine were heterogeneous, resulting in either increased or decreased evoked and spontaneous firing of many IC neurons. Given the apparent behavioral relevance of conspecific vocalizations, we expected that dopamine would increase responses of IC neurons to these sounds while decreasing responses to non-salient stimuli. Surprisingly however, dopamine decreased responses to vocalizations in the majority of neurons, and dopamine also decreased responses to all stimulus types. **Conclusions:** We found that dopamine alters firing properties in the IC, and that such modulation is independent of the presence of and/or type of stimulus. Our results demonstrate changes in the same direction for evoked and spontaneous firing, suggesting that dopamine alters the spiking properties of IC neurons in a general fashion, possibly as a type of bidirectional gain control. Understanding how dopamine modulates auditory processing is a crucial first step toward therapies for disordered dopamine signaling underlying communication- and auditory-based disorders.

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**Poster**

**306. Auditory Processing: From Midbrain to Cortex**

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**Topic:** D.06. Auditory & Vestibular Systems

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**Title:** Synaptic integration and sound processing by VIP neurons in the inferior colliculus of mice
**Abstract:** The central nucleus of the inferior colliculus (ICc) is the hub of the ascending auditory system, as it is a nearly obligatory processing center for the output of the auditory brainstem. To better understand how sounds are processed in the ICc, it is important to identify the classes of neurons that make up the ICc and determine how they function within neural circuits. By using a combination of genetic, anatomical, and physiological methods, we recently identified a novel class of stellate cells that are labeled in Vasoactive Intestinal Peptide (VIP)-IRES-Cre mice. VIP neurons in the ICc are glutamatergic, have a sustained firing pattern (214 / 237), have a stellate morphology, represent ~ 20% of stellate cells in the ICc, and 94% (81 / 86) have spiny dendrites. Via axonal tract tracing studies, we found that VIP neurons project to auditory thalamus, auditory brainstem, the periaqueductal gray, and superior colliculus. Using Channelrhodopsin assisted circuit mapping (CRACM), we found that VIP neurons receive input from the contralateral IC (n = 39) and the contralateral DCN (n = 25). Commisural inputs could be excitatory or inhibitory, or a combination of both. Excitatory commissural inputs were mediated by AMPA and NMDA receptors, while inhibitory inputs were mediated by GABAA receptors. EPSPs evoked by optical stimulation of DCN afferents were excitatory, surprisingly slow (halfwidth: 15.8 ± 9.8 ms) and had no NMDA receptor contribution. Activation of DCN afferents also elicited feedforward inhibition (FFI), which limited EPSP duration (n = 5). This FFI was blocked by tetrodotoxin, confirming it results from disynaptic transmission. Having identified VIP neurons as a distinct neuron type in the IC, we are now probing their functional role in sound processing. In *in vivo* extracellular recordings we found that VIP neurons exhibit narrow tuning curves and are only weakly driven by sinusoidal-amplitude modulated (SAM) tones. Yet, VIP neurons respond well to broadband and SAM noise, indicating that they might need to integrate inputs over a range of frequencies to be driven faithfully. This is consistent with their stellate morphology in which their dendrites cross multiple isofrequency laminae. Using two-color CRACM *in vitro*, we also are working to identify how inputs from the DCN and contralateral IC combine with FFI to shape the output of VIP neurons. These experiments are a critical step toward defining how ICc neurons integrate diverse streams of inputs and influence sound processing in the IC.
Title: Sound-evoked after-discharge: A new form of plasticity in the auditory midbrain

Authors: *A. L. BURGHARD*, C. M. LEE, D. L. OLIVER;
1Neurosci., UConn Hlth., Farmington, CT; 2Neurosci., Sch. of Medicine, Univ. of Connecticut, Farmington, CT

Abstract: The auditory system is our fastest and temporally most precise sensory system. It can decipher timing differences down to the µs scale. All the more puzzling is the existence of neurons in the auditory system that fire during sound but also continue to fire for seconds to minutes after the offset of an acoustic stimulus. The long-duration sound-evoked after-discharge (LSA) response requires an acoustic stimulus that is greater than 20 seconds in duration. LSA neurons were discovered by Ono et al. (2016) in the inferior colliculus (IC), a major hub in the auditory system. The IC receives ascending inputs from lower brainstem nuclei and descending inputs from the cortex, and sends both excitatory and inhibitory projections to the thalamus. LSA is present in both excitatory and inhibitory neurons. However, the role of LSA in auditory processing is unknown. The goal of the present study was to further study the properties of LSA. We used multi-channel single shank electrodes (16 channels/shank) to record multi-unit activity in the IC of anaesthetized mice. This approach allowed us to record from several frequency laminae in the IC simultaneously. We recorded spontaneous firing before and after the presentation of a long duration sound (≥ 60s). LSA was defined as spontaneous firing after sound offset that was two standard deviations above the mean spontaneous firing rate before the long-duration sound. We found LSA in at least 23% of the recording sites. We could identify two basic response patterns. LSA could begin immediately after the offset of the sound, or it could exhibit a build-up response that began as late as 20 s after the sound offset. Both response patterns could last for over a minute. The response type, immediate vs build-up, could not be predicted by the neural response during the long duration sound. The sound induced after-discharge behavior observed in our study could be a form of plasticity with intermediate-duration potentiation. Especially, as the observed firing after the sound offset resembles acoustically driven activity and is therefore of special interest in the context of tinnitus, a disease that is defined as the perception of a sound in the absence of an external sound stimulus.

306. Auditory Processing: From Midbrain to Cortex

Title: Monosynaptic auditory inputs to the cerebellar flocculus and paraflocculus

Authors: G. SEKERKOVA, *C.-P. RICHTER, M. MARTINA; Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Several papers suggest that cerebellar unipolar brush cells (UBCs) located in the flocculus/paraflocculus (Fl/PFl) play an important role in the generation of tinnitus. However, no evidence exist that these cells are embedded in the auditory network. Thus, we tested the hypothesis that UBCs receive auditory inputs. In a first set of experiments we performed cFOS staining on cerebellar sections form animals exposed to acoustic stimuli (90 to 110 dB SPL) and we found that several neurons the Fl/PFl showed cFos expression. These cells were by and large located in the granule cell layer and were identified as presumptive UBCs. In a second set of experiments we performed in vivo recordings in Fl/PFl to study the electrophysiological response to auditory stimulation. We found that numerous cells responded to auditory stimuli. Interestingly, in a majority of the responsive neurons the delay time for the first spike was between 3 and 4 ms, which suggests that these cerebellar neurons receive a monosynaptic input from primary auditory fibers. The spontaneous firing rate of the responsive neurons varied, suggesting potential cellular heterogeneity. However, in 16 of 21 cells in which we could establish the maximum firing frequency, this was relatively slow, suggesting that they are not granule cells. These neurons show spontaneous firing properties of UBC previously reported in other cerebellar and cochlear nuclei regions. Additionally, our tract tracing studies show that BDA labeled primary auditory fibers are not only present in Fl/PFl but they contact UBCs. In conclusion, our data suggest the existence of monosynaptic auditory inputs to UBCs of the cerebellar flocculus.

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Poster

306. Auditory Processing: From Midbrain to Cortex

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Title: Putative amygdala-brainstem synaptic mechanism important for sensory gating

Authors: *J. C. CANO¹, K. FENELON²;
¹Univ. of Texas At El Paso, El Paso, TX; ²Biol., Univ. of Massachusetts Amherst, Amherst, MA

Abstract: Sensorimotor gating (SG) is a pre-attentive inhibitory neural mechanism that filters out irrelevant sensory information. SG therefore contributes to focus attention and cognitive processes. Impaired SG is observed in patients suffering from schizophrenia, obsessive-compulsive disorder and other neuropsychiatric disorders. Clinically, SG is measured using the prepulse inhibition (PPI) of the acoustic startle reflex task. PPI occurs when a mild sound (prepulse) inhibits the startling effect of a subsequent startling sound (pulse). Uniquely positioned to relay sensory inputs from several brain regions to motor neurons, the brainstem caudal pontine reticular nucleus (PnC) is at the core of the neural circuit underlying PPI. Afferents from the pedunculopontine tegmental nucleus (PPTg) to the PnC form part of a “cortico-striato-pallido-pontine” network important for PPI. Interestingly, PPTg lesions reduce PPI without abolishing it, suggesting that other PnC-connected regions might contribute to PPI. The central nucleus of the amygdala (CeA) also sends monosynaptic projections to the PnC in rodents. We previously showed that inhibiting the CeA-PnC excitatory synapses disrupts PPI. Here, to further confirm the contribution of this alternative pathway to PPI, we photo-activated the CeA-PnC excitatory connection to mimic its physiological activation by an acoustic prepulse in mice. This resulted in a 19-30% PPI, compared to a 40-60% PPI elicited by an acoustic prepulse. We then aimed to identify the post-synaptic targets of CeA excitatory inputs in the PnC. We first determined whether CeA excitatory inputs presynaptically modulate auditory neurotransmission. Using extracellular field recordings in PnC slices, activation of CeA fibers did not significantly change the probability of neurotransmitter released by auditory fiber stimulation in PnC neurons. Then, injecting AAV-CamKIIa-mCherry in the CeA of mice that express the green fluorescent protein under the control of the Glycine transporter type 2 (GlyT2) promoter, confocal imaging revealed close appositions between CeA axonal projections and GlyT2-expressing PnC interneurons. In sum, our results suggest that CeA excitatory inputs activate PnC inhibitory neurons and this mechanism likely contributes to PPI. We thus provide some insights on the paradoxical contribution of an excitatory pathway to an inhibitory
phenomenon. Ultimately, better understanding the neural substrates of PPI is crucial to identify potential therapeutic targets for SG and cognitive impairments.

Disclosures: J.C. Cano: None. K. Fenelon: None.

Poster

306. Auditory Processing: From Midbrain to Cortex

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Wellcome Trust: WT108369/Z/2015/Z

Title: Neural circuits for somatosensory control of auditory thalamo-cortical processing

Authors: *M. LOHSE, J. C. DAHMEN, V. M. BAJO, A. J. KING;
Univ. of Oxford, Oxford, United Kingdom

Abstract: Considerable attention has been focused on the involvement of cortical areas in multisensory processing. Although visual and somatosensory influences on auditory responses can arise via direct corticocortical pathways, it is likely that some aspects of multisensory cortical processing are inherited from the thalamus. In recent years, the thalamus has received renewed interest, and several studies have suggested that sensory processing at this level may be dependent on a range of contexts, often guided by cortico-thalamic feedback. However, very little is known about the neural circuits that might implement multisensory interactions at the level of the thalamus. We have investigated this by examining the circuitry underlying the influence of tactile inputs on processing in the auditory thalamus in mice. Our in vivo electrophysiological recordings demonstrated that the mouse auditory thalamus as a whole is influenced by stimulation of the whiskers in diverse, but pathway-specific ways, with primary auditory cortex inheriting signals from the thalamus that are divisively scaled by whisker stimulation. This somatosensory suppression is dependent on a primary somatosensory corticofugal projection and is at least in part implemented via neurons in the higher-order auditory midbrain receiving input from somatosensory cortex. Furthermore, we demonstrate the presence of a parallel direct cortico-thalamic pathway from primary somatosensory cortex to the medial sector of auditory thalamus, which is capable of driving spiking activity and facilitating auditory responses. Together, these results reveal a previously underappreciated role for auditory thalamus in the implementation of multisensory processing, and the circuits which allow for such thalamic multisensory interactions.

Disclosures: M. Lohse: None. J.C. Dahmen: None. V.M. Bajo: None. A.J. King: None.
**Poster**

**306. Auditory Processing: From Midbrain to Cortex**

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**Title:** Dissection of auditory thalamocortical circuit dynamics using targeted lidocaine microinjections

**Authors:** *P. LAKATOS¹, M. N. O’CONNELL⁴, A. BARCZAK², T. M. MCGINNIS³, S. A. NEYMOTIN⁵, T. M. MOWERY⁶;

**Abstract:** While previous anatomical studies provided enough data to form the backbone of influential theories on how thalamocortical circuitry supports the transformation of sensory information on its way to conscious perception, a systematic verification of these influential theories in awake behaving animals is still lacking. The goal of our present study was to verify one of these theories in particular: the core-matrix organization of thalamic nuclei proposed by Jones (1998, Neuroscience). According to this theory, while core components of the thalamus submit detailed information about the properties of sensory stimuli, the matrix is responsible for orchestrating modulatory mechanisms and for orchestrating synchrony amongst cortical neuronal ensembles processing behaviorally relevant inputs. To verify this type of dual-purpose functional and anatomical organization, we located and selectively injected different nuclei of the medial geniculate body (MGB) with lidocaine in awake macaque monkeys. We targeted either the ventral nucleus of the MGB, which forms the auditory thalamic core and projects to the granular layer of the auditory cortex, or the dorsal and medial nuclei of the MGB, which are part of the auditory thalamic matrix and project to the extragranular layers. As anticipated, injections resulted in a dose dependent, local silencing of neuronal activity in the targeted nuclei. Our laminar auditory cortex recordings revealed that while lidocaine injections into the MGB core resulted in an attenuation of responses to auditory stimuli mostly in the granular layer, MGB matrix injections suppressed extragranular layer responses. As expected, we found marked differences between thalamic silencing’s effect on cortical responses to pure tones vs. broadband noise bursts, but response latencies to both types of stimuli significantly increased following core silencing. Additionally, cortical frequency tuning was also affected in distinct ways in the case of
core vs. matrix thalamic lidocaine injections: while core silencing sharpened, matrix silencing broadened tuning. Finally, in line with the matrix’s role in orchestrating cortical rhythmic modulatory mechanisms and synchrony, neuronal oscillations were also modulated following matrix injections. In summary, our study was able to verify and add electrophysiological detail to the theory of the core-matrix organization of thalamic nuclei.


Poster

306. Auditory Processing: From Midbrain to Cortex

Location: Hall A

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NSF GRFP DGE1745303

Title: A corticothalamic circuit for motor modulation of auditory processing

Authors: *K. K. CLAYTON1,3, R. S. WILLIAMSON2,3, G.-I. TASAKA4, A. MIZRAHI5, T. A. HACKETT6, D. B. POLLEY2,1,3; 1Program in Speech and Hearing Biosci. and Technology, Div. of Med. Sci., 2Dept. of Otolaryngology, Harvard Med. Sch., Boston, MA; 3Eaton-Peabody Labs., Massachusetts Eye and Ear, Boston, MA; 4The Edmond and Lily Safra Ctr. for Brain Sciences, The Hebrew Univ. of Jerusalem, Jerusalem, The Hebrew Univ. of Jerusalem, Jerusalem, Israel; 5The Edmond and Lily Safra Ctr. for Brain Sci., Hebrew Univ. of Jerusalem, Jerusalem, Israel; 6Dept. of Hearing and Speech Sci., Vanderbilt Univ., Nashville, TN

Abstract: Auditory cortex (ACtx) pyramidal neurons are suppressed during movement, which reduces sound-evoked activity and behavioral detection of faint sounds. Movement-related suppression of ACtx pyramidal neurons is thought to arise through projections from the basal forebrain and secondary motor cortex (M2) that engage local GABA circuits within the ACtx. While some fraction of pyramidal neuron suppression reflects monosynaptic connections from M2 onto GABA neurons, the influence of neuromodulatory inputs are often brokered by intermediaries in layer (L) 1 or L6 that directly adjust local excitability and tuning. Ntsr1-expressing layer 6 corticothalamic (L6 CT) neurons regulate sensory gain throughout the column by recruiting specialized networks of fast-spiking inhibitory neurons, suggesting that L6 CTs could be activated by motor preparatory signals to drive local GABA networks and suppress
excitatory activity. To test this prediction, we used 2-photon calcium imaging to measure activity from L6 CT neurons or L2/3 Thy1+ pyramidal neurons while mice performed a simple task that isolated the contribution of movement, sensory, and reward-related inputs. We confirmed reports that self-generated movements such as licking or running suppressed spontaneous and sound-evoked activity in L2/3 pyramidal neurons. By contrast, L6 CTs were activated immediately prior to and during movement onset. Unlike L2/3 pyramidal neurons, sound-evoked responses in L6 CTs were not attenuated during movement, and were often enhanced. Pseudo-typed rabies tracing identified substantial numbers of long-range monosynaptic inputs onto L6 CT neurons from cholinergic and non-cholinergic neurons at the border of globus pallidus and the basal forebrain. Few input neurons were identified in M2. These findings suggest that L6 CTs may act as an intermediary between long-range motor inputs from the basal forebrain and basal ganglia, but that M2 may bypass L6 CTs and connect directly onto local GABA cells within ACtx. To better identify the sequence of ACtx neural activation during movement with millisecond precision, our ongoing experiments rely on electrophysiological recordings from optogenetically identified L6 CT neurons as well as local regular- and fast-spiking neurons.


Poster

306. Auditory Processing: From Midbrain to Cortex

Location: Hall A

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Title: Cortical activation in a mouse model of the auditory brainstem implant

Authors: V. KANUMURI1, *A. ZHU1,2, A. DAVIS1, A. QURESHI1, S. MCINTURFF1, O. TARABICHI1, N. VACHICOURAS3, K. K. CLAYTON4, S. LACOUR3, M. BROWN1, D. B. POLLEY5, D. J. LEE1, A. E. TAKESIAN6;
1Massachusetts Eye and Ear Infirmary, Boston, MA; 2Howard Hughes Med. Inst., Chevy Chase, MD; 3École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; 4Eaton Peabody Labs., 5Otolaryngology, Harvard Med. Sch., Boston, MA; 6Otolaryngology, Massachusetts Eye and Ear, Boston, MA
**Abstract:** Auditory brainstem implants (ABIs) are neuroprosthetic devices that provide sound sensations to profoundly deaf patients who are not candidates for cochlear implants due to a damaged or absent cochlear nerve. Recognition of complex sounds such as speech is poor with ABIs compared to performance with cochlear implants. Efforts to improve ABI technology are hampered by a poor understanding of how ABIs recruit the central auditory pathways, particularly the auditory cortex. Here, we developed a novel ABI mouse model that enables *in vivo* imaging of auditory cortical neuronal activity in response to sound or electrical stimulation of the cochlear nucleus (CN). Mice are chronically implanted with a flexible multichannel array placed onto the CN, a contralateral cranial window is made, and transduction of cortical neurons is achieved using viral vector injection for expression of the calcium indicator GCaMP6s. Using two-photon calcium imaging, we analyzed L2/3 auditory cortical neurons in awake mice and compared their activity in response to either broadband frequency noise bursts or an electrical ABI impulse delivered to the CN. Our preliminary data suggest that the subset of auditory cortical neurons that respond to noise bursts are also activated by ABI stimulation. We then determined whether ABI stimulation induced an auditory percept by training implanted mice in a bi-modal auditory/ABI behavioral detection task. We found that mice previously trained to detect sound rapidly generalized to detecting ABI stimulation at high pulse rates. Ongoing work is determining which cortical neuron subtypes are activated by sound versus ABI-evoked representations. Together, these experiments may offer insights into improving ABI design, and ultimately lead to better outcomes in patients who must learn to interpret sound signals through these implants.


**Poster**

**306. Auditory Processing: From Midbrain to Cortex**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #: Poster #:** 306.11/K35

**Topic:** D.06. Auditory & Vestibular Systems

**Support:** JSPS KAKENHI No. 26430025

**Title:** Cell morphology-specific nicotine regulation of excitatory synapse in mouse auditory cortex

**Authors:** *T. NAGAYAMA¹, R. METHERATE², H. D. KAWAI²;¹Soka Univ., Hachioji, Japan; ²Dept. of Sci. and Engin. for Sustainable Innovation, Soka Univ., Tokyo, Japan; ³Univ. of California Irvine Dept. of Neurobio. and Behavior, Irvine, CA
Abstract: Previous in vivo studies have shown that systemic nicotine exposure enhances the responses evoked by characteristic frequency (CF) tone but suppresses those evoked by non-CF tone in primary auditory cortex (A1). These nicotinic regulations need the activation of α4β2* nicotinic acetylcholine receptors and extracellular signal-regulated kinase (ERK). Although protein kinase A (PKA) is known as an upstream effector of ERK, it is unclear whether PKA mediates these nicotinic regulations. To examine this, we recorded local field potentials (LFPs) in A1 with a 16-channel multiprobe and examined how PKA blockade affected the systemic nicotine effects by analyzing current source densities (CSDs). While nicotine exposure increased initial 3 ms (the “input” phase) and 3-20 ms (the “early intracortical” phase) current sinks of CF tone-evoked CSDs in the thalamocortical input layer as reported previously, the local injection of a PKA inhibitor, myristoylated PKI 14-22 amide (PKI), inhibited the nicotinic enhancement of both phases. As for the non-CF tone-evoked CSDs, PKI injection prevented the nicotine-induced suppression of initial 20 ms current sink (the “early” phase) but did not inhibit the 30-80 ms current sink (the “late” phase). These data suggest that there are distinct excitatory neurons and their synaptic inputs under different nicotinic regulations. We began to test this possibility by recording spontaneous and evoked excitatory postsynaptic currents (EPSCs) in layer 4 of A1 using the whole-cell voltage clamp technique. A local nicotine perfusion increased the frequency and amplitude of spontaneous EPSCs (sEPSCs) and miniature EPSCs (under tetrodotoxin) in cells with 3 or less first branches out of the apical dendrite (less-branching cells), but it decreased them in cells with 4 or more first branches (more-branching cells). The nicotinic enhancement in less-branching cells was PKA-dependent, while the nicotinic suppression in more branching cells was PKA-independent. The amplitude of the polysynaptic EPSCs evoked by stimulation at a horizontally distant (>500 μm) site was increased in less-branching cells but decreased in more-branching cells. Nicotine didn’t affect the thalamocortical monosynaptic EPSCs on either cell type. It was noted that the thalamocortical synapses were detected primarily in less-branching cells, while the horizontal inputs were detected mostly in more-branching cells. We hypothesize that less-branching cells process mainly thalamocortical and subsequent intracortical inputs, which nicotine enhances via PKA, while more-branching cells process horizontal intracortical inputs, which nicotine suppresses.

Disclosures: T. Nagayama: None. R. Metherate: None. H.D. Kawai: None.

Poster

306. Auditory Processing: From Midbrain to Cortex

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 306.12/K36

Topic: D.06. Auditory & Vestibular Systems

Support: NIH MARC-USTAR grant 2T34GM007663-32
Title: Regional expression of parvalbumin and calbindin in macaque auditory cortex

Authors: *L. A. DE LA MOTHE\(^1\), S. HUBBARD\(^1\), H. WALKER\(^1\), T. A. HACKETT\(^2\);
\(^1\)Psychology, Tennessee State Univ., Nashville, TN; \(^2\)Hearing & Speech Sciences, Psychology, Vanderbilt Univ., Nashville, TN

Abstract: In order to have a greater understanding of sensory perception and behavior, including communication, the structural and chemical organization of the brain must be established. Previous work by Kubota et al. (1994) examined the expression patterns of several calcium binding proteins including parvalbumin and calbindin in frontal cortex. While additional studies of this subset of inhibitory interneurons have expanded this work to include various species and cortical areas (Cruikshank et al., 2001; Kawaguchi and Kubota, 1997; Markram et al., 2004), our understanding of the expression of CBP within auditory cortex of primates remains to be fully characterized. The current working model of primate auditory cortex consists of three regions that correspond to levels of processing: the primary core region, a secondary belt region, and the parabelt region (Hackett, 2011). This study aims to examine expression of the CBPs parvalbumin and calbindin across the various regions of auditory cortex. Single and multi-fluorescent immunohistochemistry (IHC) were performed to visualize parvalbumin and calbindin positive cells. Samples were taken from the three regions in auditory cortex to compare distribution of these CBP. Cells labeled with parvalbumin and calbindin were plotted as well as those co-expressing both. Results indicate largely distinct populations of parvalbumin and calbindin, with few cells co-localized, clear laminar patterns, as well as decreasing expression of the CBP with increasing hierarchical regions (core, belt, parabelt).


Poster

306. Auditory Processing: From Midbrain to Cortex

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 306.13/K37

Topic: D.06. Auditory & Vestibular Systems

Support: NIH R01DC015803

Title: State-dependence of spontaneous and sound-evoked responses across layers and cell types of mouse auditory cortex

Authors: *S. JO\(^1\), T. DONOR\(^1\), E. MCCARTHY\(^2\), D. A. MCCORMICK\(^1\);
\(^1\)Inst. of Neurosci., \(^2\)Computer Sci., Univ. of Oregon, Eugene, OR
Abstract: The ability to identify meaningful auditory signals requires the ability to rapidly modulate neural responses in a manner relevant to an animal’s behavior and internal state. State-dependent variations in neural responses have been observed in auditory cortex; however, the characteristics of different cortical layers and roles of different cell types in mediating this effect are not well understood. To gain a better understanding of auditory state-dependence, we used multiple techniques, from extracellular recording to wide-field and two-photon mesoscale calcium imaging, to record neural activity in auditory cortex of head-fixed mice. We simultaneously monitored pupil size, walking speed, and whisker movement to quantify state while the mice listened to white noise, tone pips, or nothing. To observe responses on a large scale, we performed wide-field single-photon calcium imaging of superficial excitatory neurons in Thy1-GCamp6s and CaMKII-GCamp6s mice. Next, we probed the effect of state on each layer of cortex using extracellular recordings with silicon probes. Wide-field imaging and extracellular recording revealed an inverted-U relationship between pupil diameter and sound-evoked responses in superficial layers. Extracellular recordings confirmed that this relationship is also present in deep layers of auditory cortex. Furthermore, state was found to exhibit a substantial relationship with spontaneous activity. Finally, to reveal the roles of different cell types in state-dependent circuit mechanisms, we are performing cellular resolution calcium imaging of excitatory neurons, as well as parvalbumin (PV), somatostatin (SOM), and vasoactive intestinal peptide (VIP) interneurons using a two-photon mesoscope. We crossed PV, SOM, and VIP mouse CRE lines with reporter line Ai148 and/or Ai162 which are expressing GCamp6f and GCamp6s, respectively. Preliminary results suggest that the activities of individual excitatory and VIP neurons exhibit heterogenous, but significant relationships with state and movement. Our research combines multiple techniques to contribute an overall understanding of state-dependent neural mechanisms across layers and cell types of auditory cortex.

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Poster

306. Auditory Processing: From Midbrain to Cortex

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 306.14/K38

Topic: D.06. Auditory & Vestibular Systems

Support: F. Hoffmann-La Roche, Neuroscience Discovery
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Title: Modulation of glutamatergic and GABAergic circuits produce frequency and region-specific effects on auditory evoked responses
Abstract: Auditory state responses (ASSR) constitute a useful tool for evaluating cortical oscillatory activity, as they appear altered in several neuropsychiatric disorders. ASSR are oscillatory responses to rhythmic auditory stimuli. By using a tone modulated in amplitude at increasing frequencies, the chirp evoked potentials assess the response to a wide range of frequencies in a single test. Since gamma band oscillatory activity (30-120 Hz) is involved in higher-order cognition and perception, alterations in electrical activity of schizophrenia patients have been related to these symptoms of the disease (Alegre, M. et al. 2017).

We hypothesized that dizocilpine (MK-801) -which recapitulates part of the physiology responsible for schizophrenia- would disrupt the chirp and ASSR in rodents in a similar way to that described in patients. Furthermore, we hypothesized that diazepam (DZP) -shown to disrupt gamma band synchronization- would also disrupt the responses. Moreover, if chirp and ASSRs can discern distinct pharmacological modulations, the effects of MK-801 would differ from those of DZP.

We recorded electroencephalographic (EEG) responses in the rodents. In the primary auditory cortex, the effects at the 40-Hz band in ASSR were similar. The chirp under MK-801 produced a decrease in the high-gamma range while under DZP it remained stable. In the prefrontal cortex, DZP produced a decrease in the low-gamma range in both ASSR and chirp, while under MK-801 responses remained stable. Lastly, in the parietal cortex, no effect was obvious in the 40-Hz ASSR, while the chirp showed a decrease in low-gamma under DZP and in high-gamma under MK-801.

Disruptions in the glutamatergic and GABAergic circuits have region-specific effects on evoked responses. The chirp shows that disruptions are specific to the neurotransmitter system affected. We conclude that the responses to chirp and ASSR are sensitive to the fine regulation of different neuronal populations, and hypothesize that the modulation of specific cell types at a microcircuit level results in distinct responses.

Support: NIDCD R21DC016991
Bertarelli Program in Translational Neuroscience and Neuroengineering
Jacobs Foundation

Title: Diverse brain regions targeting layer 1 interneuron subtypes in auditory cortex

Authors: *C. G. SWEENEY*¹,², A. C. CASTRO¹, B. M. GLICKMAN¹, J. D. MCLENNAN¹,³, E. HE¹,³, A. E. TAKESIAN¹,²;
¹Massachusetts Eye and Ear Infirmary, Boston, MA; ²Otolaryngology, Harvard Med. Sch., Boston, MA; ³Harvard Univ., Cambridge, MA

Abstract: The primary auditory cortex (A1) receives sensory input from the auditory thalamus as well as neuromodulatory centers throughout the brain. Neuromodulatory projections powerfully filter and modify ascending auditory signals from the thalamus, thereby influencing sound perception and sound-driven behaviors. Moreover, the convergence of ascending sensory and neuromodulatory signals in A1 can induce long-lasting changes in cortical sound responses that may underlie auditory learning. Recent work from our laboratory and others implicates layer 1 (L1) of A1 as a key site for integrating both sensory signals and information about behavioral state and outcome. The interneurons that populate L1 are composed of multiple subtypes, characterized by the selective expression of distinct molecular markers, such as vasoactive intestinal peptide (VIP), neuron-derived neurotrophic factor (NDNF) and gamma synuclein (SNCG). Our recent results demonstrate that these subtypes differentially receive input from thalamic nuclei. Further, they differentially express receptors for neuromodulators such as acetylcholine, serotonin, and corticosteroids, suggesting that distinct brain regions may preferentially innervate these interneuron subtypes. Our ongoing work using 2-photon calcium imaging is revealing parallel differences in the *in vivo* activity patterns of these interneuron subtypes in response to a range of sound and other behaviorally-relevant stimuli. Together, these studies will expand upon our current understanding of the anatomical and functional diversity of L1 circuits in A1 for conveying distinct auditory and neuromodulatory information.


Poster

306. Auditory Processing: From Midbrain to Cortex

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 306.16/K40

Topic: D.06. Auditory & Vestibular Systems

Support: R01DC009607
Title: The development of circuits to L1 neurons in the mouse auditory cortex

Authors: *X. MENG¹, J. P. Y. KAO², P. O. KANOLD¹;
¹Biol., Univ. of Maryland, College Park, MD; ²Ctr. for Biomed. Engin. and Technology, and Dept. of Physiol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Layer 1 (L1) of neocortex is unique in that most L1 neurons are GABAergic interneuron and they can have profound impact on the responses of neurons in other layers, especially the neurons whose dendritic tufts reside within L1, to the sensory stimuli. During development the pattern of sensory evoked activity plays a crucial role in shaping cortical organization. Since L1 neurons are positioned to control activity across the cortical column they might play a key role. L1 neurons receive intra- and inter- cortical inputs as well as subcortical inputs. However, the development of these circuit to L1 is unknown. To address this question we performed Laser-scanning photo stimulation (LSPS) combined with whole-cell patch clamp recording of L1 neurons in thalamocortical slices from P5 to P28 mice and measured the spatial pattern of excitatory and inhibitory connections. We found that at P 5-8 L1 neurons receive excitatory input from all layers, especially L5/6 and the cortical subplate, whereas the inhibitory GABAergic inputs to L1 neurons are most from within L1, L5/6 and subplate. During development the inhibitory input from L2/3 and L4 increases. Both the excitatory and inhibitory connection from subplate decreases. Moreover, we observed that the functional circuits diversify during development. Together, our results revealed distinct laminar changes in the intracortical connections to L1 neurons over development.

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Poster

306. Auditory Processing: From Midbrain to Cortex

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

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Topic: D.06. Auditory & Vestibular Systems

Support: MRP/101/17X
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Title: Rewiring the deafferented thalamic and cortical neurons to the remaining auditory inputs: A model for treatment of tinnitus patients

Authors: *P. JENDRICHOVSKY, Y. YANG, X. CHEN, J. HE; City Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Introduction Tinnitus is the auditory phantom sensation when no external sound is present. Our working theory is that tinnitus is caused by the parasitic positive feedback oscillation in the thalamocortical circuit, neurons of which become hypersensitive due to lack of cochlear input and cause tinnitus after hearing loss. Cholecystokinin (CCK) has plasticity enabling properties towards cortical neurons. In the present study, we aim to rewire weak connections from the remaining inputs towards the deafferented ones. We hypothesize that this will suppress the hypersensitivity and therefore silence tinnitus permanently. Materials & Methods To accomplish the aim of this study, a reliable animal tinnitus induction and assessment models had to be established first. Gap-prepulse inhibition of the acoustic startle reflex (GPIAS) is used for the assessment of tinnitus, whereas unilateral noise induced hearing loss was chosen as model to induce tinnitus. Various applications of CCK4 together with sound treatment were chosen as the potential candidates for permanent tinnitus treatment. Results We obtained long term GPIAS observations from multiple mice before hearing loss induction, during two-month-long chronic tinnitus transition period and after the CCK treatment. After CCK therapy mice improved the GPIAS performance.

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Poster

306. Auditory Processing: From Midbrain to Cortex

Location: Hall A

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Topic: D.06. Auditory & Vestibular Systems

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NIH Grant DC014807

Title: Experience based changes to auditory corticostriatal E/I receptor function gates LTP and permits associative learning

Authors: N. PARAOUTY, *T. M. M. MOWERY; Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Associative learning is facilitated by cortical glutamatergic excitatory inputs onto medium spiny neurons of the sensorimotor striatum. When salient stimuli are repeatedly paired with positive or negative rewards, learning occurs as the desired behavioral response becomes
extremely reliable. While LTP is the most likely neural mechanism governing this process, the experience-based changes to synaptic properties along the corticostriatal circuit that drive synaptic strengthening remain unclear. Here we used an appetitive reinforcement operant conditioning procedure to train gerbils on a discrimination task. Behavioral performance was assessed daily and correlated with experience-based changes to synaptic properties in a corticostriatal slice preparation. Our primary aim was to determine how the changes in striatal cellular properties gate the LTP that is correlated with task acquisition. Mongolian gerbils were trained on a Go-Nogo task. Here, animals learned to discriminate a 12-Hz amplitude-modulated noise stimulus which indicated the availability of food reward from a 4-Hz stimulus which indicated no reward. Following each training session, a sensitivity measure, dprime, was computed and a corticostriatal brain slice was obtained. Inhibitory GABA_B or excitatory NMDA synaptic properties were recorded from medium spiny neurons in the auditory striatum. Theta burst stimulation of auditory cortex L5 pyramidal neurons was used to assess the probability of LTP induction in the striatum. Our data indicate that as the animals learn the task, GABA_B inhibitory receptor strength is reduced, while NMDA receptor strength is increased. Following task acquisition (d-prime >1.5), both GABA_B and NMDA receptor strengths return to baseline values, i.e., pre-task acquisition. In addition, LTP probability increases contemporaneous to task acquisition. Together these results suggest that a reduction in GABA_B receptor strength may facilitate NMDA receptor activation leading to cortically-induced LTP expression in the striatum. The short time window during which experience induces a change in E/I receptor functions permits learning to occur by allowing stimuli-evoked responses to briefly have a greater potentiation effect.

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Poster

306. Auditory Processing: From Midbrain to Cortex

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 306.19/L3

Topic: D.06. Auditory & Vestibular Systems

Support: NIH R01 DC015974

Title: Molecular heterogeneity in a peripheral auditory feedback circuit

Authors: M. M. FRANK¹, A. A. SITKO¹, W. YAN², M. H. MYOGA³, J. R. SANES², B. GROTHE³, L. V. GOODRICH¹;
¹Harvard Med. Sch., Boston, MA; ²Harvard Univ., Cambridge, MA; ³Ludwig Maximilians Univ. Munich, Martinsried, Germany
Abstract: Sensory systems rely on intricately connected networks of feedforward and feedback circuitry to encode sensory information and guide behaviors. In the auditory system, incoming sound information is transduced by hair cells in the cochlea and transmitted into the brain by spiral ganglion neurons (SGNs). Early auditory computations occur largely in the brainstem, where specialized circuits in the superior olivary complex (SOC) aggregate sensory information from both ears to mediate sound localization. The SOC also houses a group of auditory feedback cells that project back into the cochlea to target both hair cells and SGNs. These olivocochlear efferent neurons (OCNs) are known to play numerous roles in the auditory system, including protecting the cochlea from acoustic injury and aiding in speech-in-noise detection. Mammalian OCNs are typically classified into two or three major categories based on their anatomical projections. Little is known, however, about the molecular or genetic factors that distinguish these major subsets of OCNs from each other or from other brainstem neurons. As such, no markers exist to identify or manipulate subsets of OCNs, and virtually nothing is known about any heterogeneity within these major OCN subtypes. To address this gap, we’ve used a high-throughput, single-cell sequencing approach to profile the transcriptome of individual neurons in the SOC, including OCNs. Our analysis has identified several populations of SOC neurons, including two major clusters of OCNs. We’ve discovered several new markers for each of these OCN clusters, offering new insights into these key regulators of cochlear function.


Poster

307. Visual Cortex: Manipulating and Reading Neural Activity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 307.01/L4

Topic: D.07. Vision

Support: NIMH Intramural Research Program

Title: Behavioral detection of optogenetic stimulation in macaque inferior temporal cortex

Authors: *S. BOHN, A. AFRAZ;
Lab. of Neuropsychology, NIMH, Bethesda, MD

Abstract: We injected AAV5 engineered to express the opsin C1V1 (a light-activated neural depolarizing agent) to the inferior temporal (IT) cortex of a rhesus macaque. In a second surgery twelve weeks later, we verified virus expression and implanted an LED array over the transduced region. The array is composed of a 5x5 grid of LEDs, each capable of producing up to 60mW of green light (527nm). As a control, we implanted another LED array on the animal’s other IT cortex that was not transduced with virus.
The animal was trained to behaviorally detect optogenetic stimulation of its IT cortex while looking at images of different objects. After acquiring a trial by fixating on a central fixation point, an image of an object appeared on the animal’s screen for one second. Then, in half of the trials, randomly selected, a 200ms optical impulse was delivered to IT cortex via the LED array halfway through the trial. The animal was rewarded for correctly identifying whether the trial did or did not contain an optical impulse by looking at one of the two subsequently presented response targets.

The animal learned to detect optical impulses delivered to its transduced cortex significantly above chance level after 17 days of training (Chi-squared, p < .01) and improved its average performance across all images to 89% correct after 21 more days of training (Chi-squared, p < .0001). Optical impulses delivered to the control side (no virus injection) remained undetectable. We also found that the animal’s performance in detecting cortical stimulation heavily depends on the image presented during stimulation, ranging from 74% to 100% correct for images of different objects. The lowest performance (69%) was observed when only the fixation point was shown on a blank screen. Even in this condition, performance remained significantly above chance (Chi-squared, p < .01). Overall, we found a significant effect of image on detectability of the optogenetic stimulation of IT cortex (bootstrapping, p < .001).

These results show that it is possible to induce large behavioral effects using optogenetics in nonhuman primates. The results also indicate that detectability of IT cortical stimulation depends on visual input. This suggests that stimulation of IT cortex causes specific distortions to incoming visual stimuli.

Disclosures: S. Bohn: None. A. Afraz: None.

Poster

307. Visual Cortex: Manipulating and Reading Neural Activity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 307.02/L5

Topic: D.07. Vision

Title: Functional imaging of excitatory neurons in an entire column in mouse visual cortex


Abstract: Characterizing the relationship between neural function and connectivity is a central problem in visual sensory processing. In order to explore this relationship, we have recorded visual responses from pan-excitatory neurons within an 800X800 um region of primary visual cortex, spanning all visual layers from pia to white matter. This includes ~35,000 neurons per mouse in 5 mice total, collected from 750 2-photon and 35 3-photon calcium imaging planes.
spaced by ~16 um. This dataset will be used to examine the single-cell and population activity in primary visual cortex, and along with electron microscopic reconstruction from the same tissue, will serve as a valuable resource in studying the functional connectome in mouse cortex. A wide variety of visual stimuli were used to characterize neural responses, including drifting gratings, sparse noise, natural movies and natural images. We assessed multiple metrics, including receptive field profile, direction and orientation selectivity indices, reliability of response, signal and noise correlations, and sparseness of response. Here, we describe these metrics as a function of depth and explore their heterogeneity. For example, we characterize the spatial receptive field organization at different depths and quantify both spatial scatter and the degree of overlap between ON and OFF sub-fields.


Poster 307. Visual Cortex: Manipulating and Reading Neural Activity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 307.03/L6

Topic: D.07. Vision

Support: DARPA-NESD-N66001-17-C-4012
BRAIN-U01NS099720-01
NIH/NEI EY016454
NIH/NEI EY11747

Title: A bi-directional optical-genetic toolkit for reading and writing topographic neural population codes in behaving macaque cortex

Authors: *Y. Y. CHEN*¹, G. BENVENUTI¹, D. MILLER², C. T. SULLENDER², F. RADAEI², A. DUNN², C. RAMAKRISHNAN³, K. DEISSEROTH³, W. S. GEISLER¹, E. SEIDEMANN¹;
¹Ctr. for Perceptual Systems, ²Biomed. Engin., Univ. of Texas at Austin, Austin, TX; ³Bioengin & Psych, Stanford Univ. Dept. of Psychology, Stanford, CA

Abstract: In primates, visual perception is likely to be mediated by large populations of V1 neurons organized into multiple overlaid topographic maps. The distributed and topographic nature of V1’s representations raises the possibility that in some tasks, downstream areas that decode V1 signals in order to mediate perception could combine V1 signals at the relevant topographic scale—e.g., at the scale of the orientation columns. If this were the case, then the fundamental unit of information would be individual columns rather than single neurons, and to account for the subject’s behavior in a perceptual task, it would be necessary and sufficient to
consider the summed activity of the thousands of neurons within each column.

To test this topographic-code hypothesis, we developed a bi-directional optical-genetic toolkit for reading and writing topographic neural population codes in macaque cortex. First, we used viral-based methods to co-express a calcium indicator (GCaMP6f) and a red-shifted opsin (C1V1) in excitatory V1 neurons for extended periods (>1 year). Second, we developed a DMD projector that allows us to project arbitrary light patterns into the cortex. Third, we built a widefield imaging system that allows simultaneous GCaMP imaging and patterned optogenetic stimulation.

Here we focus on our initial attempts to develop a quantitative model that would allow one to determine the light stimulation necessary to recreate a desired topographic pattern of population response. Our first model has two components: a transfer function between light power and evoked neural activity, and a spatial impulse response. To estimate these components, we employed checkboard patterns with different power densities and spatial frequencies. Our results reveal that: 1) reliable response can be elicited at low power density (< 0.02 mW/mm^2); 2) above threshold, response increases linearly with power density until a saturating nonlinearity is reached; 3) the spatial impulse response contains a narrow peak (HWHH of ~0.22mm) and a widespread tail. Importantly, this method allows us to elicit responses at the scale of orientation columns.

Our long-term goal is to use this toolkit to recreate neural responses that are indistinguishable at the relevant topographic scale from those evoked by visual stimuli, and then use the animal’s perceptual report to test the extent to which these visual and optogenetic stimulations are perceptually equivalent (i.e., are neural metameres). These experiments could address fundamental questions regarding the nature of the neural code in primate cortex and could have important implications for future brain-machine-interfaces.


Poster

307. Visual Cortex: Manipulating and Reading Neural Activity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

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Topic: D.07. Vision

Support: NIH/NEI grant R01EY024662
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NIH/BRAIN grant U01NS099720
Title: Neural and perceptual correlates of nonlinear interactions between visual and optogenetic stimulation in behaving macaque V1

Authors: *S. KUMAR, S. C.-Y. CHEN, Y. Y. CHEN, G. BENVENUTI, M. P. WHITMIRE, W. S. GEISLER, E. SEIDEMANN; Ctr. for Perceptual Systems, Univ. of Texas at Austin, Austin, TX

Abstract: A central goal of visual neuroscience is to understand the relationship between neural activity and behavioral performance in visual tasks. Opto-genetics can provide powerful methods for testing specific hypotheses about this relationship. Recently, we began developing a bi-directional optical-genetic toolkit that allows simultaneous measurement and modulation of neural population activity in the cortex of behaving macaque monkeys. Specifically, we use viral-based methods to co-express, in the same population of excitatory V1 neurons, a calcium indicator (GCaMP6f) and a red-shifted opsin (C1V1). Wide-field imaging is then used to measure the GCaMP activity produced both by normal visual stimulation and/or by patterns of light projected onto the visual cortex (optostim). Our long-term goal is to use this toolkit to test specific hypotheses about the encoding and decoding of neural responses in macaque V1 by using optostim to modulate neural activity while monkeys perform specific visual discrimination tasks. As a first step, we trained a monkey to detect a small Gaussian visual target positioned at locations corresponding to either GCaMP/C1V1 expression site or a nearby GCaMP-only control site in the monkey’s V1. Our preliminary results revealed strong nonlinear (sub-additive) interactions between visual and optostim-driven neural responses. Furthermore, at the joint expression site, we found that the monkey’s detection accuracy decreased when the visual stimulus was presented together with optostim; whereas, at the control site there was no effect of optostim. This reduced behavioral accuracy at the joint expression site was consistent with the sub-additive interactions between the visually-driven and optostim-driven GCaMP responses. To further examine these nonlinear interactions, we performed additional imaging and electrophysiological experiments in passively fixating monkeys, for a wider range of visual stimuli and optostim power densities. We found: (1) strong multi-unit responses and strong GCaMP responses to optostim; (2) that both measures have a low optostim threshold, i.e., < 0.15 and 0.02 mW/mm^2, respectively; (3) that above threshold, both responses rise linearly with power density until a saturating limit is reached; (4) that normal visual stimulation and optostim nearly always combine in a sub-additive fashion. We are currently testing various quantitative models of these nonlinear interactions.

Poster

307. Visual Cortex: Manipulating and Reading Neural Activity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 307.05/L8

Topic: D.07. Vision

Title: All-optical interrogation of functional connectivity in mouse visual cortex during behaviour

Authors: *L. E. RUSSELL¹, Z. YANG¹, M. FISEK¹, A. M. PACKER², H. W. DALGLEISH¹, M. HAUSSER¹;¹UCL, London, United Kingdom; ²Univ. of Oxford, Oxford, United Kingdom

Abstract: Understanding how the structure of connectivity underlies the processing carried out by cortical circuits is a fundamental problem in neuroscience. Layer 2/3 of mouse visual cortex consists of functionally distinct subnetworks of recurrently connected neurons. Neurons sharing similar stimulus response properties (i.e. cotuned to the same stimuli) preferentially share monosynaptic connections. This specific synaptic connectivity rule may facilitate and maintain robust representations of visual stimuli even under situations when those stimuli are weak or degraded. Here we have trained mice on a visual detection task and used simultaneous two-photon calcium imaging and two-photon optogenetics to ask: 1. How does this pattern of paired connectivity extend to, and influence, activity at the population level in vivo? and 2. How does the functional signature of subnetworks impact upon the neural representation, and ultimately the behavioural salience, of weak or ambiguous stimuli? To address these questions, we performed targeted photostimulation of ensembles of either cotuned, non-cotuned or non-stimulus responsive L2/3 visual cortex neurons and observed the response of the local network as well as the animal’s performance in reporting the current visual stimulus. We find that the dominant effect of photostimulation on the local network is inhibitory, with both enhancement and degradation of the population representation of the sensory stimulus. The resulting behavioural bias follows the sign of modulation to the network response. We also find that the behavioural effect of photostimulation depends on the animals baseline performance in the task suggesting a state-dependent gating of cortical influence in guiding this behaviour. These results provide a bridge between connectomics, sensory stimulus coding and behaviour.

Poster

307. Visual Cortex: Manipulating and Reading Neural Activity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 307.06/L9

Topic: D.07. Vision

Title: Optogenetic stimulation of area V6 improves visual detection in the marmoset

Authors: *P. JENDRITZA*1,2, F. KLEIN1, P. FRIES1,3;
1Ernst Strüngmann Inst. (ESI) For Neurosci., Frankfurt, Germany; 2Intl. Max Planck Res. Sch. for Neural Circuits, Frankfurt, Germany; 3Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ., Nijmegen, Netherlands

Abstract: Understanding communication between brain areas is a fundamental challenge for systems neuroscience. Causal evidence can be obtained by targeted manipulation of neuronal activity. Therefore, we developed an approach to simultaneously record from visual areas V1 and V6, while optogenetically stimulating V6 during a visual detection task in the marmoset. We were able to monitor and manipulate neuronal activity and show that the animal’s behavior is influenced by the optogenetic stimulation. To achieve this, we implanted a 3D-printed titanium chamber that houses multiple high-density silicon probes with a total of 192 channels. To stimulate excitatory neurons with high temporal precision, we injected an adeno-associated viral vector into V6 in order to express the fast channelrhodopsin variant ‘chronos’ under the control of the CaMKIIα promoter. Recordings of single units and LFPs as well as optogenetic stimulation were reliable for several months. We were able to accurately map receptive fields in both areas and show that their location matches well the previously described retinotopic organisation of the marmoset visual cortex. Interestingly, visual stimulation induced prominent gamma oscillations in V1, reminiscent of those observed in cats, macaques and human subjects. To investigate the impact of V6 optogenetic stimulation on behavior, the marmoset was trained to perform a detection task. After maintaining gaze to a central fixation spot, the animal was rewarded for a) detecting a visual target, b) detecting an optogenetic stimulus, c) detecting a combination of visual target and optogenetic stimulus, or d) maintaining fixation in the absence of any stimulus. Our results show that optogenetic stimulation significantly increased detection performance for low contrast visual targets. Remarkably, the marmoset was also able to detect optogenetic stimulation of V6 in the absence of a visual target. Furthermore, preliminary results indicate that these effects are dependent on the frequency of stimulation. In conclusion, the combination of recordings in two connected areas, optogenetic manipulation, and behavioral report, enables us to identify causal factors that influence inter-areal communication.

Disclosures: P. Jendritza: None. F. Klein: None. P. Fries: None.
Poster

**307. Visual Cortex: Manipulating and Reading Neural Activity**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 307.07/L10

**Topic:** D.07. Vision

**Support:**
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- KTIA_NAP_12-2-2015-0006
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- VISGEN_734862
- NVKP_16-1-2016-0043
- 2017-1.2.1-NKP-2017-00001

**Title:** Stable, multi-day, functional measurement to study cellular level plasticity in mouse primary visual cortex by fast 3-dimensional imaging

**Authors:** *A. PLAUZKA*¹, M. MAROSI¹, G. SZALAY¹, K. OCSAI¹, D. PINKE¹, T. TOMPA¹, D. NAGY¹, C. CSUPERNYÁK¹, A. BOJDAN¹, A. SZEPESI¹, G. KATONA¹, B. ROZSA¹;¹\² IEM-HAS, Budapest, Hungary; \²Pázmány Péter Catholic Univ., Budapest, Hungary

**Abstract:** We investigated how 3D representation of the visual information, which is perceived and understood by the behaving animals in the primary visual cortex (V1), is changed while the animal engaged in a visual task. To reach this goal we use in vivo two-photon acusto-optic microscopy (with AAV-Syn-GCaMP6s or AAV-Syn-jRGECO1a) to record cellular responses (through different cortical layers) to visual stimuli in V1 before (baseline) and after visual training (effect). During training period, the mice learn to discriminate visual landmarks in a virtual reality (VR).

To study cellular plasticity in time we had to measure the same neuronal ensembles (up to 200 cells) during the baseline and effect period which can be apart in time (10-20 days). As neuronal responses are very sensitive to the spatial inconsistency of recording coordinates, therefore, orientation tuning and other properties of the recorded visually evoked responses could be contaminated with recording artifacts. To resolve this critical issue, we used 3D AO drift scanning microscopy, which can extend each scanning point to small 3D line-, surface- or volume-elements. With Multi-cube scanning, we are able scan small volumes (40x40x40 µm) around each cell bodies in 3D, and use this mini-volume information to carefully and precisely realign all individual scanning regions at each measurement day, significantly reducing the chance of misalignments of recording coordinates. AO scanning (ie. Chessboard scanning) of hundreds of cells at cortical depths up to 1 mm makes it feasible to examine the effect of learning in behavior experiments both at single-cell and at network scales.
We found that, in contrast to previous theories, adult mice brain is plastic as visual representation can dynamically change as a function of time at multiple temporal scales following visual learning in individual neurons.


Poster

307. Visual Cortex: Manipulating and Reading Neural Activity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 307.08/L11

Topic: H.01. Animal Cognition and Behavior

Support: MH100318

Title: The effect of optogenetic stimulation of basolateral amygdala on declarative memory in rats

Authors: *D. S. REIS¹, L. E. DIFAZIO¹, N. S. AHLGRIM², J. R. MANNS¹;
¹Psychology, ²Grad. Program in Neurosci., Emory Univ., Atlanta, GA

Abstract: Electrical or pharmacological activation of the basolateral complex of the amygdala (BLA) can enhance the consolidation of declarative memory through modulation of hippocampal activity in rodents, non-human primates, and humans. However, the specificity and precise mechanisms underlying these effects remain relatively unclear. In particular, we were interested in whether optogenetic stimulation restricted to putative glutamatergic BLA principal neurons would also facilitate memory prioritization for non-emotional declarative memories. A recent study showed that optogenetic stimulation of putative glutamatergic BLA principal neurons elicited theta-modulated gamma activity in the hippocampus, an oscillatory pattern previously associated with good memory. Here, we used the same approach to optogenetically stimulate the BLA in rats following correct responses in an object-context association task. In a subsequent novel object recognition task, we stimulated the BLA following object exploration and tested memory retention the following day. We hypothesized that optogenetic activation of glutamatergic neurons in the BLA would facilitate performance during the object-context association task and in the novel object recognition task. Our results supported the role of the BLA in facilitating the prioritization of neutral, declarative memories and demonstrated the effects of glutamatergic activation of the BLA on hippocampal-dependent memory tasks.

Disclosures: D.S. Reis: None. L.E. DiFazio: None. N.S. Ahlgrim: None. J.R. Manns: None.
Poster

307. Visual Cortex: Manipulating and Reading Neural Activity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 307.09/L12

Topic: D.07. Vision

Support: NCS-FO: A Computational Theory to Model the Neurobiological Basis of a Visuo-Cognitive Neuroprosthetic
“la Caixa” Foundation Fellowship # LCF/BQ/AA17/11610017

Title: Ultra-large field-of-view optogenetic stimulation and two-photon imaging of the foveal region of area V1 in non-human primates

1SUNY Downstate Med. Ctr., Brooklyn, NY; 2Bioengineering and Electrical Engin., Univ. of Washington, Seattle, WA; 3Cornell Univ., Ithaca, NY; 4Salk Inst., La Jolla, CA; 5Univ. of Texas at Austin, Austin, TX; 6Yale Univ., New Haven, CT; 7Peking Univ., Beijing, China; 8The Rockefeller Univ., New York, NY

Abstract: In the past decade, novel microscopy methods and genetically-encoded sensors have been revolutionary to understanding neural circuits in rodents and invertebrates. Using these methods in non-human primates (NHPs) has proven difficult due to technical hurdles that are now being overcome. NHP advances have brought the technology closer to use in humans, such as with high-quality cortical imaging windows that are both larger than an entire mouse brain—allowing for unprecedented ultra-large-scale circuit analyses over periods of years (longer than a mouse’s entire lifespan). This provides for the possibility of long-term all-optical interrogation of ultra-large neural circuits over a time period relevant to human cognitive development. Here we present a multicolor labeling of neurons in macaque area V1. We infected V1 pyramidal neurons—using cortical and thalamic convection-enhanced delivery (CED)—with adeno-associated viruses (AAVs) encoding 5 different fluorophores with different emission spectra (mTagBFP2, mCerulean, eGFP, SYFP2, and Requorin). We also infected V1 cells with a GCaMP6f/GCaMP7 transgene for calcium imaging in the same injections. We further infected ipsilateral LGN neurons with either ChR2 optogen or with the red-shifted opsin Crimson, to enable optogenetic activation of LGN inputs into V1. We imaged cells with a Bruker Ultima IV two-photon imaging microscope driven by a MaiTai DeepSee Ti:Saph laser at a wavelength of 830 nm. We captured an entire 2-cm diameter imaging window (3.14 cm² area) by tiling the window with 64 (8 x 8) 500 µm deep z-stack scans (each stack 2.8mm on a side). We stitched...
the z-stacks into a single image to visualize the whole ultra-large imaging window and performed cell counts. We quantified the color-combinations achieved and determined that our methods result in a Brainbow-like distribution of ~5,000 colors, allowing for single-cell imaging even within densely labeled areas. Optogenetic stimulation of LGN inputs into V1 was achieved, resulting in strong Ca-imaging responses from GCaMP indicators in V1 cells.


Poster

307. Visual Cortex: Manipulating and Reading Neural Activity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 307.10/L13

Topic: D.07. Vision

Support: INC NIMH T32 Fellowship

Title: Optogenetic activation of GABAergic neurons in primate V1 impairs detection performance through indirect effects on excitatory neurons

Authors: *V. E. SCERRA1, M. AVERY2, J. DIMIDSCHSTEIN3, G. J. FISHELL4, J. H. REYNOLDS1;

Abstract: Accurate stimulus detection is guided by the veridical transmission of information (signal) amongst unrelated externally- and internally-generated activity (noise). When visual detection performance suffers, is this because noise is insurmountable, or because the signal itself is attenuated somewhere along the visuomotor pathway? To address this question, we used an AAV-Dlx5/6 viral construct (Dimidschstein et al., 2016) to drive expression of an excitatory opsin, C1V1 in GABAergic interneurons in macaque primary visual cortex. We then activated interneurons via laser stimulation delivered through an optically clear artificial dura (Nassi et al., 2015) as monkeys performed a simple target-detection task. Targets were stationary Gabor patches of varying luminance contrast, of the preferred orientation of neurons at the opsin site. Responses of single-units and multi-units were recorded, and laser was randomly and unpredictably paired with the target, enabling us to examine neuronal responses and behavioral performance with and without activation of inhibitory interneurons. Behavioral performance was
impaired on laser trials, on which there were fewer correct detections, longer reaction times, and decreased perceptual sensitivity (d'), particularly for stimuli with contrasts near perceptual threshold. Nandy et al (2019) found that optogenetic activation of pyramidal neurons impairs perception, suggesting one possible interpretation of the present behavioral results: that the laser-induced increase in local interneuron firing rates act similarly, as a source of background noise, which obscures the target-evoked response, thereby impairing target detection. Alternatively, optogenetic activation of interneurons may serve to suppress and/or delay the stimulus-evoked signals that are conveyed to higher order visual areas via pyramidal projection neurons. Our neural data militate in favor of the latter hypothesis. While some neurons (putative inhibitory interneurons) were facilitated by the laser, a greater proportion of recorded neurons were suppressed by optogenetic activation. Further, in cells suppressed by the laser, trials in which a target was missed were trials in which firing rates were lower, on average, than for hits – an effect that was amplified by the laser, both in terms of firing rate, and the likelihood of missing the target. These results indicate that increased inhibition in primary visual areas impairs perceptual performance, at least in part, via suppression of the target-responsive signal conducted by pyramidal neurons.


Poster

307. Visual Cortex: Manipulating and Reading Neural Activity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 307.11/DP07/L14

ControlExtraData.DynamicPosterDisplay: Dynamic Poster

Topic: D.07. Vision

Support: Wellcome Trust grant 200501/Z/16/A
Wellcome Trust grant 200501/Z/16
BBSRC, UK: BB/R004765/1
HFSP: RGY0076/2018

Title: BonVision - An open-source software to create and control visual environments

Authors: G. LOPES¹, K. FARRELL², E. A. B. HORROCKS², T. MUZZU², M. M. MORIMOTO², A. PAPANIKOLAOU², T. WHEATCROFT², S. ZUCCA², S. G. SOLOMON²,
*A. B. SALEEM²;
¹NeuroGEARS Ltd., London, United Kingdom; ²Exptl. Psychology, Univ. Col. London, London, United Kingdom
**Abstract:** Real-time rendering of closed-loop visual environments is necessary for the next generation of experiments to understand brain function and behaviour. Currently, this is prohibitively difficult to implement for non-experts and limited to few laboratories world-wide.

We have developed a novel open-source software package, BonVision, to generate and control visual environments.

Current software is limited to either studying ‘classic’ visual stimuli with exquisite control of specific features, or exploiting game development software to create virtual reality environments. BonVision provides a single framework for classical visual stimulus presentation, and virtual or augmented reality. We achieved this by defining visual environments in an ego-centric framework, rendering scenes onto a sphere, and by treating display devices as windows looking onto that sphere. Visual environments can therefore be specified in terms of visual angle (enabling classical visual stimuli) or real-world units (enabling 3D virtual worlds). BonVision is based on reactive programming and leverages the Bonsai visual programming framework, which allows native integration with experimental hardware, including electrophysiology recording systems, cameras (e.g. eye tracking, Ca²⁺ imaging), and standard data acquisition hardware (e.g. Arduino, NI). This allows BonVision experiments to gather data from sensors such as rotary encoders, lick ports, or infrared sensors; control effectors such as water valves or motors; and update visual stimuli.

Using visual environments created and controlled by BonVision, we have successfully recorded responses of neurons in primary visual cortex of awake mouse and recovered standard tuning curves. We have also implemented 2D and 3D stimuli to investigate visually-driven behaviors: two-alternative forced choice experiments; and loom or sweep stimuli that drive escape behaviors. In addition, we have established online learning tools (bonvision.github.io) for building new stimuli, allowing easy control and replication of experiments.

BonVision enables easy implementation of closed-loop experiments and communication with behaviour and physiology measurement devices, while being open-source, easy to install, and able to run on standard windows computers including laptops.


**Poster**

**307. Visual Cortex: Manipulating and Reading Neural Activity**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 307.12/L15

**Topic:** D.07. Vision

**Title:** Large scale characterization of a visual change detection task using a high throughput two-photon calcium imaging *in vivo* pipeline
Abstract: The Allen Brain Observatory Visual Coding dataset contains the activity of more than 63,000 neurons recorded using two-photon calcium imaging across 13 transgenic mouse lines. This important dataset surveyed the visually evoked response of cortical neurons in passive viewing conditions and established a standardized database of cell metrics across all cortical layers and cell classes. Standard models of the visual cortex can be tested using this dataset (de Vries et al., 2018), however it is now established that behavior variables play a big role in neuronal activity even early in the visual pathway (Niell & Stryker, 2008). In addition, our initial survey revealed that patterns of neural activity showcase a high degree of variability across animals.

To understand this neuronal activity in the context of a behavior task, we have built and validated a visual behavior data pipeline that leverages a change detection task at scale. Using this data we can investigate differences in cellular response to a passively viewed stimulus vs. an actively attended stimulus, and see how neuronal population activity changes in response to generalization of the task to a new set of images. Here we introduce all data collected in this pipeline and discuss its challenges in the context of neurophysiological recordings. We present our protocol to optimize behavioral performance during training and rig transition, our quantification of behavior performance, and overall change in neuronal activity during training. In addition, we demonstrate the impact of variability in behavioral performance on the activity of neurons, and showcase how our data collection pipeline will allow us to capture the variability in future data released on the Allen Institute data portal.

Title: A large-scale, standardized survey of spiking activity across the mouse visual system


Abstract: The mammalian visual system is one of the most widely studied sensory systems, yet much about the function of individual visual areas and the mechanisms by which they interact remains unknown. As an initial step toward a more systematic interrogation of visually evoked spiking activity, we have extended the Allen Brain Observatory to include a standardized platform for extracellular electrophysiology in head-fixed mice. Each mouse proceeds through the same series of steps, carried out by highly trained technicians and scientists according to a set of standard operating procedures. On the day of the experiment, we use visual area maps obtained via intrinsic signal imaging to simultaneously target Neuropixels probes to all layers of primary visual cortex and 5 higher visual areas (LM, AL, RL, PM, AM). Probes are inserted up to 3.5 mm into the brain, allowing us to obtain concurrent recordings of single-unit activity from thalamic and midbrain visual areas. Each experiment begins with a receptive field mapping stimulus consisting of localized drifting gratings, followed by a series of full-field flashes. Next, we present one of three stimulus sets: a battery of natural and artificial stimuli, shown with the same parameters as those from the Allen Brain Observatory two-photon imaging rigs; many repeats of drifting gratings and natural movies; or 162,000 unique frames from nature documentary clips (plus an 18,000-frame test set). Recordings have been carried out in C57BL6/J mice and 3 transgenic lines (Pvalb-IRES-Cre x Ai32, Sst-IRES-Cre x AI32, and Vip-IRES-Cre x Ai32), to facilitate the identification of genetically defined cell types via optotagging. Each dataset passes through a rigorous quality control procedure, to ensure that data quality is consistent across experiments. Units extracted by Kilosort are mapped to structures in the Allen Common Coordinate Framework by imaging fluorescently labeled probe tracks with optical projection tomography. The final data files will be distributed in Neurodata Without Borders 2.0 format, with all sessions passing QC made publicly available via the AllenSDK and brain-map.org. This comprehensive, open dataset will make it possible to characterize spiking activity across the mouse visual system with an unprecedented level of detail.

**Poster**

**307. Visual Cortex: Manipulating and Reading Neural Activity**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 307.15/L18

**Topic:** D.07. Vision

**Title:** Adventures in motion correcting high noise multiphoton microscopy data

**Authors:** *J. M. GALBRAITH, L. KUAN, L. NG, J. WATERS, D. MILLMAN, J. LECOQ; Technol., The Allen Inst. For Brain Sci., Seattle, WA

**Abstract:** Novel multiphoton applications are often constrained by the noise robustness of data processing methods, including motion correction. Phase correlation is a popular frequency domain motion correction algorithm, and offers excellent rigid translation performance on multiphoton data of sufficient signal to noise ratio (SNR). Here, we describe a novel method for computing motion correction on data of low SNR, and applications of deep and multiplane imaging. These noisy data produce very high erroneous maxima in the phase cross-correlation matrix, and simply choosing the absolute maximum fails to compute the correct offsets in these cases. We demonstrate that biasing the cross-correlation matrix with a Kalman prediction of the motion offset offers excellent noise rejection performance in these situations where the correct peak is not the maximum peak.

**Disclosures:** J.M. Galbraith: None. L. Kuan: None. L. Ng: None. J. Waters: None. D. Millman: None. J. LeCoq: None.

**Poster**

**307. Visual Cortex: Manipulating and Reading Neural Activity**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 307.14/L17

**Topic:** D.07. Vision

**Title:** Neuropixels recordings across cortical visual hierarchy during change detection in mice

**Authors:** C. BENNETT, S. GALE, C. NAYAN, J. SWAPP, S. LAMBERT, A. CHO, J. SIEGLE, P. GROBLEWSKI, *S. R. OLSEN; Allen Inst. For Brain Sci., Seattle, WA
Abstract: Behavior and perception require coordinated activity across distributed neural circuits. Here we used Neuropixels probes to record simultaneously from multiple brain regions across the visual hierarchy in mice during active visual behavior. We trained mice on a change detection task in which natural images were continuously flashed and mice were rewarded for licking when the image identity changed. Neural activity was recorded in six visual cortical areas (V1, LM, AL, RL, PM, AM) while mice actively performed the task or were passively presented with the same sequence of images experienced during task performance. Detection of change (hit trials) was associated with an enhanced response to the image flash relative to miss trials or to image change during passive viewing. This modulation arose concurrently in V1 and higher order areas beginning ~10-20 ms after visual response onset in V1 and increased in magnitude along the visual hierarchy. Rapid modulation of visual responses to detected image change may reflect recurrent interactions between visual cortical areas involved in detection or a common feedback signal from other areas.


Poster

307. Visual Cortex: Manipulating and Reading Neural Activity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: 307.16/L19

Topic: D.07. Vision

Support: Paul Allen

Title: Comparing spike sorting quality metrics for high-density electrophysiological recordings

Authors: *G. R. Heller¹, J. H. Siegle¹, X. Jia², D. Denman¹, S. Olsen¹;
¹Neural Coding, ²The Allen Inst., Seattle, WA

Abstract: Recently developed silicon probes with active CMOS electronics, such as Neuropixels, can simultaneously sample in vivo extracellular spike waveforms across more channels than was previously possible. This higher sampling density provides more information about individual spikes, making it easier to discriminate waveforms produced by nearby neurons. However, there is still substantial variation between the quality of units recorded in the same experiment, and the field lacks a widely accepted standard for determining which units are sufficiently well isolated to include for further analysis. In this study, we calculated a suite of quality metrics for all of the units recorded in the Allen Institute Brain Observatory, which were acquired on Neuropixels probes and sorted using Kilosort. The metrics analyzed include ISI violations, nearest-neighbors contamination rate (Chung et al., 2017), Fisher’s LDA (Hill et al., 2011), isolation distance and L-ratio (Schmitzer-Torbert et al., 2005). An aggregate metric using
PCA-based dimensionality reduction of the metric space was able to explain 50% of the variance across metrics, meaning the individual metrics are somewhat redundant, but still contain unique information. We therefore examined the correlation between metrics in greater detail, as well as their relationship to subjective measures of unit quality, obtained from ratings made by trained experts. We also sought to estimate how the variability of cluster metrics (and their computation time) changes as a function of the number of spikes included. We assessed how parameters such as the percentage of spikes sampled and the size of the feature space affect the accuracy and reliability of the outcome. Finally, we applied the same quality metrics to simulated ground truth data to determine how well they track hit rate and false positive rate when these values are known.


Poster

307. Visual Cortex: Manipulating and Reading Neural Activity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 307.17/L20

Topic: D.07. Vision

Title: Tracking single cell response properties across days during performance of an image change detection task

Authors: *K. ROLL¹, M. GARRETT¹, F. LONG², L. KUAN¹, R. S. LARSEN³, N. D. PONVERT², J. LECOQ⁴, J. M. GALBRAITH⁵, S. R. OLSEN²;

Abstract: One of the unique advantages of two-photon calcium imaging is the ability to track individual neurons across multiple days to observe how cellular response properties change with experience and behavior. However, standardized tools for reliably identifying the same cells across days are lacking in the field. Here we describe the validation of a novel cell matching algorithm and its application to studying changes in response properties across days in the mouse visual cortex during performance of a behavioral task. In these experiments, the same population of cells is targeted across 7 two-photon imaging sessions. During each experiment session, mice perform a change detection task with natural images where they must lick in response to changes in stimulus identity to earn a reward. In addition to observing the behaviorally relevant stimuli, which can vary across days, mice are presented with a natural movie stimulus that is the same in every session. This repeated stimulus is used to compute cell response metrics that describe the reliability of activity patterns across days. The cell matching algorithm first creates a common coordinate space across the 7 sessions through affine registration. It then matches cells between
all the possible combinations of pairwise sessions using bipartite graph matching with degree of cell overlap and centroid distance as metrics in the cost function. Finally matching cells across all the sessions are identified by fusing pairwise matching results and resolving their conflicts. Accurate registration of the two-photon field of view from day to day is critical to the function of the cell matching algorithm, and relies on precise day to day positioning of head fixed mice under the microscope. We characterized the impact of experimental conditions on the output of the matching algorithm by relating the activity and morphology of matched cells with quantification of day to day registration. We then compared measures of cell morphology across sessions with signatures of neural activity in matched cells to evaluate the relationship between recording stability and neural response variability. Finally, we use these relationships to identify quantitative metrics that can be used to predict the quality of cell identification across sessions. These results have important implications for the interpretability of measurements of single cell response properties measured across multiple days with two-photon calcium imaging, and provide tools for validation of cell matching across sessions.


**Poster**

**307. Visual Cortex: Manipulating and Reading Neural Activity**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 307.18/L21

**Topic:** D.07. Vision

**Support:** ERC grant 682426 - VISIONby3DSTIM
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**Title:** Two-photon microscope compatible immersive virtual reality system for mice

**Authors:** *D. PINKE*¹, G. DOBOS¹, M. MAROSI¹, B. GULYÁS², B. ROZSA¹;
¹Inst.of Exptl. Med., Budapest, Hungary; ²Pázmány Péter Catholic Univ., Budapest, Hungary

**Abstract:** Introduction: The understanding of plasticity in learning requires methods that allow neuronal activity to be recorded at large and stable cell population level. However, long visual discrimination and learning protocols raise difficulties in maintaining imaging stability and performing sufficient experiments.

Aims: Our objective was to develop a behavior protocol that is rapid and robust enough to investigate cortical plasticity effectively. Designing a system that allows for a short training period is important as it helps limit other sources of change, such as the instability of surgery techniques, cell structural changes and tissue deformations. We hypothesize that learning speed is highly correlated with the immersion of the virtual reality used, which arises from perspective
correction, large field of view and binocular vision. During the development process of our own system we focused on improving these attributes.

**Methods:** We demonstrate a novel, two-photon microscope compatible virtual reality system, that covers approximately 260° degree of the mouse visual field, and provides perspective-corrected 3D environment with binocular vision. Also, we implemented a 3D imaging technique that allows the measurement of multiple cell assemblies during behavior, and tracking the activity of several hundreds of cells during the experiment.

**Results:** Our preliminary data show stable and significant learning in 3-5 day with this device when associating some of the visual clues with a conditioning stimulus. During the protocol we used a 3D two-photon laser scanning microscope which was developed in our lab, to investigate single-cell level change of the neuronal responses associated with learning.

**Conclusions:** Here we present a 3D two-photon microscope compatible virtual reality system and training protocol, that allows us to investigate visual cortex plasticity at a relatively short timescale, compared to currently long-term behavior visual discrimination protocols. Grant: EFOP-3.6.3-VEKOP-16-2017-00009

**Disclosures:** D. Pinke: None. G. Dobos: None. M. Marosi: None. B. Gulyás: None. B. Rozsa: None.

**Poster**

**307. Visual Cortex: Manipulating and Reading Neural Activity**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 307.19/L22

**Topic:** D.07. Vision

**Support:** ISF

**Title:** Population responses of backward masking in V1 of behaving monkeys

**Authors:** H. EDELMAN KLAPPER, H. SLOVIN;
Multidisciplinary Gonda Brain Res. Ctr., Bar Ilan Univ., Ramat Gan, Israel

**Abstract:** Visual backward masking (BM) is a powerful behavioral paradigm where the visibility of a brief stimulus can be dramatically reduced when followed by a second stimulus, the mask. Behavioral studies showed that the time interval between stimulus onset and mask appearance (stimulus-to-mask onset asynchrony; SOA) affects the stimulus detection rate: as the SOA becomes shorter, the stimulus visibility is reduced. Despite past research, the neuronal mechanisms underlying BM in the visual cortex are mostly unknown. To investigate this two monkeys were trained on a texture discrimination task (TDT) and were required to discriminate between vertical and horizontal targets embedded within a homogeneous patterned background. We used voltage-sensitive-dye imaging to measure population responses at high spatial (meso-
scale) and temporal resolution in the primary visual cortex (V1) while the monkeys performed TDT with BM at variable SOAs. As was previously reported, target discrimination was higher for longer SOAs and reaction times (RTs) were shorter. Population responses showed two separate temporal phases at long SOAs: an early phase that was evoked by the stimulus, and a second delayed response which was related to the mask appearance. When decreasing the SOA, the mask response shifted in time towards the stimulus evoked response, until they merged. We computed the figure-ground modulation (FG-m), i.e., the population response in the target versus the background elements sites and found a positive FG-m when the animal was performing TDT alone or TDT combined with BM. For long SOAs, where target discrimination rate was high, we found a positive and significant FG-m that developed around 120 ms after stimulus onset. However, for short SOAs, where target discrimination rate was low and closer to chance level, no significant early FG-m was found, suggesting that the mask interfered with this process. Interestingly, in short SOAs trials where RTs were longer, we found a significant positive FG-m that developed at late times. Finally, the mask influence on neuronal population responses at the target and background sites was different and depended also on the SOAs. Our results shed new light on the neuronal mechanisms underlying BM and reveal different impact of the mask on target and background regions in the visual cortex.

**Disclosures:**  
**H. Edelman Klapper:** None.  
**H. Slovin:** None.

**Poster**

**308. Visual Cortex: Plasticity**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 308.01/L23

**Topic:** D.07. Vision

**Support:** DFG SFB 889 (B03 to OS and B05 to SL)

**Title:** Increased spine dynamics in the visual cortex of PSD-95 knockout mice: Chronic two-photon imaging of neuronal morphology in the awake brain

**Authors:** A. TIPPMANN\textsuperscript{1,2}, B. JOACHIMSTHALER\textsuperscript{3}, S. TREUE\textsuperscript{2,4}, C. SCHWARZ\textsuperscript{3}, O. SCHLÜTER\textsuperscript{5,6,7}, \textbf{*S. LÖWEL}\textsuperscript{1,4,7};

\textsuperscript{1}Dept. of Systems Neurosci., Univ. of Goettingen, Goettingen, Germany; \textsuperscript{2}Cognitive Neurosci. Lab., German Primate Ctr., Goettingen, Germany; \textsuperscript{3}CIN Systems Neurophysiol., Univ. of Tuebingen, Tuebingen, Germany; \textsuperscript{4}Collaborative Res. Ctr. 889, Goettingen, Germany; \textsuperscript{5}Dept. of Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; \textsuperscript{6}Dept. of Psychiatry and Psychotherapy, Univ. Med. Ctr., Goettingen, Germany; \textsuperscript{7}Campus Inst. for Dynamics of Biol. Networks, Goettingen, Germany
Abstract: The postsynaptic signaling scaffold PSD-95 is present in excitatory synapses in the brain and hypothesized to influence their function and stability. We previously showed that in primary visual cortex (V1), adult PSD-95 knockout (KO) mice have 9 times more AMPA-silent synapses than wildtype littermates (WT), retain a lifelong and juvenile-like ocular dominance plasticity and experience-induced network changes happen faster compared to WT mice (Huang et al 2015). Thus, PSD-95 KO mice display enhanced cortical plasticity, but the neuronal circuits are less stable, suggesting that dendritic spines may be more dynamic in V1 of the KOs. To test this hypothesis, we chronically imaged spine dynamics of V1-neurons in awake head-fixed PSD-95 KO and WT mice (>P145) using two-photon microscopy through a cranial window (Joachimsthaler et al 2015). Nerve cell morphology was visualized with LifeAct-GFP via AAV-injections into V1; LifeAct labels the F-actin of neurons, which is the major cytoskeletal component of dendritic spines (Hotulainen & Hoogenraad 2010). Mice were thoroughly habituated to the head fixation under the two-photon microscope for at least 3 weeks. We repeatedly imaged the same dendrites and spines of layers 2/3 and 5 pyramidal neurons over nine consecutive days and recorded changes of dendritic spine numbers and dynamics (gained/lost spines). Our data show that PSD-95 KO mice have a higher spine turnover rate, reduced numbers of stable spines, and higher numbers of both eliminated and new spines compared to PSD-95 WT mice. Notably turnover rates of PSD-95 WT mice were similar to values previously published for visual cortex of anesthetized mice (Holtmaat et al 2005). These results support the hypothesis that the postsynaptic signaling scaffold PSD-95 is critical for stable neuronal networks in mouse cortex.


Poster

308. Visual Cortex: Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 308.02/L24

Topic: D.07. Vision

Support: NSERC

Title: Does ketamine modify the neuronal network of the rodent visual cortex?

Authors: *A. OUELHAZI1, R. LUSSIEZ2, S. MOLOTCHNIKOFF3;
1Univ. De Montreal, Montreal, QC, Canada; 2Univ. of Montreal, Montreal, QC, Canada; 3Sci. Biologiques, Univ. de Montreal, Montreal, QC, Canada
Abstract: Studies have shown that the brain reorganizes itself in response to changing stimulation and this capability continues into adulthood. The mechanisms of brain reorganization in response to varying experiences are still largely unknown. Our goal was to investigate the physiological mechanisms governing plasticity in the adult primary visual cortex, V1. We stimulated cells in V1 of anesthetized mice with eight drifting gratings. Electrophysiological recordings were made of single neuronal activity in control condition, after adaptation, and following local application of ketamine. The results show that after adaptation, V1 cells change their preferred orientation. Following ketamine application, this new orientation selectivity is not maintained and a new orientation preference emerges (different from the original). This suggests that ketamine abolished the adaptation effect and shortened its duration. The cross-correlogram analyses showed that both adaptation and ketamine affect the organization of the network inducing a new state of equilibrium. In spite of the connectional modifications between recorded cells in all conditions, control, post-adaptation and post-ketamine application, the sum of connection probabilities remained the same under all conditions, which suggests that plasticity doesn’t affect homeostasis. Finally, we show that, unlike after adaptation, ketamine decreases the OSI. We conclude that maintaining the effects of adaptation might require NMDA receptors, and blocking them changes the functional connections between V1 neurons which affects the excitation-inhibition balance and promotes the acquisition of new orientation selectivity.

Disclosures: A. Ouelhazi: None. R. Lussiez: None. S. Molotchnikoff: None.
Abstract: Non-human animal studies have shown that the structural development of the visual cortex critically depends on postnatal visual input. Brain-imaging studies in humans blind from birth have repeatedly reported a higher cortical thickness of early visual areas, which has been linked to a lack of experience dependent developmental synaptic pruning. A preliminary early study in a small group of sight-recovery individuals suggested that the process of experience dependent pruning might occurs during a sensitive phase in early development. Here we studied cortical gray matter thickness in a larger group of individuals treated for bilateral dense congenital (n = 22) with age at surgery spanning a range from 1 month to 18 years. Their data were compared to age matched sighted controls (n = 26) and congenitally permanently blind individuals (n = 10). Magnetic resonance imaging (MRI) was performed at a 1.5 T GE scanner. Cortical reconstruction and statistical analysis of surface-based cortical thickness were conducted with Freesurfer 6.0. We replicated previous findings showing an increased cortical thickness in early visual areas in congenitally blind compared to sighted participants. Individuals with a history of congenital cataracts showed an increased cortical thickness in the left primary visual cortex and additionally in a cluster in the left fusiform gyrus compared to sighted participants. We did not observe a correlation with age of surgery. These findings emphasize the critical role of visual experience for the structural development of the human cortex.


Poster

308. Visual Cortex: Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 308.04/L26

Topic: D.07. Vision

Support: NIH
Knights Templar Eye Foundation

Title: Lynx1 sharpens orientation tuning of visual cortical neurons by dampening excitatory drive and intrinsic excitability

Authors: D. KATO1,2,3, K. YAMAMURO1,2,3, *Y. GARKUN1,2,3, M. SADAHIRO1,2,3, M. SAJO1,2,3, G. C. R. ELLIS-DAVIES2, H. MORISHITA1,2,3;
1Psychiatry, 2Neurosci., 3Ophthalmology, Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Orientation selectivity is one of the hallmark functional properties of visual cortical neurons that undergo protracted postnatal maturation. While cortical inhibition is implicated for early postnatal sharpening of orientation selectivity, little is known about the mechanisms regulating the later phase of its maturation. We examined the contribution of Lynx1, an
endogenous nicotinic signaling modulator that increases in expression following adolescence, to orientation selectivity. Using in vivo two photon Ca\(^{2+}\) imaging, we found that layer 2/3 (L2/3) primary visual cortex (V1) neurons in adult Lynx1 knock-out (KO) mice have reduced orientation tuning driven by excessive responses to non-preferred visual stimuli. Whole-cell patch-clamp slice recordings from L2/3 pyramidal neurons of adult Lynx1 KO mice revealed increased excitatory but unchanged inhibitory drive as well as increased intrinsic excitability. Furthermore, conditional knock-out of Lynx1 selectively in the adult V1 neurons after maturation of cortical circuitry was sufficient to reduce orientation tuning, indicating that the functional properties of the adult V1 are actively regulated by the presence of Lynx1. Our study implicates Lynx1 as a key regulator that sharpens orientation tuning at the late phase of maturation by dampening excessive excitatory inputs and intrinsic excitability.


**Poster**

**308. Visual Cortex: Plasticity**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 308.05/L27

**Topic:** D.07. Vision

**Support:** U19 NS107464 (NIH/BRAIN/NINDS) NIMH Intramural Program

**Title:** Changes in cortical responses caused by learning novel optogenetic stimuli

**Authors:** *P. K. LAFOSSE\(^1\), S. P. DUFFY\(^2\), L. N. RYAN\(^3\), A. J. LI\(^2\), M. H. HISTED\(^4\);
\(^1\)NIMH, \(^2\)Natl. Inst. of Mental Hlth., NIH, Bethesda, MD; \(^3\)Neurosci., New York Univ., New York, NY; \(^4\)Natl. Inst. of Mental Hlth., NIH / NIMH, Bethesda, MD

**Abstract:** Repeated experience with a sensory stimulus can cause perceptual learning (Dosher and Lu, 2017), with cerebral cortical responses changing as behavioral improvement occurs. However, since sensory stimuli change the activity of neurons in many different brain areas, it has been unclear whether learning-related changes originate in the cortex. Here, to determine whether learning-related changes happen directly in local cortical circuits or if all changes occur in downstream areas, we take advantage of the fact animals can learn to base their behavior on non-natural (“off-manifold”) activity patterns evoked by direct stimulation. We trained mice to detect and report the presence of neural activity evoked by optogenetic stimulation (ChrimsonR in excitatory neurons of primary visual cortex, V1), and found large behavioral improvements as animals learned to detect this stimulus. Animals were first trained to detect a visual stimulus of varying contrast. An optogenetic stimulus was then paired with each visual stimulus. When
performance increased for the lowest contrast visual stimulus, the visual stimulus was turned off and animals performed the task based on the optogenetic stimulus alone. As animals gained experience with the optogenetic stimulus presented alone, animals’ reaction times decreased (-15.4±3.8 ms, per training session at fixed stimulation intensity, median±SEM, p<0.01, N=9 mice), and detection performance improved, increasing stimulus sensitivity by over an order of magnitude. We imaged calcium responses in V1 neurons before and after learning (before the optogenetic stimulus is presented and after animals’ detection performance for the optogenetic stimulus improved) and found changes in local cortical activity at the site of stimulation, but not at a distinct, unstimulated site. Optogenetic learning decreased visual selectivity for direction and orientation, but overall neural responses became larger. Repeated stimulation of V1 neurons without training did not induce changes in selectivity or responsivity. These data suggest local cortical circuitry adapts to support learning of an optogenetic stimulus whereby neuronal tuning is degraded, while neural responses are enhanced.


Poster

308. Visual Cortex: Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 308.06/L28

Topic: D.07. Vision

Support: NIH Grant EY12124

Title: Cortical plasticity induced by conversion of synaptic eligibility traces in vivo

Authors: *S. Z. HONG†, A. KIRKWOOD‡;
          †Mind Brain Inst., ‡Johns Hopkins Univ., Baltimore, MD

Abstract: Our brain has the ability to learn about rewarding sensory stimuli through synaptic modifications of the associated circuits (reward-based learning). These changes depend on the sensory stimuli and the associated reward which is typically delayed. This temporal difference creates a conundrum, the so called “distal reward problem”: How does the brain know which synapses are responsible for the reward among those that were active during the waiting period? A theoretical solution is ‘synaptic eligibility traces,’ silent and transient synaptic tags that can be converted into long-term synaptic strength changes by reward-linked neuromodulators. Previously in visual cortical slice preparation, we showed distinct synaptic eligibility traces for long-term potentiation (LTP) and depression (LTD), which are transformed by retrograde norepinephrine and serotonin signals, respectively. In the present work, we show evidence demonstrating the functional role of synaptic eligibility traces in the plasticity of visual responses.
in vivo using whole cell patch clamp recording and optical imaging of the intrinsic cortical signal. First, optogenetic activation of norepinephrine or serotonin projections in a temporally retrograde manner induced rapid and selective potentiation or depression of the associated visual response, respectively. Secondly, interfering with the conversion of synaptic eligibility traces prevented the rapid change of visual response by neuromodulators. Furthermore, we tested these ideas in a more "natural" setting, and found that preventing the conversion of eligibility traces also prevented ocular dominance changes induced by monocular deprivation. These results suggest that the conversion of synaptic eligibility traces by neuromodulators has a functional role in visual cortical plasticity via reward based learning mechanisms.

Disclosures:  S.Z. Hong: None.  A. Kirkwood: None.

Poster

308. Visual Cortex: Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 308.07/L29

Topic: D.07. Vision

Support: NIH Grant T32HL110952
NIH Grant R01EY012124

Title: Circuit-specific changes in the visual cortical excitation/inhibition ratio across the light/dark cycle

2Zanvyl Krieger Mind/Brain Inst., 3Mind Brain Inst., 1Johns Hopkins Univ., Baltimore, MD; 4Shanghai Institute of Organic Chemistry, Chinese Acad. of Sci., Shanghai, China; 5IRCBC, Chinese Acad. of Sci., Shanghai, China

Abstract:  Maintenance of the balance between synaptic excitation and inhibition (E/I balance) within a narrow window is thought to be crucial for cortical processing. However, changes in excitatory synaptic transmission have been reported to occur over the course of the sleep/wake cycle. Therefore, we tested whether compensatory changes in synaptic inhibition occur to maintain a stable E/I balance. We performed whole-cell patch clamp recording in mouse primary visual cortical (V1) slices obtained at different times of day. We found that in layer 2/3 pyramidal cells, spontaneous inhibitory postsynaptic currents (sIPSCs) change over the 24h light/dark cycle in a sleep-dependent manner. However, the direction of this change is the opposite of synaptic excitation, resulting in large fluctuations in the E/I balance. We next examined whether these changes were global or pathway-specific. We evoked postsynaptic responses by stimulating layer 4 (vertical pathway) or layer 2/3 (lateral pathway) and recorded
from pyramidal cells in layer 2/3. Excitatory and inhibitory responses were isolated by holding the cell in voltage clamp configuration at the reversal potential for GABA\textsubscript{A} or AMPA receptors, respectively, and the ratio between excitatory and inhibitory responses was calculated. We found that the E/I ratio fluctuated over the 24h light/dark cycle in the lateral, but not vertical, pathway. We next asked whether these synaptic changes have functional consequences for circuit output. To this end, we stimulated the lateral pathway at a range of intensities to identify the threshold for action potential generation while holding layer 2/3 cells in cell-attached configuration. We then broke the membrane seal and measured AMPA receptor currents in whole-cell voltage clamp configuration while stimulating at action potential threshold. At times of day when the E/I ratio was low, more AMPA receptor current was required to reach action potential threshold than at times of day when the E/I ratio was high. Consistent with this finding, there were no changes observed in intrinsic excitability. These observations demonstrate that 1) the cortical E/I balance is dynamic, 2) excitatory and inhibitory synaptic changes over the 24h day are circuit-specific, and 3) these changes alter circuit output, suggesting that time of day may impact sensory processing.

Disclosures: **M.C.D. Bridi**: None. **F. Zong**: None. **X. Zhang**: None. **D. Severin**: None. **K. He**: None. **A. Kirkwood**: None.

Poster

308. Visual Cortex: Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 308.08/L30

Topic: D.07. Vision

Support: ERC grant # 322742 – iPLASTICITY
Sigrid Jusélius foundation
Doctoral Program Brain&Mind
Bilateral exchange program between Academy of Finland and JSPS (Japan Society for the Promotion of Science)

Title: Optical activation of TrkB receptors in parvalbumin interneurons regulates plasticity modes in cortical networks

Authors: **F. Winkel**\textsuperscript{1}, G. Didio\textsuperscript{1}, M. LLach Pou\textsuperscript{1}, A. Steinzeig\textsuperscript{1}, M. Ryzantseva\textsuperscript{2}, J. Harkki\textsuperscript{1}, J. Englund\textsuperscript{2}, T. Taira\textsuperscript{3}, S. Lauri\textsuperscript{2}, J. Umemori\textsuperscript{1}, E. Castren\textsuperscript{1};
\textsuperscript{1}Neurosci. Ctr., \textsuperscript{2}Mol. and Integrative Biosci. Res. Programme, \textsuperscript{3}Dept. of Vet. Biosci. and Neurosci. Ctr., Univ. of Helsinki, Helsinki, Finland
**Abstract:** Neuronal plasticity is fundamental for our brain’s ability to adapt to changes in the environment but dramatically reduces after critical periods (CP) of early postnatal life. We have shown previously that the antidepressant fluoxetine induces juvenile-like plasticity (iPlasticity) in adulthood when combined with training, such as monocular deprivation during shift in ocular dominance (OD). Using the shift in OD paradigm, we question which networks and neuronal subpopulations are involved in iPlasticity in the visual cortex (VC). TrkB neurotrophin receptors are regulators of plasticity and activated by fluoxetine. They are highly expressed in parvalbumin (PV) interneurons, which seem to negatively regulate CP and OD plasticity in the VC upon maturation. Here, we first show that chronic treatment with fluoxetine reopens OD plasticity in wild-type mice but not in heterozygous PV-specific TrkB knockout mice. Then, to specifically activate TrkB-induced plasticity in PV cells, we used optically inducible TrkB (optoTrkB) constructs expressed only in PV neurons. Blue LED stimulation of optoTrkB expressed in PV neurons in the primary VC induces shift in OD in light-dependent manner and results in increased FosB intensity in optoTrkB expressing PV cells, suggesting the induction of downstream signaling and gene expression. Acute optoTrkB activation in PV cells permits long-term potentiation (LTP) in layer II/III of the VC after TBS stimulation, which is accompanied by rapid decreases in intrinsic excitability of and excitatory drive onto PV cells, resulting in disinhibition of pyramidal cells through decreased activation and output of PV cells, known to be required for OD plasticity. Furthermore, we observed a reduction in the number of perineuronal nets (PNN) surrounding PV cells and a decrease in PV and PNN intensities, suggesting dematuration and a shift towards a plastic PV network configuration state. Our data demonstrate that acute activation of optoTrkB is sufficient to trigger intrinsic changes in PV cells, accompanied by shifts in excitatory/inhibitory transmission to allow VC plasticity. These results show that TrkB activation in PV interneurons is a key regulator in OD plasticity thus offering a novel plastic mechanism for intracortical inhibition in the VC.


**Poster**

**308. Visual Cortex: Plasticity**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 308.09/L31

**Topic:** D.07. Vision

**Support:** NIH Grant R01EY025174  
NIH Grant R01DC014101
Title: Transplanted cells are essential for the induction but not the expression of cortical plasticity

Authors: *M. HOSEINI, B. RAKELA, Q. FLORES-RAMIREZ, A. HASENSTAUB, A. ALVAREZ-BUYLLA, M. STRYKER;
Univ. of California, San Francisco, CA

Abstract: Normal brain development is marked by temporally restricted windows of heightened experience-dependent plasticity known as critical periods (CPs). During a CP in primary visual cortex (V1)—postnatal day 25-35 in mouse—changes in neuronal circuitry facilitate the matching of left eye and right eye receptive fields in V1 binocular neurons. Occlusion of vision of either eye over this time, referred to as monocular deprivation, prevents binocular matching and results in structural and functional changes that reduce neural responses to the deprived eye and increase them to the eye that remains open1.

Local inhibitory interneurons in V1 play a crucial role in opening the CP for ocular dominance (OD) plasticity, which begins ~35 days after these neurons are generated in the medial ganglionic eminence (MGE). Once the CP has closed, V1 circuits are thought to remain stable throughout life. However, transplantation of newly generated parvalbumin or somatostatin interneurons from the MGE of a donor embryo into the V1 of a postnatal host opens a second brief CP ~35 days after transplantation2.

The precise contribution of inhibitory interneurons to OD plasticity is not known. While inhibitory neuron activity is crucial for inducing the CP3, the expression of the plasticity following MD no longer depends on inhibition4. Here, we investigated whether MGE transplants produce a second CP of OD plasticity by stimulating changes in host circuitry or, alternatively, by constructing a separate parallel circuit within the host tissue to inhibit deprived-eye responses and/or disinhibit non-deprived-eye responses. We transplanted inhibitory interneurons expressing either archaerhodopsin-3 or channelrhodopsin-2 that allowed us to silence or activate the transplanted interneurons after plasticity is induced by 4-5 days of MD during the second CP. Our results indicate that an OD shift induced by brief MD persists under conditions of either reduced or enhanced transplant-derived inhibition. While the manipulating of the activity of transplanted interneurons after plasticity has been induced alters the ODs of some individual neurons, it does not change the overall OD of the population. Our findings reveal that transplanted interneurons are not responsible for the expression of OD plasticity but rather facilitate its induction in the host circuitry.


Disclosures: M. Hoseini: None. B. Rakela: None. Q. Flores-Ramirez: None. A. Hasenstaub: None. A. Alvarez-Buylla: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder and serves on the Scientific Advisory Board of Neurona Therapeutics. M. Stryker: None.
Poster

308. Visual Cortex: Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 308.10/L32

Topic: D.07. Vision

Support: NSERC Grant RGPIN-2015-06215

Title: Development of immune and inflammatory proteins in human visual cortex: Biological processes and cell types

Authors: *K. ARBABI, E. JEYANESAN, K. M. MURPHY;
McMaster Univ., Hamilton, ON, Canada

Abstract: Many types of immune and inflammatory proteins are expressed in the healthy human brain, where they modulate neural development and function. In addition to their well-known immunological functions, these proteins have been implicated in circuit refinement, synaptic transmission, plasticity and homeostatic synaptic scaling. Furthermore, abnormal levels of inflammatory proteins are characteristic of numerous pathological states, including neuropsychiatric disorders (i.e. bipolar disorder). Despite recent advances, the large majority of immune proteins have not been studied for their presence and function in the brain. Here we studied lifespan changes in expression of a large collection of immune and inflammatory proteins in human visual cortex and characterized the age-related changes in the representation of cell types and biological processes. We quantified expression of immune and inflammatory markers in post-mortem tissue samples from the human visual cortex in cases ranging in age from 20 days to 80 years (n=30, female=12). None of the cases had a neurological or psychiatric disease. We measured the expression of 200 inflammatory proteins using a slide-based quantitative analysis of protein concentration (RayBiotech Quantibody 4000 array), 72 of which were reliably measured. The individual proteins followed varied developmental trajectories. We used Robust Sparse K-Means (RSKC) clustering and PCA (Balsor et al. 2019 https://www.biorxiv.org/content/10.1101/554378v1) to analyze the high-dimensional patterns in these data and found an age-related progression of clusters that we analyzed to determine their content. We used an online transcriptomics database to identify proteins with high fidelity for the four major CNS cell types (microglia, astrocytes, neurons, and oligodendrocytes) and found that proteins with high-fidelity for neurons decreased across the lifespan. In contrast, proteins with high-fidelity for oligodendrocytes were over-represented in older adults, consistent with increased turnover and remodeling of myelin in aging. We used the Gene Ontology (GO) database to identify the biological functions of over-represented proteins in each cluster. The younger clusters were dominated by processes involving inflammatory response, chemotaxis, protein phosphorylation, phosphorous metabolism, and intracellular signalling. The oldest cluster
was dominated by positive regulation of protein metabolic processes but also negative regulation of cell death. These findings describe the changing lifespan landscape of cell-types and biological functions linked with immune and inflammatory proteins in the human cortex.

Disclosures:  K. Arbabi: None. E. Jeyanesan: None. K.M. Murphy: None.

Poster

308. Visual Cortex: Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 308.11/L33

Topic: D.07. Vision

Support: NSERC Grant RGPIN-2015-06215

Title: Development of immune and inflammatory proteins in the human visual cortex: Relationship to glutamatergic and GABAergic proteins

Authors: *E. Jeyanesan, J. L. Balsor, K. Arbabi, K. M. Murphy; McMaster Univ., Hamilton, ON, Canada

Abstract: Recent studies of the human cortex are charting developmental changes for proteins that regulate plasticity, however, less is known about the development of immune and inflammatory proteins. Those proteins are found in the healthy brain where they modulate a range of complex neural processes. Animal studies have shown that specific immune and inflammatory proteins are involved in plasticity, but little is known about their roles in neural development. Here we explore this question by characterizing developmental changes for a large collection of immune and inflammatory proteins in human visual cortex. We compared those patterns with glutamatergic and GABAergic proteins that have well-known plasticity functions and used this information to find candidate age- and plasticity-related processes for the immune and inflammatory proteins. We quantified expression of immune, inflammatory and synaptic proteins in post-mortem samples from the human visual cortex (Age: 20 days to 80 years, n=30, female=12). We measured 200 immune and inflammatory proteins using a slide-based ELISA (RayBiotech Quantibody 4000 array), 72 proteins were reliably quantified. Immunoblotting was used to quantify expression of 23 glutamatergic, GABAergic and other neural proteins. The proteins had a range of trajectories, some increased, others decreased, did not change, or had one or more peaks across the lifespan. We used a data-driven approach to analyzing these data (Balsor et al. 2019 https://www.biorxiv.org/content/10.1101/554378v1). Briefly, Robust and Sparse k-means clustering (RSKC) was used to analyze the immune and synaptic proteins. Next, the median age of samples in each cluster was calculated, and both datasets had a progression of cluster-age from neonates through infants, childhood, teens, young adults to older adults. Currently, we are analyzing the content of the clusters to determine which proteins are over- or
under-represented. We are also using PCA and the protein reweightings from RSKC to identify high-dimensional features in the data that are then combined to construct immune- and synaptic-phenotypes. Finally, we are determining similarities between the patterns of the 72 immune and 23 synaptic proteins by calculating a correlation matrix. These analyses will identify groups of immune proteins that follow the developmental trajectories of well-known plasticity mechanisms and form age-specific clusters that may provide new insights about the role of immune proteins in the functioning of the human cortex.

**Disclosures:** E. Jeyanesan: None. J.L. Balsor: None. K. Arbabi: None. K.M. Murphy: None.

**Poster**

**308. Visual Cortex: Plasticity**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 308.12/L34

**Topic:** D.07. Vision

**Support:** National Institutes of Health (R01 EY019924-08)
Research to Prevent Blindness/Lions Club International Foundation
Deborah Munroe Noonan Memorial Research Fund O’Brien Award

**Title:** Reorganization of functional streams in cerebral compared to ocular visual impairment

**Authors:** *E. S. BAILIN*₁, I. DIEZ², C. M. BAUER³, J. SEPULCRE², L. B. MERABET⁴;

**Abstract:** Background: Ocular blindness is associated with dramatic structural and functional neuroplastic changes in the brain. This includes evidence of functional connectivity changes associated with the functional recruitment of occipital cortical areas for the processing of nonvisual sensory information. However, how these network changes manifest in the setting of perinatal neurological injury such as cerebral/cortical visual impairment (CVI) remain unknown. In this study, we employed a novel graph theory approach of resting state functional connectivity (rsfc) MRI data called Stepwise Functional Connectivity (SFC) to explore the organization of visual processing streams and large-scale functional networks in individuals with CVI compared to ocular visual impaired (OVI) and neurotypical sighted controls. Methods: rsfMRI data was collected using a 3T Philips Achieva scanner with a 7 minute single shot EPI sequence (TE 30 ms, TR 3000 ms) sensitive to blood-oxygen-level-dependent (BOLD) contrast. A cohort of individuals with CVI (n=5), OVI (n=12), and controls (n=26) were instructed to stay awake with their eyes closed and allow their minds to “wander”. Results: In controls, SFC analysis revealed
that the occipital pole was well segregated and the visual cortex's direct connectivity (step 1) followed along the dorsal and ventral medial streams. Subsequent steps revealed that visual cortex connectivity reached frontal areas and major cortical hubs of the brain. OVI individuals showed similar patterns of occipital connectivity however, increased connections within multimodal integration areas such as the superior parietal cortex were observed. In contrast, visual cortex connectivity in CVI subjects did not follow normal connectivity transitions, showing abnormal patterns of connectivity to other primary cortices (including auditory and motor) without a clear reach to cortical hubs. Conclusions: By delineating between how networks segregate and integrate within the brain, SFC analysis provides for more comprehensive characterization of visual connectivity organization and reorganization related to information transfer in cerebral compared to ocular based visual impairment.

Disclosures: E.S. Bailin: None. I. Diez: None. C.M. Bauer: None. J. Sepulcre: None. L.B. Merabet: None.

Poster

308. Visual Cortex: Plasticity

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 308.13/L35

Topic: D.07. Vision

Support: NIH Grant EY021580
NIH Grant EY027407

Title: Layer 4 gates the critical period for visual plasticity through nogo receptor 1

Authors: M. G. FRANTZ, T. IKRAR, G. SOKHADZE, X. XU, *A. W. MCGEE;

Abstract: Here we investigated the regulation and coordination of ocular dominance (OD) plasticity within the laminar circuitry of primary visual cortex. We performed a genetic dissection of the expression requirements for nogo receptor 1 (ngr1), a gene required to close the developmental critical period, for sensitivity to 4-day (d) monocular deprivation (MD). Deleting ngr1 in layer (L) 4, but not L2/3, L5, or L6, sustained critical-period OD plasticity in adult mice. Disinhibition mediated by reduced intracortical excitatory synaptic drive onto parvalbumin-expressing (PV) interneurons accompanied OD plasticity. Interestingly, after only 2 days of MD, OD plasticity was more extensive in L4 than L2/3 or L5 in both adult mice lacking ngr1 selectively in L4, as well as juvenile wild-type mice. We propose that developmental OD
plasticity is predominantly feed-forward, and NgR1 closes the critical period by limiting neuronal and circuit compensation for reduced sensory input.

**Disclosures:** M.G. Frantz: None. T. Ikrar: None. G. Sokhadze: None. X. Xu: None. A.W. McGee: None.

**Poster**

**309. Processing of Visual Motion**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 309.01/L36

**Topic:** D.07. Vision

**Support:** NSF Grant IIS-1607518

**Title:** Accelerating looming stimuli change the response timing of a collision-detection neuron

**Authors:** *R. B. DEWELL, G. K. KORDESTANI, F. GABBIANI;* Baylor Col. of Med., Houston, TX

**Abstract:** Visually-guided collision avoidance and escape is critical to the survival of many animals, and a wide range of animals from insects to primates have developed dedicated neurons for the detection of impending collisions. These neurons respond most strongly to objects approaching at constant velocity on a collision trajectory or their two-dimensional analogs called looming stimuli. The most common type of looming-sensitive neurons, η-type looming neurons, produce peak responses a fixed delay after a looming stimulus has reached a threshold angular size. Research into the neural computations underlying this relationship found η neurons' activity to be described by the product of the looming stimulus angular speed and the negative exponential of its angular size. However, another possible computation, denoted κ, in which the activity is determined by the product of the angular size and its negative exponential would also produce a peak response a fixed delay after the stimulus surpassed an angular threshold. The predictions for the timing of peak response made by the η and κ equations differ for non-constant velocity approaches. A well-studied looming-sensitive neuron in grasshoppers has long served as a model for understanding the neural computations underlying collision avoidance behaviors. We recorded the activity of this neuron, the lobula giant movement detector (LGMD) through its downstream relay while presenting looming stimuli of constant, increasing, or decreasing velocity to test which equation best described the response timing. We found that stimuli with positive acceleration produced later peak responses nearer to the time of collision, while stimuli with negative acceleration produced peak responses earlier before expected collision. These findings are consistent with the predictions made by the η equation. As predators could approach with changing velocity, exploring how the acceleration profile influences the neural response helps us better understand escape behavior and the neural computations underlying it.

Poster

309. Processing of Visual Motion

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 309.02/L37

Topic: D.07. Vision

Support:  NSF Grant CBET-1747506
          Margaret Q. Landenberger Research Foundation
          Edward Jekkal Muscular Dystrophy Association Fellowship

Title: A biophysical model for visual feature integration during looming detection

Authors:  S. J. O’ROURKE\textsuperscript{1}, D. P. GOODMAN\textsuperscript{1}, H. JANG\textsuperscript{1}, C. R. VON REYN\textsuperscript{2,1}, *J. AUSBORN\textsuperscript{2};
\textsuperscript{1}Sch. of Biomed. Engineering, Sci. and Hlth. Systems, Drexel Univ., Philadelphia, PA;
\textsuperscript{2}Neurobio. & Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: The early visual system distills visual stimuli into parallel visual features. How individual downstream neurons then integrate these features to form behaviorally relevant representations remains poorly understood. Here, we leverage the \textit{Drosophila melanogaster} optic glomeruli, an area of the fly brain that provides unprecedented access to visual feature encoding and visual feature integrating cell types to study how a visual feature integrating neuron, the \textit{Drosophila} giant fiber (GF), integrates separately processed visual features of a looming stimulus. Our previous work shows that the response of the GF, to drive timely escapes from rapidly approaching objects, results mainly from the summation of inputs from two cell types, LPLC2 and LC4. Both cell types project from the optic lobe (fly retina) to terminate on GF dendrites in the optic glomeruli of the central brain. LPLC2 and LC4 may each represent a different classical model for looming detection (eta and rho) encoding angular size and angular velocity of approaching objects, respectively. Many mathematical models have been derived to describe how looming responses could be formed via downstream integration of size and speed. While these descriptive mathematical models often faithfully describe the computational output of the circuits, we know little on how they could be biologically implemented. In the few cases where biophysical models have been developed, most lack knowledge of and access to the inputs to the looming integrators, and few have investigated the intrinsic properties of each cell type. Here, we use electrophysiology in tethered, behaving animals, genetic manipulations of GF ion channel expression and biophysical models of the GF to investigate the mechanisms that enable different looming stimulus features to be integrated in the GF to appropriately select and time an escape behavior. We investigate GF intrinsic properties using electrophysiology and computational modeling and identify ion channels that contribute to GF active conductances.
using RNAi. We use multicompartment models to predict channel contributions to visual feature integration. These data will enhance our understanding of the neural algorithms for generating higher order representations from extracted visual features.


Poster

309. Processing of Visual Motion

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 309.03/L38

Topic: D.07. Vision

Title: Dendritic mechanisms underlying motion detection in starburst amacrine cells

Authors: *H. E. ACARON LEDESMA¹, C. CHAN³; S. WANG³, M. Z. LIN⁴, W. WEI²;¹Biophysical Sci., ²Univ. of Chicago, Chicago, IL; ³Neurobio., ⁴Stanford Univ., Stanford, CA

Abstract: Dendritic computations play critical roles in shaping neuronal outputs and circuit functions. This is exemplified in the retinal direction-selective circuit, where starburst amacrine cells (SACs) provide directionally tuned inhibition onto direction-selective ganglion cells (DSGCs). The dendritic sectors of SACs are highly compartmentalized, each of which is strongly activated by motion in its centrifugal (outward) direction. The distal region of the SAC sector releases GABA selectively to a DSGC subtype that prefers the centripetal (inward) direction of the SAC sector, and thereby provides strong null-direction inhibition of the DSGC. The compartment-specific direction selectivity of SAC dendrites requires a sophisticated dendritic mechanism that not only converts the non-directional synaptic inputs into directionally tuned outputs during visual motion stimuli, but also establishes sufficient electrotonic isolation between sectors to maintain the independent directional tuning of each sector. Previous work from our lab has identified metabotropic glutamate receptor II (mGluR2) as an important regulator of dendritic compartmentalization. mGluR2 activation inhibits voltage-gated calcium channels on the SAC, and prevents activation of each sector in its nonpreferred centripetal direction but not in the centrifugal direction. In addition, voltage-gated $K^+$ ($K_v$) channels and passive dendritic properties have also been implicated in SAC dendritic processing. However, how these dendritic mechanisms together impact motion-evoked synaptic integration is still poorly understood. Here, we use electrophysiological recordings and subcellular functional imaging to explore the concerted action of multiple dendritic mechanisms underlying SAC dendritic computation. Our data suggest a synergistic interaction of mGluR2 signaling and $K_v$ channels in the perisomatic compartment of the SAC in regulating sector-specific dendritic activation and synaptic release.
**Disclosures:**  

**Poster**

**309. Processing of Visual Motion**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 309.04/L39

**Topic:** D.07. Vision

**Support:**  
National Science Foundation Research Fellowship Program  
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NEI RO1 EY024016  
NINDS RO1 NS109990

**Title:** Occlusion responses lead to fine spatial discrimination despite aberrant velocity information in direction-selective ganglion cells

**Authors:** *J. DING, A. CHEN, W. WEI, S. PALMER;  
Univ. of Chicago, Chicago, IL

**Abstract:** How neural systems infer object trajectories in complex environments where signals can be interrupted or conflated with other sources is an essential question in sensory processing, yet little is known about the circuit mechanisms underlying these computations. The natural visual world is full of solid objects that occlude each other; here we investigate how occlusion impacts motion processing. We focus on the responses of retinal direction-selective ganglion cells (DSGCs), which fire maximally to visual stimuli moving in their preferred direction and are almost completely inhibited in their null direction. We created a visual stimulus in which a moving bar disappears behind an occluder centered on the receptive field of the DSGC. The occluder is larger than the total dendritic span of the DSGC. With electrophysiological recordings, we show that these cells fire an unexpected burst of spikes to a bar exiting the occlusion area and moving in the cell’s null direction. One might expect this aberrant null direction response to degrade the fidelity of stimulus estimation. However, while the spiking of the cell to its non-preferred direction certainly hampers velocity estimation, it provides extra information about the bar’s position, as assessed in a population model through maximum likelihood decoding of the stimulus position. This model incorporates biologically relevant parameters such as the distribution of inter-soma spacing and relevant trial-to-trial and cell-to-cell variability. Together, these experimental and modeling results reveal how complex features such as interrupted motion are encoded by retinal circuitry and may facilitate downstream readout of important stimulus features.

**Disclosures:** J. Ding: None. A. Chen: None. W. Wei: None. S. Palmer: None.
Title: Receptive field properties of the developing ferret lateral geniculate nucleus

Authors: *A. K. STACY*¹, N. A. SCHNEIDER¹, D. A. BRESSLER¹, L. S. HAYASHI¹, S. D. VAN HOOSER²;
²Biol., ¹Brandeis Univ., Waltham, MA

Abstract: Visual experience is critical for normal development of particular receptive field (RF) properties in primary visual cortex (V1). More specifically, visual experience is necessary for the development of direction selectivity in V1 of the ferret, while orientation selectivity is already present in ferret V1 at the onset of visual experience. However, little is known about receptive field properties of the lateral geniculate nucleus (LGN) inputs to V1 at the onset of visual experience in terms of orientation and direction selectivity, latency, and sustainedness/transience. Moreover, we do not know how visual experience affects the development of these RF properties. In order to address these gaps in knowledge, we performed multichannel, in vivo recordings of LGN cells in visually naïve ferrets, and made measurements before and after exposure to 6 hours of a moving sinusoidal grating stimulus known to induce direction selectivity in ferret V1. In our preliminary observations, most LGN cells are not significantly tuned for orientation or direction at the onset of visual experience, or following exposure to the motion stimulus, suggesting that individual LGN cells are not playing a large role in the increase of direction selectivity in V1 with visual experience. This indicates that direction selectivity must be computed at a later stage in the visual pathway, providing insight into the visual processing of motion detection.

Title: Development of direction selectivity in the ferret motion pathway and effects of stimulation

Authors: *A. A. LEMPEL*¹, A. S. KAVUTURU¹, K. J. NIELSEN²;
¹Neurosci., ¹Johns Hopkins Univ., Baltimore, MD

Abstract: The ferret has become a major animal model for visual development due to its early parturition. In addition, motion processing in early visual structures of the ferret parallels that in primates, with direction-selective signals first found at the level of primary visual cortex (V1). Previous studies have therefore taken advantage of the ferret to investigate the development of V1 motion processing. These experiments demonstrate development of V1 direction selectivity during the first week after the onset of visual experience, which begins with eye opening around postnatal day 30. In addition, previous experiments also suggest a role of visual experience in the development of V1 direction selectivity. Recently, we have established the ferret as a model for studying the development of motion processing in cortical areas beyond V1. In particular, our work suggests that visual area PSS is a higher-order motion area comparable to area MT in the primate motion pathway. In this study, we make use of this finding to study the development of direction selectivity across multiple motion pathway stages. In a first set of experiments, we performed single unit recordings to determine V1 and PSS direction selectivity at different ages. In contrast to a strictly hierarchical development, our data show emergence of V1 and PSS direction selectivity during the same time period after eye opening. To more directly test the impact of visual experience on the development of direction selectivity in V1 and PSS, we then exposed visually naïve ferrets to drifting gratings for up to 8 hours while recording V1 and PSS responses. Prolonged exposure to drifting gratings indeed resulted in the emergence of V1 and PSS direction selectivity. In contrast, exposure to static stimuli did not induce the emergence of direction selectivity in either area. Finally, inhibition has been suggested to play an important role in shaping direction selectivity in ferret V1. We therefore studied how visual experience might impact the development of inhibitory circuits in V1 and PSS. To this end, we analyzed the laminar distribution of V1 and PSS parvalbumin (PV) expression in animals that had been exposed to moving gratings, static gratings, or were visually naïve. Intriguingly, this analysis revealed that exposure to moving stimuli resulted in early maturation of PV expression in both V1 and PSS. In conclusion, our results point to important parallels in how direction selectivity
develops in early and later stages of the motion pathway, including similar developmental timelines, impact of visual experience, and possible contributions of inhibitory circuit development.

**Disclosures:** A.A. Lempel: None. K.J. Nielsen: None. A.S. Kavuturu: None.

**Poster**

309. Processing of Visual Motion

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 309.07/L42

**Topic:** D.07. Vision

**Support:** This research was supported by the Scientific and Technological Research Council of Turkey (BIDEB 2221 Program).

**Title:** Erp evidence for persistence of object-based selection over unpredictable changes in object attributes

**Authors:** E. N. CATAK1,2, *H. KAFALIGONUL1,2, M. OZKAN3, G. R. STONER1,4; 1Natl. Magnetic Resonance Res. Ctr. (UMRAM), 2Interdisciplinary Neurosci. Program, Bilkent Univ., Ankara, Turkey; 3Dept. of Psychological and Brain Sci., Dartmouth Col., Hanover, NH; 4Salk Inst. for Biol. Studies, La Jolla, CA

**Abstract:** There is growing evidence that attention directed to one attribute of an object enhances the processing of other attributes of that object. The transparent-motion design introduced by Valdes-Sosa et al. (2000) has been adapted to study both perceptual mechanisms (e.g., Mitchell et al. 2003) and ERP correlates (e.g., Khoe et al. 2005) of such object-based attention. In this paradigm and its variants, subjects judge brief translations of one of two superimposed dot fields. It has been found that translations of dot fields that are cued (endogenously and/or exogenously) are judged more accurately than translations of the other field. By introducing motion direction and color swaps of the dots at the onset of translation, Stoner and Blanc (2010) discovered that the cueing advantage was specific to the individual cued dots. Their findings implicated early visual areas (e.g., V1) with receptive fields small enough to discriminate between the spatially intermixed dots. To explore the cortical bases of these behavioral effects, we designed an EEG experiment based on the Stoner and Blanc paradigm (i.e., delayed-onset cueing, with and without motion and/or color swaps). The behavioral results (N=14) confirmed that the cueing effect was specific to the cued dots though the cueing effect was significantly reduced by motion swaps. A cluster-based permutation analysis of the ERPs revealed an early (200-240 ms) spatiotemporal cluster (p=0.054) and a later (250-350 ms) cluster (p=0.009). Both clusters were centered over occipital and parieto-occipital scalp sites. To determine whether the clusters identified by this analysis were modulated by cueing and/or
swapping, we performed a two-way repeated-measures ANOVA with cueing (cued vs. uncued) and swapping (no-swap, motion, color, motion and color) as factors on the averaged ERP amplitudes. We found a significant effect of cueing for both time-ranges. For the early 200-240 ms time-range, we found a significant cueing effect (p=0.007), as well as an effect of swapping (p=0.039) such that swapping attributes decreased the average ERP amplitudes. For the late (250-350) time-range, we found robust modulation by cueing (p<0.001) and that swapping had no significant effect. While our ERP analyses did not reveal the involvement of early stages of visual processing, our findings demonstrate that the cueing effect, even in the presence of motion and color swaps, is correlated with modulation of later processes.


Poster

309. Processing of Visual Motion

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 309.08/L43

Topic: D.07. Vision

Title: Posterior entopallium lesions may produce a spatial integration deficit that affects pigeons’ discrimination of human behavior

Authors: M. A. J. QADRI1, *S. L. KELLOGG2, G. ROTHMAN1, H. ADAMSON4, M. H. TRAN3, A. M. KELLER1, D. I. BROOKS1, T. SHIMIZU3, R. G. COOK1; 1Psychology, Tufts Univ., Medford, MA; 2Psychology, 3Univ. of South Florida, Tampa, FL; 4Max Planck Inst. for Human Cognitive and Brain, Leipzig, Germany

Abstract: The mammalian visual system demonstrates a greater reliance on thalamofugal processing over tectofugal processing, though the avian visual system shows the reverse. While separate processing streams and their functions have been identified and examined extensively in mammals, such segregation has received less attention in the visual system of birds. Previous research implicates the avian entopallium in tasks of motion and shape perception. Lesions of the anterior entopallium have been shown to impact form processing, while lesions of the posterior entopallium have impacted motion processing. To further explore this functional segregation using object-based stimuli, six of eight pigeons (Columba livia) were successfully trained on an action recognition task that required the pigeons to conditionally respond based on whether a digital human actor was static or moving and whether the actor’s behavior demonstrated a martial arts or Indian dance sequence. These pigeons were then given bilateral electrolytic lesions targeting either the anterior portion of the entopallium or posterior portion of the entopallium. After recovery, these pigeons’ performance on the visual action recognition task was assayed and revealed a detriment only in the case of the posterior entopallium lesion on motion processing. Subsequent post-lesion acquisitions suggested that the posterior portion of
the entopallium contributed to spatial processing while the anterior portion of the entopallium contributed to temporal processing. While the spatial processing deficits seemed to persist over the subsequent courses of training and testing, the temporal processing deficits showed initial decrement and rapid recovery. These data suggest that the posterior entopallium may be critical for processing the spatial aspects of object or agent motion, and that the anterior entopallium may be relevant for temporal processing. Future studies should further evaluate the temporal and spatial processing of these separate visual streams.


Poster

309. Processing of Visual Motion

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 309.09/L44

Topic: D.07. Vision

Support: NSERC Discovery Grant 418331

Title: Surround suppression in deep models of complex motion processing

Authors: *O. SADAT REZAI, B. P. TRIPP;
Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Inhibitory surrounds are ubiquitous in the visual cortex, and thought to be important both for calculating higher-order fields and for suppressing goal-irrelevant information. Spatial suppression measures strongly correlate with IQ in humans, hinting at a broadly important role of surrounds in neural computation, but the specific contributions of surrounds to perceptual decisions are unclear. Computational models have provided insight, but they have focused on simple information processing and/or idealized visual scenes. To fill this gap, we focused on the role of surrounds in models of naturalistic processing of naturalistic scenes. We began with a detailed model of activity in primate middle temporal area (MT), because details of the MT representation of visual motion are closely related to surround suppression of motion perception. We used the MT activity model as input to deep networks that performed naturalistic functions, specifically self-motion estimation (visual odometry) and gesture recognition. In each case, adding inhibitory surrounds to the MT model improved performance moderately. It is somewhat surprising that the effect was similar in each case, because gesture recognition involves complex motion discontinuities, while odometry involves large self-induced motions, which surrounds have been proposed to cancel out. We then compared the learned structure of direction-selective and non-selective components of the surround to data from the MT literature. We found that the
learned surrounds were distinct. Whereas non-selective surrounds are more distant from the excitatory center in MT, this difference did not appear in the task-optimized model surrounds. However, performance did not degrade when we manually incorporated biologically realistic surrounds. This suggests that the particular spatial structure of biological surrounds may belong to a larger manifold of structures that are similarly effective for these naturalistic tasks.

Disclosures: O. Sadat Rezai: None. B.P. Tripp: None.

Poster

309. Processing of Visual Motion

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 309.10/L45

Topic: D.07. Vision

Support: ANR-15-CE37-0011

Title: Representation of multiple strokes apparent motion in awake monkey V1

Authors: *K. BLAIZE¹, S. CHEMLA², A. REYNAUD³, M. DI VOLO⁴, A. DESTEXHE⁵, F. Y. CHAVANE²;
¹CNRS & Aix-Marseille Université, Marseille, FRANCE, France; ²CNRS & Aix-Marseille Univ., Marseille, France; ³McGill Univ., Montreal, QC, Canada; ⁴Unité de Neurosciences, Information et Complexité, Ctr. Natl. de la Recherche Scientifique, FRE 3693., Gif sur Yvette, France; ⁵Unité de Neurosciences, Information et Complexité, Ctr. Natl. de la Recherche Scientifique, FRE 3693., Gif Sur Yvette, France; ⁶European Inst. for Theoretical Neurosci., Paris, France

Abstract: The apparent motion illusion is evoked when stationary stimuli are successively flashed in spatially separated positions. For large spatial and temporal separations, the so-called long-range apparent motion, we still have a poor understanding of how the visual system integrate and represent motion information. In a recent work, we used Voltage-Sensitive Dye Imaging (VSDI) in primary visual cortex (V1) of the awake monkey to image the cortical representation of two-stroke long-range apparent motion (Chemla et al 2019). We revealed that intra-cortical connections are crucial to shape the representation of illusory motion within V1 retinotopic map and keep track of the stimulus along the motion trajectory. More precisely, we showed that the two-stroke apparent motion (AM) illusion induce multiple wave interactions, generating a precise encoding of the stimulus velocity that is shaped by a suppressive wave propagating in a direction opposite to that of the AM stimulation. Using a neural field model, we showed that this suppressive wave is the expected emergent property of a dynamic recurrent gain control fed by the intra-cortical network. In the present work, we wanted to extend this study to apparent motion stimuli composed of multiple strokes in the same or opposite directions. Using
VSDI in the awake monkey we measured either a reinforcement of V1 activation when the stimuli were in the prolongation of the initial two-stroke AM, or a strong suppression when the third stimulus was presented in a direction opposite to the initial AM trajectory. We used our previously developed model (Zerlaut et al 2018, Chemla et al 2019) to test whether such results are expected by the dynamic recurrent gain control hypothesis. To conclude, these results show that the intra-cortical circuitry implement a gradual encoding of the illusory stimulus taking into account the spatio-temporal contiguity. Our understanding of the low-level processing involved in motion representation are a crucial step to guide further experiments that will link the V1 neuronal dynamics with the visual perception of motion.


Poster

309. Processing of Visual Motion

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 309.11/L46

Topic: D.07. Vision

Title: Predicting the future from the past in visual object motion: Optimal representations of mixed stochastic/deterministic trajectories

Authors: *V. SACHDEVA¹, A. WALCZAK³, T. MORA⁴, S. E. PALMER²; ²Organismal Biol. and Anat., ¹Univ. of Chicago, Chicago, IL; ³Ecole Normale Superieure, Paris, France; ⁴Lab. de Physique Statistique, Ecole Normale Supérieure, Paris, France

Abstract: Making predictions about the future state of the external world confers benefits to biological systems that can translate to increased fitness. However, physical constraints, such as limited memory and finite neural population sizes, combined with energetic constraints, prevent the brain from constructing a representation that retains perfect knowledge of the past. Hence, any learned or evolved predictive encoding strategy must both compress the information of the current and past stimulus, yet still represent the future as faithfully as possible. For some classes of stimulus statistics, the optimal tradeoff can be computed directly. The retina has been shown to construct a representation that approaches this theoretical bound on the future information for a stimulus evolving with the dynamics of a stochastically driven, overdamped harmonic oscillator. This suggests that the retina has evolved to solve the so-called information bottleneck problem for this physical model with a particular set of motion parameters. Here, we compute the optimal predictive strategy for a variety of motion models, including those where there is a long-tailed correlation structure in the noise. We demonstrate that the encoding strategy that enables optimal prediction changes as a function of the damping coefficient. For overdamped harmonic motion, prediction requires high precision in measurements of the past object position.
For underdamped harmonic motion, oscillatory motion is produced, resulting in a need for predictive strategies that make precise measurements of both position and velocity simultaneously. This is especially true at prediction timescales comparable to the periodicity of the harmonic oscillator. We note that the presence of long-tailed correlations in the stimulus result in optimal predictive strategies requiring integration over a range of past measurements of the stimulus, and we can compute the optimal memory kernel for these dynamics. We also consider the conditions under which a particular predictive strategy can be generalized to a wider family of models while maintaining minimal performance loss, and which predictive strategies are highly tuned to specific types of motion. These representations can be compared to experimental measurements of predictive information in the brain, determining what types of motion the brain might have evolved to optimally predict.

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connection weights and MSTd activations, we were able to reconstruct MT activity patterns (the reconstructed MT and the input MT neuronal firing rates have a correlation of 0.68±0.11), and recover the flow field stimuli with high fidelity. The MSTd neurons appeared to have 3D translation and rotation selectivity resembling neurophysiological data, and each neuron spanned only a subregion of the visual field, preferring a mixture of translational and rotational flow components. The model accurately captured the 3D visual response properties of MSTd and indicated that STDP-H is indeed performing a form of dimensionality reduction.

Disclosures:  K. Chen: None. M. Beyeler: None. J.L. Krichmar: None.

Poster

309. Processing of Visual Motion

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 309.13/M2

Topic: D.07. Vision

Support: NSF EPSCOR Award #1632738

Title: Motion induced blindness increases with distance from moving background

Authors: *S. SALEKI, N. HELLER, P. CAVANAGH, P. U. TSE; Psychological and Brain Sci., Dartmouth Col., Hanover, NH
Abstract: A stationary target positioned among moving objects can fade in and out of perception (Motion Induced Blindness, MIB, Bonneh, Cooperman, & Sagi, 2001). We used a version of the stimulus with only two linear arrays of moving spots rather than a full background and varied the spacing between the linear arrays and the test target to see if MIB was influenced by spacing to the moving background elements. Participants fixated a dot in the lower half of the screen and viewed two horizontal, linear moving arrays of gabor patches (each array consisting of 14 gabors spanning display) positioned above the fixation point. During each trial, the arrays had 2 or 4 visual degrees displacement between them and a single target dot was presented halfway between the two arrays. In each trial, the internal texture of the gabors was either moving faster than the gabor (congruent), moving with the gabor (static), or moving more slowly than the gabor (incongruent). These different internal motions changed the apparent speed of the linear motion. Participants reported the moment of disappearance and reappearance of the target object over trials that lasted 20s each. The duration of disappearance was longer (more MIB) for the larger spacing between the moving arrays. Moreover, disappearance was longer for the incongruent internal gabor motion condition compared to the static or congruent internal motion conditions. Here, we showed that MIB occurs when a yellow stationary dot is positioned between only two linear arrays of moving gabor patches on a gray background and increases with distance from the background motion, and with the perceptually slower motion.

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Poster

309. Processing of Visual Motion

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 309.14/M3

Topic: D.07. Vision

Support: NSF EPSCoR Award #1632738

Title: Functional links between motion-onset visual evoked potentials and perception

Authors: *M. OZKAN1, G. R. STONER2,3, H. KAFALIGONUL3,4;  

Abstract: An important question in visual neuroscience is how low-frequency cortical activity and population responses are related to different aspects of motion. Previous studies have shown that visual evoked potentials (VEPs) around 160-200 ms (N2 component) after the onset of motion are driven by coherent motion perception (Kuba, et al., 2006; Niedeggen and Wist, 1998). Although this motion specific N2 component has been associated with the activity of
human area MT+ (Nakamura and Ohtsuka, 1999), it is still not clear how local information is integrated in the subdivisions of MT+ and how the N2 peak activity relates to behavioral performance. To shed light on these important issues, we focused on the relationship between the N2 component amplitude and coherence thresholds. Our EEG experiment included 3 types of random-dot motion (translational, rotational and radial) with coherence levels ranging from 5% to 80%. To control the coherence level and determine the position of every dot in each random-dot frame, we used the white noise motion algorithm developed by Britten et al. (1992). Observers engaged in a 2AFC (two-alternative forced-choice) direction discrimination task. In accordance with previous studies (e.g., Patzwahl and Zanker, 1999), N2 amplitude significantly increased as the coherence level was increased. Our results also indicated a significant two-way interaction (coherence level x motion type) such that radial motion produced overall larger amplitudes. More important, the average N2 amplitudes were significantly correlated with the individual thresholds (i.e., coherence level corresponding to 75% correct responses) for translational motion. This correlation was absent in the radial and the rotational motion types. However, these motion types elicited less between-subject variance in sensitivity as compared to the translational motion ($SDs$ for the radial, rotational, and translational thresholds: 1.7%, 3.3%, and 14.4%, respectively). Thus, the relationship between the sensitivity to these motion types and the overall N2 amplitude still remains open to further investigation. Taken together, our results suggest that the N2 component is significantly dependent on the physical coherence level of the different motion types studied here, and the overall N2 amplitude is correlated with participants' sensitivity to translational motion.

Disclosures: M. Ozkan: None. G.R. Stoner: None. H. Kafaligonul: None.

Poster

309. Processing of Visual Motion

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 309.15/M4

Topic: D.07. Vision

Title: Multistable heterogeneous flicker has neural, functional and aesthetic effects

Authors: *M. GRABOWECKY, M. MENCELOGLU, S. SUZUKI; Psychology, Northwestern Univ., Evanston, IL

Abstract: Spatially heterogeneous flicker is ubiquitous in natural dynamics. Phenomena such as falling rain, flames, waving grass, shimmering, falling and wavy water surfaces, rustling leaves, and so on are also typically pleasant to observe. One way in which spatially heterogeneous flicker is special is that it is multistable, allowing sensory activation that provides a good fit to momentary biases of the visual system. This activation may result in the perception of spontaneous motion and the calibration of motion detectors. In this sense, heterogeneous flicker
may provide particularly “natural” signals to the visual system, engaging fluent processes with minimal engagement of top-down control. In two exploratory experiments we used EEG to investigate this possibility, presenting observers with 16-element displays that varied in the number of independently flickering regions, and thus also varying in the amount of perceptual multistability. Consistent with our hypothesis, increasing multistable flicker (relative to control flicker with matching local and global temporal statistics) commensurately reduced posterior EEG beta power. This beta-band activity has been implicated in long-range neural interactions that are associated with top-down influences that impose constraints on sensory signals and potentially perceptual interpretations. Further, the degree of multistability, the amount of beta-power reduction, and the aesthetic rating of the presented flicker were closely associated (Fisher-Z transformed correlation coefficient $r_z$, Expt. 1 $r_z \text{mean} = 0.490$, $r_z \text{sem} = 0.117$, $t_{17} = 4.186$, $p=0.00062$; Expt.2 $t_rz \text{mean} = 0.401$, $r_z \text{sem} = 0.098$, $t_{17} = 4.089$, $p=0.00077$). Thus spatially heterogeneous, multistable flicker reduced posterior EEG beta power relative to spatially homogeneous flicker controls that were matched in terms of the dynamics of local luminance changes, luminance changes at any location, and spatially averaged luminance changes. These results are consistent with the idea that the pleasantness of spatially heterogeneous flicker in nature may derive from its multistability that affords fluent and self-calibrating visual processing. The resulting sensory recalibration may be an underlying mechanism of observed restorative effects of exposure to nature.


Poster

309. Processing of Visual Motion

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 309.16/M5

Topic: D.07. Vision

Support: NSF CAREER-1652617
Alfred P. Sloan Foundation

Title: Dynamic scaling of motion in natural scenes

Authors: *J. M. SALISBURY, S. E. PALMER;
Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: Some of the most important tasks of visual and motor systems involve estimating the motion of objects and tracking them over time. Such systems evolved to meet the behavioral needs of the organism in its natural environment, and should therefore be adapted to the statistics of motion it is likely to encounter. Experimental neuroscientists, working in systems ranging from the retina to the pursuit eye movement system, have begun to explore this relationship...
using stochastically moving stimuli; however, it is not at all clear what properties make motion naturalistic. By tracking the movement of individual points in videos of natural scenes using a simple optical flow-based algorithm, we begin to identify common properties of natural motion trajectories. As expected, objects in natural scenes move in a persistent fashion, with velocity correlations lasting hundreds of milliseconds depending on the composition of the particular scene. This can be attributed to the significant mass--and therefore inertia--of the objects we record. More subtly, we find that the observed velocity distributions are highly non-Gaussian and can be modeled as a scale mixture of Gaussians, a property that velocity in natural scenes shares with, for example, wavelet coefficients describing natural images and sounds. Extending this model to the time domain leads to a dynamic scale mixture model, consisting of a Gaussian process with relatively fast dynamics (here, a two-dimensional velocity vector evolving over time) multiplied by a positive scalar quantity with slower dynamics. Dynamic scaling of (angular) velocity arises naturally as a consequence of changes in object distance from the camera, and may approximate the effects of changes in other parameters governing the motion in a given scene. The dynamic scale mixture model allows us to estimate the scale process using maximum a posteriori estimation, decomposing the stimulus into a product of approximately Gaussian- and gamma-distributed components. This modeling and estimation framework may have implications for the neurobiology of sensory and motor systems, which need to cope with these fluctuations in scale in order to represent motion efficiently and drive fast and accurate behavior.

Disclosures: J.M. Salisbury: None. S.E. Palmer: None.

Poster

309. Processing of Visual Motion

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

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Topic: D.07. Vision

Support: The study was funded by a grant to ST from the Deutsche Forschungsgemeinschaft (DFG) for the Research Unit 1847 “Physiology of Distributed Computing Underlying Higher Brain Functions in Non-Human Primates”

Title: Size matters (sometimes): Increasing stimulus size impairs motion perception for gratings, but not random-dot patterns

Authors: *B. WILD¹, A.-K. KENKEL¹, S. TREUE²;
¹German Primate Ctr. (DPZ), Goettingen, Germany; ²German Primate Ctr., Goettingen, Germany
Abstract: Modern accounts of motion processing in the primate brain emphasize a hierarchy of different regions involved, especially primary visual cortex (V1) and the middle temporal area (MT). Different experimental approaches suggest that MT is both necessary and sufficient for motion perception. A recent study (Liu & Pack, 2017, Neuron, 95, 436-446), however, shows that monkeys can perceive the motion of a drifting sinusoidal grating after MT has been inactivated. After prolonged training with random-dot patterns (RDPs), however, inactivating MT impairs performance for both gratings and RDPs, suggesting that the training with RDPs shifts the readout of motion information from other brain areas (e.g., V1) to MT. This hypothesis receives further support from behavioral findings: testing motion perception with gratings of different sizes shows that performance decreases once the stimulus size exceeds a critical value, presumably because bigger stimuli stimulate the suppressive surround of neuronal receptive fields (Tadin et al., 2003, Nature, 424, 312-315). In Liu & Pack’s study, the optimal stimulus size, for which performance reaches its peak, increased after training with RDPs, providing further evidence that read-out had shifted to an area with larger receptive fields (e.g., from V1 to MT). We investigated this phenomenon in more detail in human subjects. 15 observers discriminated the motion of drifting gratings and RDPs of different sizes and contrast levels. We find the previously described impairment in performance with increasing stimulus size for gratings, but not for RDPs. Other than in the study by Tadin et al. (2003), these effects were independent of stimulus contrast. A possible explanation for these results is that the perception of gratings, but not RDPs, depends heavily on neurons with a clear center-surround structure. An alternative explanation would be that the size-effect in gratings is not caused by inhibitory subregions of visual neurons but is related to the stimulus and task-design: detecting the motion of a drifting grating might be easier if one can see the grating’s edge, where individual dark or bright cycles appear or disappear as they drift into or out of the aperture. The grating’s edges become less visible with increasing stimulus size when a subject fixates the grating’s center. In RDPs, on the other hand, motion can be detected based on a single dot, independent of stimulus size. Our data document a highly flexible network for visual motion processing, where alternative processing paths and/or adaptive local neuronal networks contribute to enable perceptual performance.

Disclosures: B. Wild: None. A. Kenkel: None. S. Treue: None.

Poster

309. Processing of Visual Motion

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 309.18/M7

Topic: D.07. Vision

Support: NIH Grant EY05729
         NIH Grant 5T32LM012414-03
Title: Complex properties of visual motion during natural locomotion

Authors: *K. MULLER*, J. S. MATTHIS, M. M. HAYHOE;

1Univ. of Texas At Austin Inst. For Neuros, Austin, TX; 2Ctr. for Perceptual Systems, 3Univ. of Texas at Austin, Austin, TX

Abstract: Properties of neural processing often reflect the statistics of natural images. For example, disparity tuning distributions in V1 reflect disparities encountered during a range of naturalistic tasks (Liu et al, 2008). Therefore we might expect that the tuning for visual motion reflects the statistics of the retinal motion signal experienced by observers during natural locomotion. However, the precise nature of these statistics has not been explored. We measured these motion patterns using a Pupil Labs mobile eye tracker to record eye position, and high-resolution scene video. Subjects’ gaze patterns reveal a series of fixations, stabilizing the image through the vestibular ocular reflex. We estimated retinal motion input by first computing visual motion relative to the head-fixed camera using the DeepFlow optical flow estimation algorithm on the scene video (Weinzaepfel et al 2013), and then transforming it into the retinal coordinate frame and removing the motion transients generated by saccades. The resulting motion statistics are complex and shaped by the direction of gaze, the motion of the body, and environmental geometry. The overall distribution of speeds is skewed slow, with over half the distribution being less than 6 degrees per second. Due to the distortion introduced by the tilted ground plane, there are upward and downward directional biases in motion direction. Additionally, average speeds increase with retinal eccentricity, in all polar directions. Inspection of the first four components of retinal flow obtained through PCA reveal interpretable components of rotation, expansion, and translation in two directions. The distribution of scores for rotation is bimodal, generated by gaze locations both to the left and right of the body’s instantaneous momentum as a result of body sway and turns. Likewise the expansion component distribution is bimodal, due to the gait-linked modulation of head velocity, leading to differing amounts of expansion and contraction relative to an expansive mean flow field. These different components have behavioral drivers, for example angle off of path is related to degree of rotation in the corresponding direction. While a number of studies have attempted to link neural responses in MSTd to self-motion, the complexity of the natural optic flow patterns which depend on both gaze and gait makes it hard to interpret the cells' precise role in coding self-motion. Thus investigation of neural responses to natural optic flow patterns may provide new insights into the neural coding of self-motion.

Disclosures: K. Muller: None. J.S. Matthis: None. M.M. Hayhoe: None.

Poster

309. Processing of Visual Motion

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 309.19/M8
Localized intracranial visual motion response drives fast BCI spelling

D. Liu, X. Xu, D. Li, J. Li, Z. Ling, B. Hong
Tsinghua Univ., Beijing, China; PLA Gen. Hosp., Beijing, China

Abstract: Currently, scalp EEG based brain-computer interface (BCI) is still the mainstream of BCI spelling system, with remarkable accuracy and speed on normal subjects (X. Chen et al., 2015). However, incapable of providing signal with consistent good quality over long-term use, BCI based on scalp EEG can hardly gain its position in clinical applications. Meanwhile, recent studies have shown that intracranial EEG (iEEG) based BCIs were able to achieve long-term stability (Jarosiewicz et al., 2015). However, for a practical intracranial BCI, minimizing the invasiveness of the electrode implantation while maintaining the performance is crucial. In this study, we used only three adjacent channels which matched most with the fMRI defined visual motion middle temporal (MT) region on one single Stereo-Electroencephalography (SEEG) electrode to implement an online BCI for fast typing. When subjects attended the virtual button containing moving bars, prominent responses were elicited by visual motion stimuli at the electrodes within the fMRI defined MT region, which were composed of motion-onset visual evoked potentials (mVEPs) around 200 ms post-stimulus and a power increase at the high gamma (70-100 Hz) frequency range (Zhang et al., 2013). In the online BCI experiment with four surgical epilepsy patients, combining both features and using a posterior probability based stopping strategy, the system achieved highest information transfer rate (ITR) of 61 bits/min, which was equivalent to 12 characters per minute. Our findings demonstrate the feasibility of implementing a minimally invasive intracranial BCI with the help of precise localization of visual motion area.

Disclosures: D. Liu: None. X. Xu: None. D. Li: None. J. Li: None. Z. Ling: None. B. Hong: None.

Poster

310. Sensorimotor Transformation: Behavior and Neuroprocessing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 310.01/M9

Topic: D.08. Visual Sensory-motor Processing

Support: Research Foundation - CUNY, NY
The Psychology Department - Brooklyn College, CUNY, NY
Title: Using a flash stimulus to investigate the underlying visual and motor transformation of body patterning in intact, living squid

Authors: *S. P. HADJISOLOMOU*, K. KLOSKOWSKI, A. CHAVARGA, R. EL-HADDAD, I. ABRAMOV;

Social and Behavioral Sci., American Univ. of Kuwait, Salmiya, Kuwait; Psychology, Brooklyn Col. of the City Univ. of New York, Brooklyn, NY; Fawzia Sultan Hlth. Network, Salmiya, Kuwait

Abstract: The coleoid cephalopods can switch body patterns with unparalleled speed for camouflage and communication. While there are numerous studies on the physiology of the chromatophore system, the temporal dynamics of rapid chromatophore activation and coordination underlying body patterning are still mostly unknown. One way to study the complete sensorimotor transformation is by using an intact, living animal. The camouflage control system can be investigated by using a sudden, brief, and intense visual stimulus such as a flash of light to activate the startle reflex. The visual information from the sudden stimulus triggers the optic lobes to send signals to the central nervous system and, in turn, activate the giant axons controlling the chromatophore radial muscles. We used eight squid in this exploratory study to measure the coordination of chromatophore activity at the single organ level by using a light-flash stimulus to trigger body pattern changes. Two cameras captured images of the whole animal as well as close-ups of specific body regions (fin, mantle, head, and arms). The change-point analysis identified 4065 chromatophores from 185 trials with significant chromatophore surface area changes triggered by the stimulus. We measured the temporal dynamics of chromatophore responses as the magnitude of surface area changes, latency, and duration. Our findings indicate the latency of the response was at 50 milliseconds (± 16.67 milliseconds) on average, for both types of responses (expansion and retraction) and across all body regions. While the chromatophore surface area increased 155.06% on average during expansions, the retractions only caused a change of 40.46%. Further differences were observed between body regions; while the chromatophores on the mantle, head, and arms had surface area changes of 159% and 168%, respectively, the fin had a change of 116%. Our study is the first which characterizes the immediate temporal dynamics of individual chromatophore organ responses to a visual stimulus in an intact, living cephalopod. Collectively, the contributions of the methods and results described here will be valuable to help understand how cephalopods can employ hundreds of thousands of chromatophore organs in milliseconds to achieve rapid, dynamic camouflage.

Poster

310. Sensorimotor Transformation: Behavior and Neuroprocessing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 310.02/M10

Topic: D.08. Visual Sensory-motor Processing

Support: NIH Grant R01MH085927
NIH RISE Training Program 5R25GM061151-16
NIH 5R25MH059472-18

Title: Dissecting sensory information processing onto interneurons using calcium imaging in freely moving animals navigating a thermal gradient

Authors: *E. G. Cabezas-BOU1,2, J. Hawk1, A. N. Lauziere3,4, H. Shroff3, L. Shao1, B. A. Mohler2, I. E. Adeyefa-Olasupo1, D. A. Colon-Ramos1; 1Neurosci., Yale Univ., New Haven, CT; 2Biol., Univ. of Puerto Rico, Rio Piedras Campus, San Juan, PR; 3NIBIB, Natl. Inst. of Hlth., Bethesda, MD; 4Dept. of Mathematics, Univ. of Maryland, College Park, MD; 5Dept. of Genet. and Genome Sci. and Ctr. for Cell Analysis and Modeling, UConn Hlth. Ctr., Farmington, CT

Abstract: How interneurons process sensory inputs to generate appropriate motor responses is a fundamental question in neuroscience. In C. elegans the interneuron AIIY has an important role in a navigational behavior called thermotaxis, where the nematode detects temperature and decides which direction to move based on previous experience. Paralyzed worm preparations have been useful in understanding how AIIY responds to thermal stimuli, but it is difficult to determine the behavioral relevance of this activity without simultaneously monitoring behavior. Our goal is to define calcium activity patterns in AIIY during thermotaxis behavior in freely moving C. elegans. By specifically labeling AIIY with the GCaMP6 indicator we can detect and measure calcium signals, while tracking the worm as it navigates a thermal gradient. With this tool we are able to dissect compartmentalized calcium activity with subcellular resolution in the AIIY neurite and correlate activity patterns with precise thermosensory experience. Combining this approach with molecular genetics allows us to characterize how temperature preference is encoded at the synaptic level within the AFD-AIIY synapse. We also correlate neural activity with body posture, velocity, dorsal-ventral flexions and direction over a thermal gradient to characterize the role of interneuron AIIY in specific locomotor decisions during thermotaxis. With this approach we are investigating how mutants with defects in thermotaxis behavior affect AIIY thermal responses. Our findings will contribute useful insight into how processing of thermal stimuli across a single synapse produces thermotaxis behavior.

Poster

310. Sensorimotor Transformation: Behavior and Neuroprocessing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 310.03/M11

Topic: D.08. Visual Sensory-motor Processing

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Title: Evolutionary origin of visual and somatosensory processing in the vertebrate cortex

Authors: *S. M SURYANARAYANA, J. PÉREZ-FERNANDEZ, B. ROBERTSON, S. GRILLNER;
Dept. of Neurosci., Karolinska Institutet, Stockholm, Sweden

Abstract: The lamprey lateral pallium (LPal), an area corresponding to cortex of mammals, has a three-layered laminated cytoarchitecture with a molecular layer and outer and inner cellular layers. GABAergic interneurons represent 22% of the total number of cells. Pyramidal tract-like (PT-type), intertelencephalic (IT-type) and thalamo-recipient cells (layer 4 equivalent) are located in the cellular layer and extend their dendrites towards the molecular layer (Suryanarayana et al., 2017). The LPal is also conserved in terms of efferent connectivity. Glutamatergic and monosynaptic projection neurons target all major motor centers in the midbrain, brainstem and rostral spinal cord (Ocaña et al., 2015). With respect to sensory mapping, primary visual input, relayed via thalamus, is represented in the dorsomedial LPal in a retinotopic fashion. Distinct neuronal subpopulations are activated by stimulation of different parts of retina. Whole-cell recordings during optic nerve/chiasm stimulation revealed EPSPs followed by inhibition. Thalamic neurons prelabelled from the LPal visual areas send their dendrites into the optic tract and stimulation of the optic tract elicited EPSPs and action potentials during whole-cell recordings in these neurons. Somatosensory information from the dorsal column nuclei is also relayed via thalamus, and represented in a ventromedial area in LPal, distinct from the visual areas. Thalamic neurons projecting to the somatosensory areas of LPal form a subpopulation distinct from those projecting to visual areas. Stimulation of the
dorsal column and the trigeminal nerve elicited field potentials in adjacent areas. The thalamus is a major relay of sensory input to LPal. This study reveals the presence of distinct modality specific sensory and motor areas in the phylogenetically oldest group of vertebrates. Our overarching conclusion is that basic aspects of sensory-motor organisation in the forebrain had evolved when cyclostomes diverged from the lineage leading up to mammals.


Poster

310. Sensorimotor Transformation: Behavior and Neuroprocessing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 310.04/M12

Topic: D.08. Visual Sensory-motor Processing

Title: Role of forebrain for zebrafish prey capture

Authors: *I. GROSSRUBATSCHER¹, A. D. HOAGLAND², M. KLINGER³, A. R. ROMO², S. PARIJA², E. ISACOFFF²;
¹Helen Wills Neurosci. Inst., ²UC Berkeley, Berkeley, CA; ³Univ. of California, Berkeley, Berkeley, CA

Abstract: Experience strongly influences behavior, but little is known about how experience-dependent changes in neural activity are implemented at a network level to improve behavioral performance. Here, we use prey capture behavior in larval zebrafish as a model to study the role of experience in improving behavioral outcome. In larval zebrafish, prey capture is a complex behavior consisting of a stereotyped sequence of events: 1) ocular convergence, 2) approach and orientation towards the prey with J-bends of the tail, and 3) strike and engulfment of the prey. Earlier results in the lab have shown that prior experience with live prey increases capture success. Simultaneous behavior monitoring and wide-field calcium imaging in head-restrained animals showed that experienced fish exhibited greater activity in the forebrain during eye convergence. Moreover, Granger Causality analysis demonstrated a strengthened functional link from the optic tectum to the telencephalon during eye convergence in experienced fish. These findings suggest that the forebrain may play a role in modulating prey capture. The forebrain in zebrafish contains the telencephalon, which is homologous to the hippocampus and amygdala of other vertebrates, and the habenula, which is conserved across vertebrates and is involved in reward and avoidance learning. We find that pharmacogenetic and optogenetic disruption of the zebrafish forebrain compromises prey capture behavior. This supports the hypothesis that the forebrain operates as an experience-dependent switch that enhances the impact of information transfer from visual to motor-related areas during prey capture.
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**Poster**

**310. Sensorimotor Transformation: Behavior and Neuroprocessing**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 310.05/M13

**Topic:** D.08. Visual Sensory-motor Processing

**Support:** Howard Hughes Medical Institute

**Title:** Sensorimotor processing in the zebrafish dorsal raphe nucleus

**Authors:** *T. KAWASHIMA, Z. WEI, A. S. ABDELFATTAH, J. S. MARVIN, L. L. LOOGER, L. D. LAVIS, E. R. SCHREITER, M. B. AHRENS; HHMI Janelia Res. Campus, Ashburn, VA

**Abstract:** The serotonergic system affects brain-wide neural systems in motor learning, passive coping, and other behaviors. Underlying this widespread modulation of neural function are fast computations in the dorsal raphe nucleus, of which very little is known. To gain mechanistic insight into such millisecond-timescale computation, we measured the subthreshold membrane potential and spiking of serotonergic neurons during motor learning tasks in zebrafish by using a chemogenetic voltage indicator Voltron\(^1\) and an advanced imaging processing pipeline\(^2\). We also measured glutamatergic and GABAergic input into serotonergic neurons using neurotransmitter indicators iGluSnFR\(^3\) and iGABAStnFR\(^4\). Consistent with previous observations\(^5\), serotonergic neurons responded to visual sensory signals during swim bouts with action potentials. Although the spiking dynamics contained little information about motor variables, surprisingly, right after swim bout initiation, the subthreshold voltage of serotonergic neurons showed strong hyperpolarization which depended on motor vigor. This hyperpolarization occurred during simultaneous GABAergic and glutamatergic inputs into serotonergic neurons during swimming. Post-swim release from inhibition was accompanied by the release of glutamate onto serotonergic dendrites encoding visual feedback from swimming, which may contribute to motor learning. These results show that sensorimotor computations in the raphe nucleus are implemented by a temporally-precise, behavior-locked sequence of inhibition and excitation that together allow serotonergic neurons to encode the consequences of actions.

1. Abdelfattah, Kawashima et al., BioRxiv, 2018
2. Buchanan, Kinsella, Zhou et al., BioRxiv, 2018
3. Marvin et al., Nature Methods, 2018
4. Marvin et al., BioRxiv, 2018
5. Kawashima et al., Cell, 2016

Poster

310. Sensorimotor Transformation: Behavior and Neuroprocessing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 310.06/M14

Topic: D.08. Visual Sensory-motor Processing

Support: National Institutes of Health R01 EY028915
WSU Research Grant
Research to Prevent Blindness Grants

Title: Alpha7 nicotinic acetylcholine receptors in bipolar cells play a role in the optomotor response in mice


Abstract: The underlying mechanism of motion detection in the retina remains contested. Certain third-order neurons, starburst amacrine cells (SACs) and direction-selective ganglion cells, contribute to retinal direction selectivity and are considered key parts of the motion detection mechanism. Bipolar cells however, have largely been excluded. We recently found that specific types of bipolar cells express α7 nicotinic acetylcholine receptors (α7 nAChRs). Since SACs are the only retinal neurons that release acetylcholine, it is likely these bipolar cells play a role in motion detection. We knocked out α7 nAChRs in type 7 bipolar cells and tested the ability of these mice to detect motion by measuring their optomotor response (OMR). We generated transgenic mice in which α7 nAChRs were eliminated from type 7 bipolar cells using Cre-loxP technology and Cre-DOG (dependent on GFP). We first generated mice that carry a floxed Chrna7 (α7 nAChR gene) locus (homozygous) and express GFP in only type 7 bipolar cells (Gus-GFP). We injected adeno associated virus (AAV) which packaged Cre-DOG into the intra-vitreal space of male and female target mice 4 to 7 weeks old. C57BL/6J mice, transgenic mice lacking Chrna7-loxP or Gus-GFP, and non-injected mutants were used as controls. OMRs were measured and contrast sensitivity was assessed. A looming test was run and pupillary light reflexes were examined to demonstrate the preservation of visual response in mutant mice.

Two months after AAV injection, OMR and light reflex were measured. α7 nAChR knockout mice (n=10 eyes) had a significantly lower contrast sensitivity (p<0.01) than control mice, including C57 mice (n=23), non-injected transgenic mice (n=11), and injected mice lacking either Chrna7-loxP (n=14) or Gus-GFP (n=8). In contrast, no significant difference was found among control mice. α7 nAChR knockout mice demonstrated no significant change in response
to looming stimuli after injection (p>0.5). All transgenic mice retained normal pupil light response. Mice in which α7 nAChRs were knocked out of type 7 bipolar cells demonstrated significantly decreased optomotor response measurements. We conclude that α7 nAChRs in type 7 bipolar cells likely play a key role in tuning the motion detection pathway through bipolar cell outputs.

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Poster

310. Sensorimotor Transformation: Behavior and Neuroprocessing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 310.07/M15

Topic: D.08. Visual Sensory-motor Processing

Support: BNI ICM P09-015-F
CONICYT DOCTORADO NACIONAL 21150176

Title: Improvement of visual discrimination during active behavior requires precise sensorimotor contingencies

Authors: *M. CONCHA-MIRANDA1,2, J. RIOS1,2, J. BOU1,2, J. VALDÉS1,2, P. MALDONADO1,2;
1Neurosci., Univ. De Chile, Santiago, Chile; 2BNI, Santiago, Chile

Abstract: Active sensing refers to the process by which animals perceive their environment through self-initiated motor acts. During active sensing, movements elicit changes on the sensory organs that are temporarily locked to the initiation of the action. To the date, it has been shown that motor acts can modulate sensory processing in different modalities, and through a variety of actions. But, is the brain able to take advantage of the precise time-locking that occurs during active sensing? We conjecture that if stimuli presentation is evoked by a self-initiated motor act sensory discrimination would improve and that this improvement requires precise time coordination between the motor act and the sensory stimulation produced. We studied this phenomenon by training rats to locate the position of a brief light stimulus, either when it was elicited by a warning light [passive condition (PC)] or when it was generated by a lever press [active condition (AC)]. We found that during the AC rats had a significantly higher percentage of correct responses when compared with the PC. Furthermore, reaction times and omissions were reduced during AC. To test if this improvement required precise coordination between action and the corresponding sensory stimulation, rats were subjected to a variation of the AC were the stimulus was uncoupled from the motor act, by introducing a random delay after the lever press. For the latter condition, the probability of detecting the side of the light stimulus was
negatively correlated with the time lag between the motor act and the evoked light. We also found differential modulation of functional connectivity between motor and sensory areas during these three tasks. These experiments show that the mechanism that underlies sensory improvement during active behaviors have a constrained time dynamic, where the peak performances occur during the motor act, decreasing proportionally to the lag between the motor act and the stimulus presentation. This result is consistent with the evidence already found in humans, of a precise time dynamic of the improvement of sensory acuity after a motor act and reveals an equivalent process in rodents. Our results support the idea that perception and action are precisely coordinated in the brain.

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Disclosures:  

Poster

310. Sensorimotor Transformation: Behavior and Neuroprocessing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 310.08/M16

Topic: D.08. Visual Sensory-motor Processing

Support: NIH Grant P20 GM103650

Title: Behavioral responses evoked by novel motion stimuli change over development and correlate with immediate early gene expression patterns in the midbrain and cortex

Authors: *K. M. ALLEN, N. M. PROCACCI, J. L. HOY;  
Univ. of Nevada, Reno, Reno, NV

Abstract: Orienting responses evoked by visual motion are present early in life. Successfully honing these responses during development ultimately shapes both complex visuomotor behavior and the regulation of spatial attention in the adult. However, the mechanisms underlying the development of motion processing and spatial orienting behaviors in the mammalian brain are unclear. Similar to a wide range of experimental models, adult mice exhibit distinct orienting responses to specific features of visual motion. This presents an opportunity to study the cellular and molecular mechanisms underlying the development of these basic visuomotor orienting responses in the mammalian brain. Here, we begin to address this goal by quantifying how juvenile mice respond to novel, naturalistic virtual stimuli that evoke approaches in adult mice. We demonstrate that adult mice display two behavioral responses to motion stimuli: approaching and freezing. While juvenile mice approach motion stimuli as often as adults, they rarely freeze. This behavioral difference corresponds to cellular activity measured by immediate early gene (IEG) activation in the periaqueductal gray (PAG) and specific layers of primary visual cortex
(V1). Ongoing experiments seek to understand whether the activity of either midbrain and/or cortical circuits are causal to developmental differences in responses to motion. Ultimately, this work will facilitate our understanding of the mechanisms underlying the development of spatial orientating behaviors in the mammalian brain.

**Disclosures:** K.M. Allen: None. N.M. Procacci: None. J.L. Hoy: None.

**Poster**

**310. Sensorimotor Transformation: Behavior and Neuroprocessing**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 310.09/M17

**Topic:** D.08. Visual Sensory-motor Processing

**Support:** NIH Grant P20 GM103650

**Title:** Experience alters the connectivity between hypothalamus and periaqueductal gray during critical periods of development in the mouse

**Authors:** *R. OLVERA*¹, K. ALLEN², A. AYALA³, J. L. HOY⁴; ¹Biol., Univ. of Nevada Reno, Reno, NV; ²Univ. of Nevada, Reno, Cool, CA; ³Biol., ⁴Univ. of Nevada, Reno, Reno, NV

**Abstract:** The proper development of neural circuits that underlie responses to salient environmental stimuli is essential for an organism’s survival. For example, visuomotor circuits that underlie the innate ability to rapidly obtain prey or flee threat are highly conserved across species. Innate behaviors should be evoked robustly, but also in the appropriate context; e.g. it is beneficial to become more sensitive to stimuli that signal food when hungry, while amplify avoidance responses and decrease sensitivity to food signals when sated. To respond to stimuli in a conditional manner, the brain couples internal states, such as hunger, to circuitry that robustly transforms sensory stimuli into seeking or avoidance behaviors. In more complex organisms, state-dependent modulation of sensory processing may depend on early experience. Here, we provide evidence that early life experience alters the structural connectivity between leptin (nutrient) sensing neurons in the ventromedial hypothalamus (VMH) and those that directly gate avoidance behaviors in the periaqueductal gray (PAG) in the adult. By studying a mouse model of prey capture and using a Cre transgenic approach, we identified a specific projection from VMH to dPAG that is selectively weakened in mice given prey capture experience as juveniles. The density of these projections did not change in adults exposed to the same experiences. Thus, this structural connectivity may be particularly flexible during sensitive periods of development in the mouse. Ongoing studies seek to understand how this structural change induced by juvenile experience influences behavioral responses to sensory stimuli as well as foraging behavior in the adult. We are also working to understand how this connectivity directly influences visually-
guided predation behavior in both adults and juveniles. This work will ultimately further our understanding of how developmental experience shapes the brain to conditionally transform visual stimuli into context-appropriate motor output.


Poster

310. Sensorimotor Transformation: Behavior and Neuroprocessing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 310.10/M18

Topic: D.08. Visual Sensory-motor Processing

Support: NIH F31 NS103305-02
R01NS0779518

Title: Long-range inhibition mediates decision making in the superior colliculus

Authors: *J. ESSIG, J. B. HUNT, G. FELSEN;
Dept. of Physiol. and Biophysics, U. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: Fast and accurate decision making is required for generating purposeful behaviors in a complex environment. Despite numerous innovative experimental and theoretical approaches, how internal states and external stimuli are integrated by neural circuits to produce behavior continues to be elusive. The superior colliculus (SC) is at the center of sensorimotor decision making for survival-based orienting movements. Since the SC has been extensively characterized anatomically, physiologically and functionally across many species, it is poised to serve as a powerful model system to illuminate how activity in neural circuits give rise to behavior. Here, we examine how GABAergic neurons in the SC shape decisions. Mice were trained to select a left or right reward port based on a binary odor mixture and to wait for a ‘go tone’ before initiating a movement, thus giving us access to neural activity during the decision (i.e., the ‘delay epoch’). We first confirmed that SC output promotes contralateral choices by optogenetically exciting glutamatergic SC neurons during the delay epoch and by inhibiting the SC with muscimol. As expected, excitation elicited a contralateral choice bias and inhibition elicited an ipsilateral choice bias. Informed by anatomical and physiological data obtained from slice experiments, we initially hypothesized that GABAergic neurons inhibit adjacent SC motor output neurons (akin to common motifs in cortical circuits) and therefore predicted that these neurons would be most active before, and promote, ipsilateral choices. However, optogenetic identification and activation of GABAergic neurons revealed that they are active before contralateral choices, and driving their activity during the delay epoch elicited a contralateral choice bias. Histological analysis revealed GABAergic neurons projecting to various locations in the midbrain, including the opposite colliculus. We are currently testing if our results are
mediated by commissural GABAergic neurons by optogenetically silencing their terminals during the delay epoch. Together, our findings point to a functional role for long-range inhibitory interactions in the SC, which provide a substrate capable of mediating winner-take-all dynamics during decision making.

**Disclosures:** J. Essig: None. G. Felsen: None. J.B. Hunt: None.

**Poster**

**310. Sensorimotor Transformation: Behavior and Neuroprocessing**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 310.11/M19

**Topic:** D.08. Visual Sensory-motor Processing

**Support:** HHMI

**Title:** Visual feature representation in *Drosophila*

**Authors:** *N. C. Klapoetke*¹, A. Nern¹, E. M. Rogers¹, G. M. Rubin², M. B. Reiser³, G. M. Card¹;

¹HHMI Janelia Res. Campus, Ashburn, VA; ²Howard Hughes Med. Inst. Janelia Res. C, Ashburn, VA; ³HHMI / Janelia, Ashburn, VA

**Abstract:** Visual systems combine lower-level sensory signals (e.g. light intensity) to generate higher-order representations for guiding natural and voluntary behaviors. In *Drosophila*, it is thought that encoding of higher order visual features such as another fly or potential predator may be supported by ~20 classes of lobula visual projection neurons, which convey visual information from the optic lobe to the central brain. Using in-vivo two-photon imaging of these visual projection neurons in response to a wide range of visual stimuli, we show that they have diverse size and motion selectivity. We propose potential mechanisms that enable these neurons to have diverse feature representations and how they may be used to visually guide natural behaviors.

Motor context-dependent dynamic neural processing for fine-scale control of locomotion

**Authors:** *T. FUJIWARA, T. CRUZ, E. CHIAPPE; Champalimaud Fndn., Lisbon, Portugal

**Abstract:** Locomotion is a dynamic process that relies on an accurate estimate of self-motion to adjust to the unpredictable properties of the environment. Such a robust self-motion estimation is thought to depend on the integration of multiple sensory feedback, such as self-generated visual flow and motor-related mechanosensory signals. In addition, this integration must occur flexibly, i.e., depending on motor context, since the properties of sensory feedback depend on the structure of locomotion. However, the nature of such a dynamic multi-modal processing underlying locomotor performance remains poorly understood. Previously, we found that in *Drosophila melanogaster*, a class of progressive motion-sensitive visual cells, the HS cells integrate direction-selective motor-related and visual signals congruently to encode self-rotations during walking (Fujiwara et al., 2017). Here we found that a class of regressive motion-sensitive cells, the H2 cells, also integrates direction-selective, motor-related and visual signals congruently. Like HS cells, H2 cells are thought to contribute to course control. Further, we observed that at high forward walking speed, HS and H2 cells are modulated differentially, with an increase in overall activity in HS cells, and a decrease in direction selective responses in H2 cells. These modulations occur at a fine time scale, and reveal a flexible control of visuomotor interactions for an internal estimate of self-motion that depend on motor context (here defined as a function of forward speed). Furthermore, conditional unilateral silencing perturbations of neural activity, showed that HS activity contributes to locomotion control while H2 does not when the fly walks at high forward speed, further supporting the motor-context dependent contribution of these neurons to the control of locomotion. Therefore, our study reveals an underlying dynamic mechanism for fine-scale motor control that depends on the interaction of multi-modal sensory feedback with motor-related internal signals, and that guarantees flexible
control of the contribution of neural activity to behavior.

Disclosures: T. Fujiwara: None. T. Cruz: None. E. Chiappe: None.

Poster

310. Sensorimotor Transformation: Behavior and Neuroprocessing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 310.13/M21

Topic: D.08. Visual Sensory-motor Processing

Support: HHMI

Title: A gradient of synaptic connectivity underlies directional control of fly escape

Authors: *M. Y. PEEK1, S. NAMIKI2, P. BREADS1, J. POLSKY1, S. ALGHAILANI1, E. TENSHAW1, W. R. WILLIAMSON1, R. PAREKH3, G. M. CARD1;
1HHMI Janelia Res. Campus, Ashburn, VA; 2Univ. of Tokyo RCAST, Tokyo, Japan; 3Janelia Res. Campus/HHMI, Ashburn, VA

Abstract: To investigate the neural basis of directional, visually-evoked behavior, we dissected the circuit mechanisms underlying the control of looming-evoked fly escapes. Utilizing a collection of cell type-specific lines, we identified a set of nine descending neurons that receive input from the same looming-speed-encoding visual projection cell type (LC4) as the giant fiber, a well-studied descending neuron controlling rapid escape. By annotating and analyzing these connections in an electron microscopy dataset, we discovered gradients in the number of synapses between the 55 retinotopically-arranged LC4 neurons, and four downstream descending neuron partners. The synaptic connectivity gradients are unique for each descending neuron cell type and anatomically suggest distinct regions of higher sensitivity to looming stimuli within the visual field. Furthermore, for two descending neurons, optogenetic activation generated shifts in body posture that result in directional escapes opposite to regions of predicted high sensitivity, indicating that these descending neurons map the approach direction of looming stimuli to appropriate directional escape motor programs by a synaptic count mechanism that weighs the sensitivity to looming features across the visual field.

Poster

310. Sensorimotor Transformation: Behavior and Neuroprocessing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 310.14/M22

Topic: D.08. Visual Sensory-motor Processing

Support: HHMI

Title: Action selection and feature detection in neuronal circuits for escape and landing in *Drosophila*

Authors: *J. M. ACHÉ*¹, S. NAMIKI¹, C. R. VON REYN², M. SUMATHIPALA¹, P. BREADS¹, E. ROGERS¹, K. M. BRANSON¹, G. M. CARD¹;

Abstract: To survive, animals must detect and respond to sensory cues in a context-specific manner. Even innate sensorimotor responses are flexible, such that an identical cue can elicit different actions in different situations. How the brain achieves this context-dependent flexibility is unclear. In *Drosophila*, visual looming stimuli elicit an escape takeoff when the fly is standing, but a landing response during flight. To unravel the mechanisms enabling this flexibility, we dissect the neuronal circuits for escape and landing by combining *in-vivo* electrophysiology, neurogenetics, and EM circuit reconstruction.

First, we show that a population of visual projection neurons anatomically specialized to detect looming (LPLC2) mediate the size of visual looming objects to the giant fiber (GF) escape neuron. We establish LPLC2 as necessary for GF-mediated escape, and find LPLC2 and a second population of visual projection neurons (LC4) directly presynaptic to the GF via EM reconstruction. Critically, LPLC2 silencing eliminates the size component of the GF looming response, leaving only the velocity component, whereas LC4 silencing eliminates the velocity component but leaves the size component intact. Selection and timing of the GF-driven escape response is therefore determined by the summation of inputs from two populations of visual feature-detecting neurons.

To understand how the selection of escape or landing is achieved, we conducted an optogenetic activation screen of 130 descending neuron (DN) lines and identified two DN types whose activation drove landing responses. Silencing either DN significantly reduced visually-evoked landing responses, suggesting they are intrinsically important to the control of landing. *In-vivo* patch-clamp recordings revealed that the landing DNs encode visual stimuli and control leg extensions in a graded fashion while the fly is flying. Critically, their visual responses are severely attenuated when the fly is not flying. This gating occurs by separate mechanisms (neuromodulation and motor feedback) in the two landing DN types. Thus, landing DNs are...
gated out when the fly is not flying, such that landing responses cannot be elicited by a looming stimulus. The GF visual responses, in contrast, do not require flight, which enables escape takeoff in standing flies.

Our findings show that sensorimotor flexibility is achieved by state-dependent coupling of sensory networks of the brain to motor networks in the ventral nerve cord.


**Poster**

310. Sensorimotor Transformation: Behavior and Neuroprocessing

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 310.15/M23

**Topic:** D.08. Visual Sensory-motor Processing

**Title:** Development of ecologically-relevant visual stimuli for study of *Drosophila* behavioral response phenotypes

**Authors:** *C. MORROW, T. ORAM, P. BREADS, G. CARD; HHMI Janelia Res. Campus, Ashburn, VA

**Abstract:** In the wild, *Drosophila melanogaster* live in visually complex surroundings in which they must identify approaching conspecifics and predators, and differentiate them from environmental clutter, to select the appropriate behavioral response. While the mechanisms by which many neurons in the early visual pathway encode ethologically relevant object features, such as looming motion, have been recently elucidated, it remains unclear how these visual features are integrated to build perceptions of complex visual objects. We utilized the recently-developed FlyPEZ platform, which provides a means to study behavioral responses to visual stimuli with high-throughput and high spatiotemporal resolution, to display a variety of stimuli based on objects *Drosophila melanogaster* would encounter in the wild. In some cases, these stimuli are developed through 3D kinematic modeling of conspecific or predator behaviors, which are then mapped to the observing animal’s visual field. These modeled behaviors are digitized and can be presented to animals on the FlyPEZ to identify behavioral phenotypes associated with the animal’s response. By observing the differences in behavioral response phenotypes, and identifying the neurons involved in the behaviors, we can gain an understanding of the mechanisms by which flies differentiate between these stimuli.

**Disclosures:** C. Morrow: A. Employment/Salary (full or part-time); HHMI Janelia Research Campus. T. Oram: A. Employment/Salary (full or part-time); HHMI Janelia Research Campus. P. Breads: A. Employment/Salary (full or part-time); HHMI Janelia Research Campus. G. Card: A. Employment/Salary (full or part-time); HHMI Janelia Research Campus.
Poster

310. Sensorimotor Transformation: Behavior and Neuroprocessing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 310.16/M24

Topic: D.08. Visual Sensory-motor Processing

Support: Howard Hughes Medical Institute

Title: Should I stay or should I go?: Mechanisms of survival decisions in Drosophila

Authors: *T. B. ORAM, J. M. ACHE, G. M. CARD;
HHMI Janelia Res. Campus, Ashburn, VA

Abstract: In nature, animals simultaneously face both homeostatic and acute challenges to their survival. For example, a hungry fruit fly requires food to replenish energy and nutrient stores, but feeding on a fallen apple exposes it to the threat of predation. If a predator attacks, the fly must then choose whether to stay on the apple and risk being eaten, or fly away and risk starvation. The fly’s survival thus depends on its ability to integrate environmental stimuli and representations of internal state to produce a rapid response. Here, we studied the neural mechanisms of conflicting-threat resolution in the context of hungry flies responding to predator-mimicking looming stimuli. In response to these stimuli, one appropriate escape behavior in flies is a takeoff (TO). TO sequences can be classified as either a fast, unstable short-duration takeoff (short mode) or a slow, stable long-duration takeoff (long mode). When a fly perceives a looming stimulus, it must decide whether to take off, and if speed or stability is more critical. The Drosophila Giant Fiber (GF) descending neuron is necessary and sufficient for short mode takeoff; alternate descending pathways are implicated in control of long mode takeoff. To determine whether hunger state affected a fly’s decision to take off, we presented starved flies with the choice of staying on a food-bearing platform or escaping from a looming stimulus. We found that, compared to sated flies presented with the same choice, starved flies rarely chose to leave a food source when confronted with a looming stimulus (sated, with food: 29% TO; starved, with food: 9% TO). Moreover, the presence of food did not significantly affect sated flies’ decision to take off (sated, no food: 35% TO). We also found that starvation changed the likelihood a fly would elect a short mode versus a long mode takeoff (starved: 29% short mode; sated: 14% short mode). This suggests that hunger state modulates a fly’s decisions of both whether and how to take off. Finally, when we inhibited GF activity in starved flies using a GF-specific split-GAL4 driver to selectively express Kir2.1 channels (GF x Kir2.1), the takeoff percentage in response to looming stimuli decreased from that of starved wild-type flies in which GF activity is intact (WT, starved: 25% TO; GF x Kir, starved: 5% TO). The TO percentage was unaffected by inhibition of GF activity in sated flies (WT, sated: 39% TO; GF x Kir, sated: 36% TO), suggesting that starvation selectively inhibits the non-GF descending pathways
coordinating long mode takeoff. Thus, hunger state and immediate food cues regulate survival decisions through the differential, context-dependent modulation of descending escape pathways.


Poster

310. Sensorimotor Transformation: Behavior and Neuroprocessing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 310.17/M25

Topic: D.08. Visual Sensory-motor Processing

Support: Howard Hughes Medical Institute

Title: Tracing all presynaptic inputs to the Giant Fiber descending neuron of Drosophila melanogaster in an electron microscopy dataset

Authors: R. PAREKH\(^1\), E. TENSISHAW\(^1\), J. POLSKY\(^1\), S. ALGHAILANI\(^1\), J. S. LAURITZEN\(^1\), D. BOCK\(^2\), *G. M. CARD\(^1\);

\(^1\)HHMI Janelia Res. Campus, Ashburn, VA; \(^2\)Univ. of Vermont, Burlington, VT

Abstract: In the fly, Drosophila melanogaster, visual looming stimuli that mimic an approaching predator prompt the fly to take evasive action. Among other possible escape maneuvers (freezing, turning, etc.), the fly chooses between a short-duration takeoff, which is fast but unsteady, and a long-duration takeoff that risks capture but is more controlled. We previously showed that the timing of a single spike in the Giant Fiber (GF) descending neuron, relative to activity in other descending neurons, determines whether the takeoff executed is short or long. Several inputs to the GF are already known, including Johnston’s Organ neurons, the Giant Commissural neurons, and two visual projection neuron types that determine GF spike timing in response to looming stimuli. However, in addition to immediate visual cues, other sensory and contextual information may be integrated by the GF, affecting its spike timing. Thus to understand how spike timing in the GF is determined, ideally one would identify all of its inputs. Using a serial section TEM (ssTEM) dataset of the female adult Drosophila brain and CATMAID manual annotation software, we identified the single GF neuron in each hemisphere, traced GF dendrites to completion, and traced each unbranched GF axon to its EM volume exit at the neck connective. We tagged every postsynaptic site on the right GF. From these marked synapses, we manually traced the major neurites of each presynaptic neuron. We found 694 neurons presynaptic to the right GF. Of these, 297 were identified based on known morphology (108 JO neurons, 107 LPLC2, 55 LC4, 24 descending neurons, 3 interneurons of the giant commissure). We grouped the remaining 397 neurons into over 100 types using criteria such as morphology, fiber bundle, soma location, synapse location, neuropils innervated, and associated sensory modality. We uncovered innervation patterns in the input neurons including GF branch-
specificity, sensory modality preferences, and synapse count differences within a cell type onto the two GFs. Our traced presynaptic neurons comprise a library of GF input neuron skeletons that can be compared to light microscopy images of GAL4-line expression patterns to facilitate future experiments assessing the contribution of each input type to GF responses and escape behavior in behavioral and electrophysiological experiments with transgenic flies. This work forms the first comprehensive report of all inputs to the GF from EM data. Given the role of GFs in rapid sensory-motor responses, having a complete collection of identified upstream partners will help in understanding the complex circuit underlying motor behavior involving the GFs.


**Poster 310. Sensorimotor Transformation: Behavior and Neuroprocessing**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 310.18/M26

**Topic:** D.08. Visual Sensory-motor Processing

**Support:** NINDS R01NS079518
NINDS R01NS084996
University of Colorado Physiology Department Collaborative Pilot Award

**Title:** Investigating the functional organization of cerebellotectal projections in orienting behavior

**Authors:** *T. YAMAUCHI, T. DOYKOS, A. PERSON, G. FELSEN; Dept. of Physiol. and Biophysics, Univ. of Colorado Sch. of Med., Aurora, CO

**Abstract:** Well executed orienting behavior is fundamental to an animal’s ability to select and acquire targets of interest. This class of behaviors is controlled by a network of several interconnected brain regions, but how activity is coordinated across these regions at the level of neural circuitry is not understood. The cerebellum (Cb), important for motor control, and the superior colliculus (SC), important for target selection and the resulting orienting movement, are key hubs of this network and may play complementary roles in orienting behavior. Anterograde tracing methods across multiple species have consistently shown monosynaptic projections from major Cb output structures, the cerebellar nuclei (CbN), to motor regions of the SC, intermediate and deep layers (int/dpSC). However, the cell type specificity and rudimentary function of these cerebellotectal projections have yet to be examined. Using pseudotyped G-deleted rabies-mediated retrograde tracing methods in Gad2 -cre and VGlut2-cre mice, we assessed the innervation patterns of individual CbNs to excitatory (eSCN) and inhibitory (iSCN) SC neurons. We found the major source of cerebellotectal projections were provided by lateral (LN) and
interposed (IN) nuclei, whereas the medial nucleus (MN) provided fewer projections overall. All CbNs demonstrated stronger projections to eSCNs relative to iSCNs across int/dp SC. While MN specifically targeted rostral eSCNs, LN and IN contacted both rostral and caudal eSCNs with a stronger projection to rostral eSCNs relative to caudal eSCNs. To interrogate the basic function of CbN input to SC, we recorded SC activity in anesthetized mice while stimulating ChR2-mCherry expressing neurons in the IN. Optogenetic activation of IN revealed short-latency drive of rostral and caudal SC neurons. Consistent with our retrograde tracing, histological analysis of these mice revealed synaptic terminals expressing mCherry throughout int/dpSC showing robust labeling in rostral SC. Together, these data provide insights into the functional organization of cerebellotectal circuitry and allow for the examination of how Cb drive to the SC may influence target selection and motor plans.


Poster

310. Sensorimotor Transformation: Behavior and Neuroprocessing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 310.19/M27

Topic: D.08. Visual Sensory-motor Processing

Title: Hippocampus and MST cortex integrate self-movement and location in driving monkeys

Authors: *C. J. D. DUFFY1, W. K. PAGE2;
1Neurol., Penn State Hlth., Hershey, PA; 2Neurol., Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: INTRODUCTION: MST cortex is thought to play a critical role in the processing of self-movement, and the hippocampus is thought to support location sensitivity for cognitive mapping. Here we test whether monkey MST and hippocampus show such distinct roles during active driving by awake monkeys. METHODS: We trained a monkey to steer a room-sized, rail-mounted sled between a randomly selected series of targets drawn from eight locations distributed around the room. Successive steering target locations were designated on a small computer screen to guide the monkey’s joystick steering between locations. Single neuron responses (SNRs) and local field potentials (LFPs) were recorded from MST cortex and the ipsilateral hippocampus as the monkey drove to successive goal locations. RESULTS: Hippocampal and MST LFP spectra showed similar sensitivities to room position and self-movement speed and direction. Likewise, SNRs in hippocampus and MST showed location and movement sensitivities with single neurons in both areas showing a wide range of preferred location and movement sensitivities. Re-analyzing LFP and SNR activity with reference to the current target location revealed that both hippocampus and MST mainly represented the monkeys’ current goal location and best direction from its current location to the goal location.
CONCLUSIONS: Our findings suggest that hippocampus and MST cortex share a great many response properties related to navigation. In particular, during active driving to designated locations, both hippocampus and MST are sensitive to where the monkey is driving to in each driving trial.

Disclosures: C.J.D. Duffy: None. W.K. Page: None.

Poster

310. Sensorimotor Transformation: Behavior and Neuroprocessing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 310.20/M28

Topic: D.08. Visual Sensory-motor Processing

Support: NIH K99-EY028229
R01-EY05729

Title: Retinal optic flow and the visual control of locomotion in real-world terrain

Authors: *J. S. MATTHIS\textsuperscript{1}, K. S. MULLER\textsuperscript{2}, M. M. HAYHOE\textsuperscript{3};
\textsuperscript{1}Biol., Northeastern Univ., Boston, MA; \textsuperscript{2}Ctr. for Perceptual Systems, \textsuperscript{3}Univ. of Texas at Austin, Austin, TX

Abstract: A long history of research on the visual control of locomotion has explored the role of optic flow in the regulation and guidance of human walking. It was generally agreed that the head-centered Focus of Expansion (FoE) lies in a stable location in the walker’s direction of travel, and that humans use this feature to control heading. We used optic flow estimation algorithms to measure head-centered optic flow recorded from the head-mounted camera of a mobile eye tracker of subjects walking in real-world environments. Contrary to the traditional view, we found natural head oscillations during locomotion cause the FoE to move constantly at high velocities within the walker’s field of view. Thus strategies that suggest the FoE is used for heading could not be implemented on the basis of naturally occurring optic flow. In contrast, we found that retinal optic flow contains information that may be used for the control of locomotion. Fixation nulls motion at the fovea, resulting in regular patterns of outward flow across the retina that encode information about the walker’s movement relative to the fixated location. Analyzing retinal flow fields using the curl and divergence operators from vector calculus reveals features that are directly applicable for the control of locomotion. For instance, the sign of retinal curl (which corresponds clockwise/counter clockwise rotation) indicates whether the walker will pass to the left or right of the point they are fixating. In addition, the walkers’ instantaneous, world-centered velocity vector may be derived directly from the divergence across the retinal flow field. These features provide a much richer and more stable source of information for the control of locomotion than the FoE. Furthermore, this information can be extracted directly from
retinotopic visual motion, so a coordinate transform into head-centered coordinates is unnecessary.

**Disclosures:**  **J.S. Matthis:** None.  **K.S. Muller:** None.  **M.M. Hayhoe:** None.

**Poster**

**310. Sensorimotor Transformation: Behavior and Neuroprocessing**

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**Program #/Poster #:** 310.21/M29

**Topic:** D.08. Visual Sensory-motor Processing

**Support:** NSF 1632849

**Title:** The stronger EEG signature of motor preparation for real objects versus images is modulated by graspability

**Authors:**  **G. T. FAIRCHILD**\(^1\),  **F. MARINI**\(^2\),  **J. C. SNOW**\(^1\);
\(^1\)Psychology, Univ. of Nevada Reno Dept. of Psychology, Reno, NV;  \(^2\)Swartz Ctr. for Computat. Neurosci., UCSD, San Diego, CA
Abstract: The human brain evolved for a world of real, physical, three-dimensional (3-D) objects, yet the vast majority of scientific studies of visual cognition have relied on impoverished stimuli presented as two-dimensional (2-D) images - stimuli that do not afford manual interaction. We recently investigated how the action affordances provided by real objects affect cortical brain dynamics by recording electroencephalography (EEG) while observers viewed real-world objects or closely matched 2-D images of the same items. Real objects elicited stronger event-related μ-band (8-13 Hz) desynchronization, attesting to greater automatic motor preparation. This larger μ desynchronization for real objects vs. images persisted both throughout the 800-ms window of stimulus presentation, and ~700 ms beyond stimulus offset. In the current study, we extended this work by examining whether similar patterns of μ desynchronization were apparent when the same stimuli were presented unoccluded (as in our previous study), or behind a clear acrylic glass barrier that eliminated the potential for manual interaction with the (real) objects. Without the barrier, we replicated our previous finding of stronger μ desynchronization for real objects than images both during and after stimulus presentation. Importantly, the barrier attenuated the difference in μ desynchronization between real objects and images during the period of stimulus presentation, while later μ desynchronization differences between stimulus formats after stimulus offset were relatively unaffected. These results suggest that a physical barrier that limits the potential for in-the-moment manual interaction with a real object attenuates the early (but not later) differential in cortical motor preparation signals for real objects vs. images. The early versus late μ desynchronization differences between real objects and images may reflect different underlying cortical processes related to graspability - an early process that is influenced by immediate actionability and a later process that reflects the inherent graspability of real objects.

Disclosures: G.T. Fairchild: None. F. Marini: None. J.C. Snow: None.

Poster

310. Sensorimotor Transformation: Behavior and Neuroprocessing

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Topic: D.08. Visual Sensory-motor Processing

Support: NIH (NINDS-NS078127)
The Sloan Foundation
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Title: The impact of perceptual, cognitive and motor demands on the fidelity of internal time estimates

Authors: *A. AKKAD*, T. V. PARKS, A. C. FERGUSON, M. JAZAYERI;  
1McGovern Inst. for Brain Res., Cambridge, MA;  
2Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Perceptual, cognitive and motor aspects of interval timing are often examined in isolation. For example, sensory anticipation tasks have focused on the perceptual aspects of timing, whereas studies in the motor system have focused on the motor aspects. Natural behavior, however, depends on a confluence of multiple aspects of interval timing. For example, the performance of a musician or an athlete depends critically on the ability to estimate time intervals using multiple temporal cues, retain information about time intervals over delays, and plan multiple movements in accordance with different temporal contingencies. Here, we developed a novel composite timing task for monkeys to investigate the mechanisms by which the nervous system integrates the perceptual, cognitive and motor aspects of interval timing. The task consists of 5 epochs: 1) Fixation: monkeys fixate a central spot and a peripheral saccadic target is presented. 2) Entrainment: monkeys see a visual metronome; i.e., a ring that alternates its position once or twice between the target and fixation points. The tempo is sampled from a prior distribution ranging between 659 and 1139 ms. 3) Memory: monkeys continue fixating during a variable delay period. 4) Production: monkeys shift their gaze toward the target immediately after a ‘go’ cue, and make alternating saccades afterwards between the target and fixation points aiming to reproduce the tempo of the visual metronome in the entrainment epoch. 5) Feedback: monkeys receive reward whose magnitude was adjusted based on the magnitude of error.

Monkeys’ behavior exhibited four important characteristics. First, produced intervals exhibited a characteristic bias toward the mean of the sample distribution, indicating that animals relied on their prior knowledge to optimize their responses. Second, performance improved when the entrainment period consisted of two compared to one alternation. This improvement was manifested by a decrease in both bias and variance. Third, performance was weakly affected by the delay period suggesting that animals maintained a reasonably stable memory of the estimated interval. Fourth, performance during the production epoch deteriorated when animals produced the desired interval twice compared to once; i.e, the second produced interval was more variable and more biased relative to the first. These results provide a rich dataset for understanding the perceptual, cognitive and motor factors that influence the fidelity of temporal information in the nervous system. Electrophysiological experiments are underway to probe the underlying neural mechanisms.

Disclosures: A. Akkad: None. T.V. Parks: None. A.C. Ferguson: None. M. Jazayeri: None.
Poster

310. Sensorimotor Transformation: Behavior and Neuroprocessing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 310.23/M31

Topic: D.08. Visual Sensory-motor Processing

Support: NSF Grant IIS-1524647

Title: Information dynamics in embodied multifunctional recurrent neural networks

Authors: *M. CANDADAI, E. J. IZQUIERDO;
Program in Cognitive Sci., Indiana Univ., Bloomington, IN

Abstract: Understanding how information about external stimuli is transformed into behavior by the nervous system is one of the central goals of neuroscience. This involves characterizing in detail the flow of information through a complete brain-body-environment system: from the task-relevant environmental information, to the sensory stimulus, through the sensory neurons, interneurons, motoneurons, all the way to action. There are a number of experimental challenges to this endeavor, which include access to all variables of interest in the brain-body-environment system over many repeated trials and under different conditions. To address these shortcomings, we adopt a complementary computational modeling approach. We utilize optimization techniques to generate an ensemble of recurrent dynamical neural network models, embodied, and situated in environments, where they perform the behaviors of interest. All relevant variables are simultaneously recorded, and this data is then analyzed using information theory to understand their operation. Specifically, we apply a set of information theoretic tools that are based on a multivariate generalization of mutual information, which extends Shannon’s traditional concepts in key important ways: We unroll information over time and across different trials and tasks; and we consider how information is spread across different variables in the system, thereby measuring redundant, unique, and synergistic information. We use these tools to understand the flow of information in neural circuits at three levels: task-relevant information; sensorimotor information; and neural information. In the current work, we report on the analysis of neural circuits trained to perform multiple visually-guided tasks, including object categorization and affordance perception. Results reveal key insights into the principles of operation of multifunctional neural circuits. First, task-relevant information is represented in a distributed and spatiotemporally dynamic manner within the circuit. Second, synergistic information about sensory stimuli and motor actions reveals neurons that are performing crucial computations in the circuit. Third, the transfer of information within the circuit tends to form task-dependent effective networks. Altogether, our work demonstrates how the tools of multivariate information theory can be used to characterize the information flow throughout a complete sensorimotor circuit and the unique insights that such analyses can provide. Ultimately,
the principles derived from our analysis can be used to drive new experiments and the theoretical framework can be used to analyze existing data.

**Disclosures:** M. Candadai: None. E.J. Izquierdo: None.

**Poster**

310. Sensorimotor Transformation: Behavior and Neuroprocessing

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 310.24/M32

**Topic:** D.08. Visual Sensory-motor Processing

**Support:** NIH Grant U19NS104653

**Title:** Complex social behaviors in larva and juvenile zebrafish are controlled by simple visual cues and are affected by single gene mutations

**Authors:** *R. HARPAZ, A. C. ASPIRAS, A. BAHL, M. C. FISHMAN, F. ENGERT; Harvard Univ., Cambridge, MA

**Abstract:** Dysfunctional social behavior can be detrimental to the survival of individuals in most animal species. Despite its importance, the links between genes, neural circuits and adaptive social behavior are largely unknown. The zebrafish model system, provides a unique opportunity to study the genetic and neural basis of social behavior. Here, we aimed to characterize collective social behavior in larva and juvenile fish, infer how visual social cues drive swimming responses and their underlying genetic control. We analyzed group swimming behavior (5-10 fish) of wildtype larva and juvenile fish and compared it to groups of fish carrying single mutations in genes associated with autism and schizophrenia. We found that from age 7 to 21 days fish transition from swimming in highly dispersed spatial distributions to tight groups. Mutations in the immp2l gene, and in the homer1b gene resulted in a significant increase in group cohesion already at age 7-days that persisted over development. To extract the visual cues that drive fish movement decisions in a group we reconstructed the visual scene of each fish during movement decisions. We found that the visual angle subtended by neighbors on each eye strongly modulated the turning direction and bout rate of the fish. In mutant fish, we found clear alterations in responses to these visual cues, matching the observed increase in group cohesion. Presenting free swimming fish with simple fictive social stimuli to each eye, mimicking the visual occupancy experienced when swimming in a group, elicited similar swimming responses. Finally, we used the identified responses to visual cues to inform mathematical models of fish social interactions. Simulating group swimming behavior using these models we show that the identified social interactions are sufficient to explain behavioral findings for both wildtype and mutant fish over development. Our results link complex collective behaviors to simple social cues already at 7-day old larva.
zebrafish and show that single gene mutations can alter these innate behaviors. Next, these findings will allow us to explore the neural circuits underlying social and collective behavior using optical imaging to acquire whole-brain activity recordings while larva fish engage in fictive social interactions. Comparisons between wildtype and mutant fish with altered social responses will potentially help identify the crucial circuit elements affected by these mutations and their relation to maladaptive social behavior.

**Disclosures:** R. Harpaz: None. A.C. aspiras: None. A. Bahl: None. M.C. Fishman: None. F. Engert: None.

**Poster**

310. Sensorimotor Transformation: Behavior and Neuroprocessing

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 310.25/M33

**Topic:** D.06. Auditory & Vestibular Systems

**Title:** Effects of motor timing training on golf swing motion in Parkinson’s disease

**Authors:** *J. KIM*¹, Z. LEMKE¹, A. L. RIDGEL¹,²;
¹Exercise Physiology., Kent State Univ., Kent, OH; ²KSU Brain Hlth. Res. Inst., Kent, OH

**Abstract:** Individuals with Parkinson’s disease often show deficits in motor timing, specifically during tasks that require rhythmic motor patterns such as gait and finger tapping. However, it is not clear if rehabilitation training that focuses on improving motor timing can improve rhythmicity during coordinated movements. In this study, we utilized a computer-based rehabilitation tool, called Interactive Metronome (IM), which trains individuals to improve motor timing by reacting to an auditory or visual reference cue. Changes in motor timing and coordination were measured using a golf swing motion. The purpose of this study was to examine if IM training with golf swing motion improves motor timing in Parkinson’s disease. Participants completed 12 IM sessions, three times weekly training for 4 weeks. The aim of each session was to perform the golf swing motion to match the audible beat. Visual feedback was given to the participants to encourage them to hit the ‘target zone’ (±15 ms) during the golf swing. Motor timing was assessed using the Long Form Assessment (LFA) which evaluated timing and accuracy during fourteen movement tasks of the hands and feet. A wireless kinematic sensor system was utilized to measure movement acceleration and pitch during the backswing and down swing. Paired t-test were used to compare pre- and post-intervention measures. There was no significant difference in motor timing as measured with LFA, but the motor timing improved after the intervention (pre: 131±76.4 vs post: 54.5±0.7 ms). There was a significant difference between pre-post training in the thigh pitch on the back swing (X pitch) [t=2.766, p=.012] and the down swing (Y pitch) [t=2.93, p=.009]. Although there was no significant difference, acceleration of the pelvis during the back swing improved from 8.5±8.7 to 4.1±2.8
These results suggest that IM training can improve motor timing and acceleration during the golf swing in Parkinson’s disease. In light of these findings, future studies will also examine if MT training promotes improved motor timing and golf swing mechanics in individuals who show impaired range of motion.

**Disclosures:** J. Kim: None. Z. Lemke: None. A.L. Ridgel: None.

**Poster**

**311. Motor Control and Rehabilitation in Primates and Humans**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 311.01/M34

**Topic:** E.04. Voluntary Movements

**Support:** Grant 90RES5013 from the U.S. Department of Health and Human Services, Administration on Community Living, National Institute on Disability, Independent Living and Rehabilitation Research

**Title:** Different speeds and retention periods for motor adaptation to different types of unexpected impedances for target reaching by intact human subjects

**Authors:** *K. OH*¹,², W. Z. RYMER²,¹;

¹Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; ²Shirley Ryan AbilityLab, Chicago, IL

**Abstract:** Impedance-based training has been widely used to enhance recovery of motor performance in patients with neurological disorders. Using an internal model formulated in the cerebellum, humans can estimate load magnitude and load characteristics imposed on their limbs, correct deviations from the planned movements, and plan revised movement trajectories. Even for unexpected changes in load, intact subjects can successfully adapt within a few trials, and could show that movements converge to a specific trajectory. Nevertheless, how swiftly humans can adapt to different types of mechanical impedances and which type of adaptation lasts longer still remain unclear.

In this study, we compared adaptation speed and retention period for motor adaptation between different types of mechanical impedances imposed on via a haptic device during outward reaching in the horizontal pane. We added a rotational spring (1 N-m/rad) referenced to the elbow joint, a mass (5 kg), or a damper (10 N-s/m) each implemented by using the HapticMaster multi degree of freedom robot. Four intact subjects participated in this experiment. A randomly chosen impedance was introduced unexpectedly after 20 trials with a baseline impedance, and an average of movement velocity profiles from last three trials among 10 trials was used to represent a fully adapted velocity profile. The minimum number of trials required for adaptation to reduce a deviation from the fully adapted velocity profile to 15% was then calculated. After testing all three types of impedances, the second phase with the same protocol
was conducted to check if the previous experience could help subjects adapt more promptly. For a given inertial load, our subjects showed relatively smaller number of trials for adaptation (2.3 trials) as compared to the spring (5.3 trials) and the damper (4.7 trials). Interestingly, even after 15 minutes of the first phase and 5 minutes of rest, only spring condition showed the decreased number of trials for adaptation from 5.3 to 2.3 in the second phase. The results imply that both the time required for formulating and updating an internal model for a given load and correcting movements and the retention period for the adaptation may vary with different types of mechanical impedances. Our study may be utilized to identify time-efficient protocols to quickly modify a movement trajectory with relatively long-lasting effects. We still need to analyze if different speeds of motor adaptation correlate with different exposure rates of each type of impedance in daily life, or with magnitude of impedance, or other factors.

**Disclosures:** K. Oh: None. W.Z. Rymer: None.

**Poster**

**311. Motor Control and Rehabilitation in Primates and Humans**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 311.02/M35

**Topic:** E.04. Voluntary Movements

**Support:** NIDILRR Grant 90REGE0005-01
              NICHD Grant 1R01HD072080
              NSF Grant 1632259

**Title:** Visualizing high-dimensional motion and muscle signals

**Authors:** *F. RIZZOGGLIO*1,2,3, A. A. PORTNOVA2,3, D. DE SANTIS2,3, E. ROMBOKAS4, M. CASADIO1, F. A. MUSSA-IVALDI2,3; 1DIBRIS, Univ. of Genova, Genova, Italy; 2Northwestern Univ., Chicago, IL; 3Shirley Ryan Ability Lab., Chicago, IL; 4Univ. of Washington, Seattle, WA

**Abstract:** Body Machine Interfaces (BoMIs) convert high-dimensional body signals into low-dimensional control commands that allow their users to operate external devices. To this end, nonlinear methods for dimensionality reduction, such as Autoencoder (AE) networks, are used to learn a mapping that embeds the low-dimensional latent space as a manifold $M$ into the high-dimensional data (body space, $y$) so that it captures the probability density of $y$. However, interpreting these methods is difficult because of their nonlinear character compounded with the problem of visualizing data distributions in high dimensions. We applied cartographic algorithms, used for representing higher-dimensional maps in 2D, to visualize the nonlinear latent space of electromyographic (EMG) and kinematic data from individuals performing a variety of body and hand movements. To construct a coordinate chart, we superimposed a grid
over the 2D latent manifold obtained after training a nonlinear AE on input data. The AE decoder was applied to the grid to obtain its embedding $M$. First, Euclidean distances within local neighborhoods of $M$ were measured. Then, a matrix ($R$) of global distances was determined by computing the geodesic between each pair of intersection points in $M$. Finally, the original grid was redrawn by displacing all the intersection points so as to match the corresponding distances in high dimension (as specified in $R$). Hence, Euclidean distances between points lying on the distorted grid reflect those between body space data lying on the manifold $M$. Visualization of the latent space was modeled by the nonlinear AE with 2 coding units (CUs) in the latent manifold. Movement/EMG data were fed into the AE encoder to obtain corresponding points in the 2D coordinate chart. This visualization applied to the muscle and kinematic data allowed us to observe distortions in the latent grid of a nonlinear AE as well as to establish the separability of different tasks, muscle synergies, and movements in the latent space. We expect that this technique can be also used to assess learning, for example, by studying how distortions evolve during a learning process. In addition, we aim to understand how visualization of the latent space can aid the user in improving the performance of their controller.


Poster

311. Motor Control and Rehabilitation in Primates and Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 311.03/M36

Topic: E.04. Voluntary Movements

Support: EU Horizon 2020 MSCA REBoT G.A. No. 750464
NIDILRR Grant number 90REGE0005-01
NICHD grant 1R01HD072080

Title: Hybrid EMG-IMU BOdy-Machine Interfaces for promoting voluntary control of muscles after spinal cord injury

Authors: *D. DE SANTIS$^{1,2}$, M. T. PERLMAN$^1$, F. MUSSA-IVALDI$^1$;
$^1$Northwestern Univ., Chicago, IL; $^2$Inst. Italiano di Tecnologia, Genova, Italy

Abstract: High-level injuries to the spinal cord (SCI) compromise function of the upper limb and result in severe deterioration of the quality of life. Studies have shown that learning novel motor skills triggers a process of remodeling at different levels of the Central Nervous System. In particular, there is evidence that corticospinal plasticity is very localized to the muscles engaged in leaning the skill. Hence, the question arises of how to involve in skilled activities muscles for which residual voluntary control is limited by SCI.
Body-machine interfaces (BoMIs) allow their users to perform skillful tasks via artificially remapping available movements after SCI into a suitable control space. They are effective in promoting continued practice, improving upper body strength and help restoring independence. However, one of the major drawbacks of movement-based BoMIs is the lack of control over which muscles contribute to the observed motions. Here we present a novel approach for engaging targeted muscles into skilled activities while operating a movement-based BoMI. EMG signals from muscles of interest are collected while the user learns to operate a IMU-based BoMI. After an initial phase of familiarization with the IMU-BoMI, we evaluate the cross-correlation between a target EMG channel and all IMU signal pairs. The target muscle is then remapped onto the IMU signal with the highest correlation and minimum delay among the possible combinations. Moreover, the degree to which a certain muscle contributes to the selected input of the BoMI is modulated by a weighting factor that scales the contribution of the IMU signal and its EMG-derived reconstruction.

Following this approach, the IMU-BoMI is transformed into a Hybrid EMG-IMU BoMI in its input space, while the mapping from body-motion to control signals itself is left unaffected. In order to evaluate the efficacy of our method in increasing the activity of targeted muscles (Biceps or Triceps brachii), we asked unimpaired volunteers to practice a reaching task with the BoMI using their upper arms and forearms for one session of about 1 hour. Preliminary results show that manipulation of the weighting factor is effective increasing the contribution of the targeted muscle over its antagonist through time.

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Poster

311. Motor Control and Rehabilitation in Primates and Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 311.04/M37

Topic: E.04. Voluntary Movements

Support: Craig H. Neilsen Foundation

Title: 2-dimensional cursor control via EMG from upper cervically-innervated muscles can be learned rapidly via deep learning driven game play

Authors: *R. J. COTTON;
Physical Med. and Rehabil., Shirley Ryan Abilitylab & Northwestern, Chicago, IL

Abstract: Accessing a smartphone is an important part of modern life, but for many people with quadriplegia this can be a significant challenge. Decoding neural population activity is a promising research direction, although requires invasive recordings from the brain. Decoding more easily accessible signals, such as electromyography (EMG) from muscles remaining under
volitional control is another option. However, it also has significant challenges including system performance, refinement for intact muscles, calibration, and portability. Here I describe work to reduce these barriers.

The control system was implemented with wearable sensors using a smartphone application. Specifically, a miniature battery-powered, Bluetooth-enabled, 8-channel differential biopotential amplifier was developed for surface EMG acquisition. The sensor connects to an accompanying Android application for data recording and real-time processing. Multichannel EMG activity was mapped to control signals using a neural network. First a latent space representation is learned from spontaneous movement to find the lower dimensional manifold capturing the spatiotemporal EMG activity. Following this, subjects play a game on the smartphone where they attempt to follow a cursor. This provides a supervised learning signal to train additional output layers that map from the latent space to the control signals. The EMG activity and target location collected during training is streamed from the smartphone to a GPU-powered server allowing real-time training of the network and feedback to the subject. Finally, the optimized network can run the control inference in real time on the phone. The system was demonstrated on a neurologically intact test subject by recording from muscles available in many quadriplegic patients (trapezius, rhomboids, deltoids, sternocleidomastoid).

One advantage of this neural network driven supervised learning is that the movements of the subject do not have to be predetermined and the user can select motions that feel easy or natural. This may benefit people with disabilities who each have unique impairments. In addition, the game-based training procedure with real-time feedback allows the user to quickly see which movements are effective and which are less effective as the network learns the EMG patterns. This addresses some challenges for quadriplegic patients to control a phone via EMG: it uses wireless and portable hardware with a training approach that allows more natural movements and can be rapidly trained.

Disclosures: R.J. Cotton: None.

Poster

311. Motor Control and Rehabilitation in Primates and Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

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Topic: E.04. Voluntary Movements

Support: Italian Multiple Sclerosis Foundation (FISM, 2013-Cod.2013/R/5)
Marie Curie Integration Grant (FP7 PEOPLE-2012-CIG-334201)
Ministry of Science and Technology, Israel (Joint Israel-Italy Lab in Biorobotics Artificial Somatosensation for Humans and Humanoids)
**Title:** Interaction between position and force control in bimanual tasks in unimpaired subjects and people with multiple sclerosis

**Authors:** *G. BALLARDINI*¹, V. PONASSI¹, E. GALOFARO¹, G. CARLINI¹, L. PELLEGRINO¹, F. MARINI², M. MULLER³, C. SOLARO³, P. MORASSO², P. GIANNONI¹, I. NISKY⁴, M. CASADIO¹;


**Abstract:** Proprioceptive deficits are frequently associated with neuromotor impairments that strongly affect daily-life activities, interfering with motor control, learning, and recovery. Despite this, these deficits are poorly understood, less investigated and treated than motor impairments.

Here we aim to assess proprioceptive deficits through the observation of position and force control performance during bimanual tasks, because most daily-life activities require coordinating the motion and the force produced by both hands. Moreover, several neurological diseases induce coordination problems and often affect the two arms differently. In our study participants had to (1) reach a target position with both their hands while holding objects with equal or different weights and (2) exert an isometric force, pushing equally with the two arms in the upward direction, while their hands were maintained in a fixed position at the same or at different heights. Our primary outcome was the difference between the two hands at the end of each trial, in terms of position (task 1), or force (task 2). First, we tested a population of unimpaired subjects and then we provided a first proof of concept that this set-up could be used also for people with Multiple Sclerosis (MS) with a low to moderate level of impairment.

As for the unimpaired participants (20 subjects for task 1; 25 for task 2), we found that the difference in hand positions was greater when the two hands held different weights; instead, the ability to exert forces was influenced by the position of the left hand, regardless of the right hand position and without effects of symmetric or asymmetric arm configurations.

As for MS participants (7 people with MS and different levels of impairment and 7 matched controls) we found that the ability to exert symmetric forces with the two hands was significantly altered in all subjects with respect to healthy controls, independently of the hand configuration. Conversely, their ability to control the position decreased only for subjects with higher level of impairment. These findings, if confirmed on a wider population, could be relevant for the early detection of the disease onset, and to assess how the proprioception and motor control changes in the progress of the disease.

The next step is to modify the device for providing different somatosensory feedback and investigating the influence on performance and training outcomes.

Motor learning of novel dynamic environments for robotic rehabilitation using an exoskeleton

Authors: *C. Pierella*¹, S. Guelfi², N. Rossignol¹, M. Casadio², S. Micera¹; ¹Ecole Polytechnique Federale De Lausanne (EPFL), Lausanne, Switzerland; ²Univ. of Genova, Genova, Italy

Abstract: Stroke is one of the most prevalent causes of impairment in many countries and its incidence continues to rise. The possibility to modify the usually pathological patterns of upper-limb coordination in stroke survivors remains a central issue and an open question for neurorehabilitation. Current therapies for rehabilitation perform assisting movements to help the patient recover some smoothness and regain some control of the impaired limb. Recent works provide evidence that error-enhancing strategies of rehabilitation might prove beneficial for a more efficient recovery. In particular, exposing the patient to a new dynamic environment, i.e. force field while working with a planar robot, is indeed expected to activate motor learning strategies that promote neuromotor adaptation and reorganization. This work exploit the potential of exoskeletons to address the problem of understanding the way the human central nervous system deals with natural upper-limb redundancy for common activities, motor learning and coordination in novel environments. We recruited healthy subjects and, while most studies focused on 2D force fields at the end-effector (EE) of the device, we investigated the possible differences when subjects had to adapt to a 3D force field either at the EE of the exoskeleton or at one of the 2 joints, elbow or shoulder. Subjects worked with ALEX, an upper limb exoskeleton, and were asked to perform a center-out reaching task. Kinematic and muscular activity was recorded during the full training. Results show that all the subjects, with training, were able to adapt to the perturbation introduce by the force field. Moreover the force field applied at the EE and the ones applied at the joints generated different compensatory movement and motor strategies of adaptation at the kinematic and muscular level. This is a first step into gaining a deeper insight on the control mechanisms in healthy subjects and then transfers them to understand and predict behavior of stroke subjects and their recovery. Future perspective will translate this knowledge into robot-based subject-specific rehabilitative approaches suited to tackle the problem of motor coordination in stroke.

Poster

311. Motor Control and Rehabilitation in Primates and Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 311.07/M40

Topic: E.04. Voluntary Movements

Support: Ministry of Science and Technology, Israel (Joint Israel-Italy lab in Biorobotics “Artificial somatosensation for humans and humanoids”) GM is supported by the 'regione liguria' PhD scholarship

Title: Robot-based static and dynamic balance assessments for planning age-tailored training protocols

Authors: *G. MARCHESI¹, A. CANESSA¹, A. DE LUCA², L. DE MICHELI³, A. PILOTTO⁴, F. VALLONE⁴, A. CELLA⁴, M. SPINELLI⁵, A. LEO⁵, C. SANFILIPPO², V. SQUERI², J. SAGLIA², M. CASADIO¹;
¹Univ. of Genoa, UNIGE, Genoa, Italy; ²Movendo Technol. srl, Genoa, Italy; ³Rehab Technologies, IIT, Genoa, Italy; ⁴Dept. Geriatric Care, Orthogeriatrics and Rehabilitation, E.O. Galliera Hosp., Genoa, Italy; ⁵ASST Grande Ospedale Metropolitano Niguarda, Unità Spinale Unipolare, Milan, Italy

Abstract: Aging increases the probability of falling in healthy subjects. Risks of falling are associated with balance deficits and with the inability to counteract unexpected perturbations or instability of the support surface. Currently, there are protocols and devices that allow studying postural control under static and dynamic conditions by using robotic force platforms. Our goal is to define a set of different exercises and parameters that allow quantifying balance deficits and detecting their onset. We tested 160 healthy subjects with ages ranging from 20 to 90. Participants performed both static and dynamic exercises with the Hunova device (Movendo srl, Genoa): in the static test, they stood on a fixed platform both with eyes open and closed. Then they performed two dynamic tests with eyes open. In the first test the platform moved in proportion to the body sway, hence subjects were actively controlling the platform motion. In the second test, the platform tilted in a preprogrammed way around a central pivot, hence the subjects had to compensate the platform motion. During all these tests, the Hunova measured the inclination of the platform and the displacement of the center of pressure, while the trunk movements were recorded with an inertial measurement sensor placed on the sternum. For a comprehensive evaluation of task performance, we computed parameters from both the center of pressure and the trunk oscillations. Then, with advanced classification algorithms we defined the combination of parameters that better discriminates postural performance in subjects with
different ages and that allows detecting the onset of balance deficits. Our protocol was well tolerated by all subjects, independent of their age. Hence, this study can be a starting point for the definition of rehabilitative protocols aiming at preventing falls in older adults, by detecting the onset of balance problems and specifically targeting the skills that firstly deteriorate with age.


Poster
311. Motor Control and Rehabilitation in Primates and Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 311.08/M41

Topic: E.04. Voluntary Movements

Support: NSF Grant 1632259
Binational United-States Israel Science Foundation Grant 2016850
Israeli Science Foundation Grant 823/15

Title: A dysfunctional consequence of visuo-motor adaptation

Authors: I. PLENZIO\textsuperscript{1,2}, K. OH\textsuperscript{3,4}, F. RIZZOGLIO\textsuperscript{1,3,4}, I. NISKY\textsuperscript{5}, E. H. WALKER\textsuperscript{3}, R. A. SCHEIDT\textsuperscript{2,3}, M. CASADIO\textsuperscript{1}, *F. A. MUSSA-IVALDI\textsuperscript{3,4},
\textsuperscript{1}DIBRIS, Univ. of Genova, Genova, Italy; \textsuperscript{2}Marquette Univ., Milwaukee, WI; \textsuperscript{3}Northwestern Univ., Chicago, IL; \textsuperscript{4}Shirley Ryan Ability Lab., Chicago, IL; \textsuperscript{5}Ben-Gurion Univ., Beer-Sheva, Israel

Abstract: The brain determines positions and movements of body parts from inputs arising at least from two different systems: visual and proprioceptive. Interactions between the two sensory modalities have been described in experiments inducing conflict between vision and proprioception during some motor tasks. The dominance of vision over proprioception has been widely reported in the literature and it has been showed that incorrect visual feedback on the position of the hand can bias the perception of hand location.

In this study, we investigated the visuo-proprioceptive conflict when applying contact forces, by developing a new experimental protocol and finding a model capable of predicting the effect of a rotation of the visual field on the estimate of hand position through the control of a contact force. During the experiment, users performed reaching task while holding the end effector of a planar manipulandum. At the end of each reaching movement the end effector was locked, and subjects
were instructed to reproduce, in direction and magnitude, a specified force that they learned during a previous training phase. Subjects wore a virtual-reality headset, preventing them to observe the actual configuration of their arm. We then imposed a rotation of the visual field and allowed subjects to adapt by performing repeated movements. At the end of adaptation, subjects learned to compensate the rotation by moving the hand to a position that differed from the one presented in the display. We observed how this difference affected the contact force that they produced. We considered two different hypotheses: 1) The brain relies on the (unrotated) proprioceptive estimate of hand position to apply the correct force at the end of the reach, resulting in no effect of visual rotation on force error (Proprioception Dominance); 2) The brain relies on the visual estimate of hand position to produce the desired force and generates the same torques it would have applied if the hand were actually in the displayed location (Vision Dominance). Results obtained from a cohort of 11 unimpaired subjects show an overall superiority of the vision dominance model with respect to its competitor. As subjects adapted the hand motion to match the visual display, they effectively learned to discount the truthful proprioceptive information about the configuration of their arm and as a consequence a consistent systematic error emerged in the applied force and amplitude of the applied force vectors with respect to the planned values. We conclude that the dominance of vision over proprioception in the production of free hand movements carries a penalty for force control, a critical component of skillful manipulation.


Poster

311. Motor Control and Rehabilitation in Primates and Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 311.09/M42

Topic: E.04. Voluntary Movements

Support: NIH Grant F31-NS100520

NIH Grant 2R01-NS053606

Title: Proxy process models enable identification of the time course of motor learning from sparse observations

Authors: *P. N. PARMAR*1,2, J. L. PATTON1,2;
1Arms + Hands Lab., Shirley Ryan Ability Lab., Chicago, IL; 2Bioengineering, Univ. of Illinois at Chicago, Chicago, IL

Abstract: Enhanced neurorehabilitation using robotic and virtual-reality technologies require a computational framework that can readily assess the time course of motor learning in order to
recommend the best conditions for training. Error-feedback plays an important role in the acquisition of motor skills for goal-directed movements by facilitating the learning of internal models. In this study, we investigated changes in movement errors during sparse and intermittent “catch” (no-vision) trials, which served as a “proxy” of the underlying process of internal model formations. We trained 15 healthy subjects to reach for visual targets under eight distinct visuomotor distortions, and we removed visual feedback (no-vision) intermittently. We tested their learning data from no-vision trials against our so-called proxy process models, which assumed linear, affine, and second-order model structures. In order to handle sparse (no-vision) observations, we allowed proxy process models to either update trial-to-trial, predicting across voids of sparse samples or update sample-to-sample, disregarding the trial gaps. We exhaustively cross-validated our models across subjects and across learning tasks. The results revealed that the second-order model with trial-to-trial update best predicted the proxy process of visuomotor learning.

Disclosures: P.N. Parmar: None. J.L. Patton: None.

Poster

311. Motor Control and Rehabilitation in Primates and Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 311.10/M43

Topic: E.04. Voluntary Movements

Support: AHA Grant 18PRE34080333

Title: Effects of brief exposure to velocity-based perturbations on movement performance in stroke survivors

Authors: *Y. ABDEL MAJEED\(^1\), S. AWADALLA\(^2\), J. L. PATTON\(^3\);
\(^1\)Bioengineering, Univ. of Illinois At Chicago, Chicago, IL; \(^2\)Sch. of Publ. Hlth., Univ. of Illinois at Chicago, Chicago, IL; \(^3\)Sensory Motor Performance Prog, Rehab Inst. Chicago, Chicago, IL

Abstract: The first step towards improving clinical outcomes for stroke survivors is understanding the relationship between movement metrics and clinical performance. Our efforts to model change in clinical outcomes using movement data suggested that velocity- or speed-based training has the highest potential to improve clinical outcomes. First, we needed to find the most effective method of increasing patient speed. We devised a crossover paradigm in which each patient is exposed to three types of velocity-based forces: negative viscosity, positive viscosity, and breakthrough. The breakthrough condition involved positive viscosity up to the 70th percentile of a patient's speed during baseline, after which viscosity was removed as a 'reward' for reaching faster speeds. We evaluated the effects of each of these force types using changes in two primary metrics, maximum speed and maximum perpendicular distance, before
and after exposure to each force type. Here, we present the results of this exploration. We are using the most effective force type in this paradigm as the basis for a longer-term intervention, where our main outcome measure is patient performance on standard clinical assessments.

**Disclosures:** Y. Abdel Majeed: None. S. Awadalla: None. J.L. Patton: None.

**Poster**

311. Motor Control and Rehabilitation in Primates and Humans

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 311.11/M44

**Topic:** E.04. Voluntary Movements

**Support:** NIH Grant R01NS053606 – 05A1

**Title:** Movement distribution variables that best predict clinical assessment in stroke

**Authors:** *Z. A. WRIGHT*\(^1,2\), J. L. PATTON\(^2,1\), F. C. HUANG\(^2\);
\(^1\)Bioengineering, Univ. of Illinois at Chicago, Chicago, IL; \(^2\)Arms + Hands Lab., Shirley Ryan AbilityLab, Chicago, IL

**Abstract:** We investigated probability distributions of several movement variables to find the best predictors of upper limb impairment during self-directed motor exploration. Our past work analyzed endpoint and joint kinematics (position, velocity, acceleration), which relate to some clinical descriptions of impairment that include, range of motion and interjoint synergies. However, motor impairments often manifest in terms of weak neural drive or changes in muscle force production, which are perhaps more directly attributed to kinetic variables (i.e. relating force and energy to motion; including endpoint force, joint torque and joint power). We hypothesized that kinetic variables would be most important for predicting individual differences in impairment level, measured using Fugl-Meyer scores (UEFM). Stroke survivors (n = 22) were asked to perform a motor exploration task with affected limb, moving at various speeds and directions, while trying not to repeat the same patterns. For each participant, we computed two-dimensional probability distributions (20 bins x 20 bins) of each variable (nine in total). Since there are a large number of bins (400 bins per variable), many of which are likely mutually correlated, we first applied principal component analysis across all distribution variables. We then performed multiple regression analysis (with LASSO regularization) using the reduced feature set (21 principal component scores) to predict UEFM scores. To evaluate model performance, we used the PRESS statistic which is a modified coefficient of determination (R2) calculation for leave-two-out cross-validation. With all variables included in our model (full model), the variance explained was 29.7 ± 11.3% (mean ± standard deviation R2). Next, we performed three separate runs of the PCA-LASSO method removing a single variable domain per run, and then compared the differences in predictive performance with respect to the results...
of the full model. We found that model performance decreased the most when we removed endpoint kinematic variables (change in $R^2$, -46.0%; mean ± standard deviation $R^2$, -16.3 ± 10.4%) followed by joint kinematic variables (change in $R^2$, -30.0%; mean ± standard deviation $R^2$, -9.7 ± 11.2%). Removing kinetic variables modestly increased model predictions (change in $R^2$, 10.3%; mean ± standard deviation $R^2$, 40.0 ± 8.6%). Despite overall modest predictive performance, these results indicate that distributions of kinematic variables are most important for predicting clinical UEFM scores.

**Disclosures:** Z.A. Wright: None. J.L. Patton: None. F.C. Huang: None.

**Poster**

311. Motor Control and Rehabilitation in Primates and Humans

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 311.12/N1

**Topic:** E.04. Voluntary Movements

**Title:** Velocity error augmentation in learning a visual distortion task

**Authors:** *F. C. HUANG*¹, J. L. PATTON², C. FRITSCHE³;
¹Shirley Ryan Abilitylab, Chicago, Illinois, IL; ²Sensory Motor Performance Prog, Rehab Inst. Chicago, Chicago, IL; ³Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland

**Abstract:** This study tested a novel form of assistance for motor skill training: Velocity Error Augmentation (VelEA). Our task paradigm considered error as the lateral deviation of the hand moving along a path between a start to a stop target. Our augmentation scheme makes use of robot rendered forces, designed to be proportional to only the components of velocity that point perpendicularly away from the straight line representing the ideal path. From a causality point of view, error in velocity precedes errors in position space. Hence, we hypothesized that augmentation of velocity error would, in particular, facilitate adaptation of planning, or feedforward aspect of control. As a secondary hypothesis, we expected that augmentation forces could help adaptation when visual feedback is not reliable. In order to test VelEA, we developed a 3-dimensional point-to-point reaching task using a 3-DOF robot and stereo feedback visual display. The protocol consisted of four phases: baseline, training, testing, and washout. As a novel visuomotor environment, we presented a 60-degree visual rotation during the training and testing phases. We tested twenty neurologically intact subjects, randomly assigned to two groups (force and control). During the training phase, the force group received forces based on velocity error augmentation, while the control group did not receive forces. Our results were striking in that while the direct effects of training with forces were strong, the results in learning were quite similar. Our analysis showed that both groups adapted to the novel environment and exhibited characteristic after-effects. The groups were comparable in pre/post change for maximum perpendicular error. However, the force group significantly reduced depth error (p=0.0008),
whereas the control group exhibited only marginally significant reductions (p=0.053). These preliminary results suggest that VelEA can benefit motor skill training in cases with limited visual feedback. It is also possible that the velocity-based forces in this study promoted increases in co-contraction, which diminished or masked the effects of learning. Another possibility is that the nervous system places a greater priority on spatial error than on higher-order movement variables. This study provides an important foundation for the study of velocity error augmentation. Further study is needed for how to better facilitate adaptation specifically such higher order variables with forces or other forms of augmentation.

**Disclosures:** F.C. Huang: None. J.L. Patton: None. C. Fritsche: None.

**Poster**

**311. Motor Control and Rehabilitation in Primates and Humans**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 311.13/N2

**Topic:** E.04. Voluntary Movements

**Support:** KAKENHI

**Title:** The integrative role of the M1 for the motor sequence learning

**Authors:** *Y. H. Hamano1, S. K. Sugawara2,3, M. Fukunaga2,3, N. Sadato2,3; 1Natl. Inst. For Physiological Sci., Okazaki, Aichi, Japan; 2Natl. Inst. for Physiological Sci., Okazaki, Aichi, Japan; 3SOKENDAI, Hayama, Japan

**Abstract:** Primary motor cortex (M1) is known to be crucial in motor learning. Our previous study demonstrated that distinct motor engrams are formed depending on the training modes, specifically left anterior intraparietal sulcus with the speed pressure training (maximum mode, as quickly accurately and as possible) and bilateral dorsal premotor areas and M1 through visually cued training without speed pressure, (constant mode) (Hamano et al. 2019). It is unknown if the motor engrams generated by different learning procedures are integrated into the M1. Evaluation of the motor engram in M1 is difficult because their active states are hard to discriminate from the motor execution per se. As preparatory activity is known to reflect the parameters of the upcoming movement, we hypothesized that the retrieval of motor engrams generated by different learning modes is reflected as learning related increase in the preparatory activity of M1. To test this hypothesis, we evaluated the preparatory activity during the learning of sequential finger tapping with the non-dominant left hand using 7T MRI. The task was consisted of maximum mode and constant mode alternatively with the rest epoch right after each epoch of both modes for 30 minutes duration of time. Participants performed the sequential finger tapping task as quickly and as possible during the max mode, and as accurately as possible with the 2 Hz paced sequence specifying visual cue during the constant mode. At the beginning of each epoch, the
instruction message appeared for 2 secs to instruct participants to do either maximum or constant mode. We estimated the preparatory activity map related to each instruction. Then, we calculated the increased activities across the training in each mode. We found a training-related increase in preparatory activity in the network covering bilateral anterior intraparietal sulcus and inferior parietal lobule extending to right M1 during the maximum mode, and right M1 during the constant mode. Conjunction analysis showed the preparatory activity in right M1 commonly increased in both training modes. Present findings indicate that right M1, as the last effector of the motor output, integrates the distinct motor engrams generated by different training modes.


Poster

311. Motor Control and Rehabilitation in Primates and Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 311.14/N3

Topic: E.04. Voluntary Movements

Title: Altered alpha and beta oscillations in parietal and occipital cortices in chronic jaw pain

Authors: *W.-E. WANG, R. HO, S. A. COOMBES;
Univ. of Florida, Gainesville, FL

Abstract: Motor- and pain-related processes separately induce a reduction in alpha and beta power over sensorimotor cortex. When movement and pain occur simultaneously, the effects on alpha and beta power are additive, but this has only been demonstrated in healthy adults when the pain eliciting stimulus and the movement are spatially and temporally overlapping but are independent of one another. Hence, very little is known about the cortical processes underlying motor-evoked pain. In the current study, we combined high-density electroencephalography with a paradigm in which motor-evoked pain was induced during a visually-guided jaw force task. Eighteen human participants with chronic jaw pain and sixteen control participants produced jaw force at 2% and 15% of their maximum voluntary contraction (MVC). We report 2 novel findings. First, compared to controls, task performance in the chronic jaw pain group was associated with an increase in motor-evoked jaw pain, an increase in motor variability, and an increase in motor error. Second, rather than being additive, motor-evoked pain attenuated the modulation of alpha and beta power, and this was most evident over parietal and occipital regions. Our findings provide the first evidence of the neural basis of motor-evoked jaw pain, and are in line with previous studies that link chronic pain with deficits in attention and visuomotor brain networks.

Disclosures:  W. Wang: None. R. Ho: None. S.A. Coombes: None.
Title: Neural correlates of decreased functional connectivity related to sensorimotor control dysfunction in persons with multiple sclerosis

Abstract: Sensorimotor control of visually guided arm movements uses visual feedback to relate current limb position with a desired location. To compensate for long feedback delays, an internal prediction of movement is used to enable corrective movements. In persons with Multiple Sclerosis (PwMS) who have upper extremity dysfunction, a mismatch between the predicted and actual visual feedback delay during goal-directed movement has been reported. The goal of this research was to identify neurological correlates of the visual delay mismatch using electroencephalography (EEG) to characterize brain activity while subjects performed a reach and hold task.

Seven PwMS (5 male, 33-58 years) and 6 gender and age-matched controls (4 male, 32-60 years), all right handed, performed a reach and hold task using a 1-D passive wrist manipulandum while 64-channel EEG was recorded simultaneously. During the task, participants were asked to continually place a user-controlled cursor onto a target. The target would pseudorandomly move once every 3.0-5.5 seconds to a new position throughout ten, 50 second trials. Cross-correlation between the target displacement and the participant’s response was used to estimate the visual feedback delay of each participant. The predicted visual delay was quantified using a submovement interval analysis of the velocity data. After typical EEG preprocessing, task related magnitude squared coherence (MSC) was calculated for all electrode pairs in the alpha [8 12] Hz and beta [14 30] Hz frequency ranges during target reach and stabilization to measure the synchronization between brain regions.

On average, PwMS had a larger mismatch between the predicted and actual visual feedback...
delay. Compared with controls, PwMS had a decreased local MSC (average MSC of the adjacent electrodes) over parietal and motor regions for alpha frequencies and over motor regions for beta frequencies. In the alpha band, the visual delay mismatch in PwMS was well correlated with the MSC between contralateral parietal electrode (CP5) and ipsilateral parietal electrodes across subjects. More extensive correlations were seen in the beta band between occipital-parietal (O2:CP5), occipital-motor (O2:C3), and parietal-motor (CP5:C6) electrodes. These results suggest upper extremity dysfunction with visual delay mismatch is associated with a reduction in functional connectivity within sensory integration areas of the brain. Future studies will examine how the changes in cortical connectivity impact cerebellar-cortical processing of goal directed movements in PwMS.


Poster

311. Motor Control and Rehabilitation in Primates and Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 311.16/N5

Topic: E.04. Voluntary Movements

Support: Faculty of Health Sciences, University of Tromsø, Norway 2016/5455
Norwegian Health Association (Nasjonalforeningen for folkehelsen) 2016/4882
Norwegian Health Association (Nasjonalforeningen for folkehelsen) 2014/888

Title: Associations of cortical thickness and surface area with dexterity measures in mild cognitive impairment

Authors: *S. A. CASTRO-CHAVIRA¹, O. VASYLENKO², T. R. VANGBERG³, M. M. GORECKA³, K. K. WATERLOO¹, C. RODRIGUEZ-ARANDA¹;
¹Dept. of Psychology, Univ. of Tromsø, Tromsø, Norway; ²Dept. of Psychology, ³Univ. of Tromsø, Tromso, Norway; ⁴Univ. Hosp. of North Norway, Tromsø, Norway

Abstract: Novel clinical research showed that manual dexterity deteriorates in Mild Cognitive Impairment (MCI). However, no experimental data corroborating this finding exists. Because cerebral atrophy is documented in MCI, the present study aims to explore the association between dexterity declines as measured by a detailed kinematic analysis and gray-matter cortical thickness and surface area in patients with MCI and healthy older controls. Both dominant and non-dominant hand abilities were investigated. Method: 16 right-handed MCI patients (mean age 70.4 y, 10 women) and 20 right-handed healthy elderly (mean age 70.4 y, 13 women) were tested with the Purdue Pegboard test and video recorded with Vicon Motus to calculate movement...
times (MT) and kinematic parameters for reaching, grasping, transport and inserting. T1-weighted MR brain images, acquired on a 3T MR scanner, were processed using the FreeSurfer pipeline. The associations of cortical surface area and thickness with MTs and kinematics, including path length and linear velocity, were analyzed with the general linear model controlling for age and gender, and results were corrected for multiple comparisons. Behavioral results: A main effect of hand was observed across all dexterity measures. For MTs, group differences were found for reaching (p<0.000) and grasping (p<0.01), while interactions existed between hand and group (p<0.01) in reaching and transport. Mean linear velocity (MLinV) showed interactions on reaching (p<0.001) but no group differences. For path length, there were significant interactions during grasping (p<0.001) and inserting (p<0.01). For grasping, only path length differed between the groups (p<0.001). MRI results: No group differences were found on cortical thickness or surface area. However, we found an interaction between group and gender in the left lateral orbitofrontal cortex. A positive association existed between cortical thickness and MLinV during transport of pins on right hand in left frontal and parietal regions (11 clusters, 2.88<z<5.92) and a positive correlation between cortical surface and MLinV for transport was found on left hand, which differed between MCI and controls in the right pars triangularis. This correlation had a steeper slope for controls. Conclusions: The MCI subjects had dexterity declines in terms of larger MTs for both hands and disproportionate path lengths for the left hand. Although groups did not differ on MLinV, this measure was the only one showing a correlation that differentiated the groups where larger cortical thickness corresponded to faster hand movements.


Poster

311. Motor Control and Rehabilitation in Primates and Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 311.17/N6

Topic: E.04. Voluntary Movements

Support: NSF Grant FP00014638

Title: Action planning emerges from competition between value and motor representations

Authors: *J. M. FINE, S. BOEGE, M. SANTELLO;
Arizona State Univ., Phoenix, AZ

Abstract: Individuals must often choose how to move among potential alternatives before a motor goal is specified. This is experimentally examined using a ‘go before you know’ task, wherein individuals start reaching towards multiple potential targets before a target is specified.
Initial movements are often aimed midway between targets when they are equally likely. Early theories posited this phenomenon occurred via dorsal pre-motor (PmD) areas representing multiple motor plans that compete for expression and are ‘incidentally’ averaged. Averaging can be biased by both differential target-reward or -spatial separation. These averaging effects indicate motor plans compete for expression in a value and sensorimotor space. An alternative explanation is that midway reaching results from goal-uncertainty, decisions are indifferent to sensorimotor contingencies, and only a single motor plan is represented. Herein, we tested a third alternative: initial reach decisions are driven by ‘purposeful’ (Bayesian) averaging of motor plans: initial reach plans emerge from an adjudication between control policies weighted by their expected utility denoted by target value and risk. We tested this in humans performing a “go before you know” reach task with two potential targets. We scaled relative monetary reward and risk of targets. By defining ‘risk’ as the target size scaled to individual’s baseline motor variability, we could generate an expected utility of each target. Behaviorally, initial reach angles were strongly and linearly related to the relative expected utility (REU) of potential targets ($R^2=0.85$), even though targets were equally likely to be specified. REU effects on initial angles were independent of reaction time ($r=0.05$), delays in initiation were unrelated to motor plan expression. This indicates decisions involved an averaging competition process in both value and motor spaces. We examined source-based EEG of sensorimotor, frontal, and parietal cortices to estimate neural planning processes involved in this purposeful averaging. Supporting our hypothesis, PmD planning activity encoded both value and risk, and scaling of its activity (power) was contingent on the relative expected target utility, rather than a target with maximum utility. Our behavioral and neural results support a purposeful averaging mechanism in motor planning.


Poster

311. Motor Control and Rehabilitation in Primates and Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 311.18/N7

Topic: E.04. Voluntary Movements

Support: NASA 80NSSC17K0021

Title: Resting-state functional connectivity and ophthalmic changes associated with a spaceflight analogue environment

Authors: H. MCGREGOR¹, J. LEE², N. GADD³, I. KOFMAN³, Y. DEDIOS³, J. BLOOMBERG⁴, A. MULAVARA³, *R. D. S. SEIDLER³; ¹Univ. of Florida, Gainesville, FL; ²DLR, Cologne, Germany; ³KBRWyle, Houston, TX; ⁴NASA Johnson Space Ctr., Houston, TX; ⁵Univ. of Florida, Gainesville, FL
Abstract: Following spaceflight, astronauts exhibit functional remodeling of the brain, impairments in sensorimotor performance and, in approximately 30% of cases, ophthalmic abnormalities collectively known as Spaceflight Associated Neuro-ocular Syndrome (SANS). Astronauts are exposed to microgravity and elevated CO₂ levels onboard the International Space Station. It is unclear how microgravity and elevated CO₂ combine to affect the brain and behavior. We investigated this issue using 6° head-down tilt bed rest with elevated ambient CO₂ (HDTBR+CO₂) as a spaceflight analog. This bed rest intervention was the first of its kind to induce signs of SANS in a subset of the healthy subjects. Here we present differential profiles of brain and behavioral changes following bed rest for those subjects who developed signs of SANS compared to those who did not. We examined brain function in terms of resting-state functional connectivity. We assessed sensorimotor behavior using dynamic posturography to assess balance, and a rod and frame test to assess visual sensory bias. Eleven healthy subjects participated in this study. Subjects underwent a 14-day baseline phase followed by 30 consecutive days of HDTBR in 0.5% ambient CO₂. By the end of the HDTBR+CO₂ phase, 5 of the 11 subjects had developed optic disc edema, a sign of SANS. After bed rest, subjects underwent a 14-day recovery phase which involved physiotherapy. Resting-state fMRI and behavioral measures were each acquired twice during the baseline phase, twice during the HDTBR+CO₂ phase, and twice during the recovery phase. We found that those subjects who developed signs of SANS during HDTBR+CO₂ exhibited different functional connectivity patterns prior to bed rest compared to subjects who did not develop signs of SANS. Subjects who exhibited functional connectivity between the insula, motor, and memory brain areas at baseline were those who went on to develop signs on SANS during HDTBR+CO₂. Following bed rest, subjects who developed signs of SANS exhibited increased functional connectivity between primary visual and premotor cortices, as well as decreased functional connectivity between posterior cingulate and primary visual cortices. These connectivity changes are suggestive of bed rest-associated multisensory reweighting. Supported by NASA 80NSSC17K0021.


Poster

311. Motor Control and Rehabilitation in Primates and Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 311.19/N8

Topic: E.04. Voluntary Movements

Support: NRF-2016R1D1A1B03936326
NRF-2017M3C7A1047227
Title: Neural mechanisms of obstacle avoidance: Obstacle avoidance differs from planning and execution of collision-free trajectory

Authors: *J.-K. RYU¹², H. JOO³, S. KIM¹⁴, M. SEO³, K.-M. LEE⁵³;

Abstract: Humans and animals can move safely and efficiently in very complex environments. The ability to avoid obstacles plays a vital role in successful movements in complex environments as well as the ability to approach the target. Although it is widely known that parietal cortex plays an important role in moving towards the target, much remains to be unknown about the neural mechanisms of obstacle avoidance.

The purpose of this study is to examine the neural mechanisms of obstacle avoidance and to show that obstacle avoidance cannot be explained by the planning and execution of collision-free paths, which is often referred to as waypoint passing.

Forty-eight healthy adults participated in the study and all procedures were performed with the approval of Seoul National University IRB. The participants used a joystick in the fMRI scanner to make the cursor displayed on the screen to arrive at the target point as accurately and quickly as possible. Experimental conditions consisted of four types: direct movement, obstacle avoidance, waypoint passing, and stopping. In the direct movement condition, participants moved the cursor to the target point as fast as possible. In the obstacle avoidance condition, participants moved the cursor to the target point avoiding collision with the obstacle. In the waypoint passing condition, the participants moved the cursor to the target point through the intermediate waypoint. In the stop condition, participants waited without moving the cursor. All conditions were presented in random order. The collected fMRI data were analyzed by SPM 8 software, and the neural activities related to obstacle avoidance were examined through the contrast between obstacle avoidance, waypoint passing, and direct movement conditions.

The analysis showed that the neural activity of the right superior parietal cortex was larger in the condition of avoiding obstacles than the condition of the waypoint passing. This neural activity did not appear to be significant in the stopping condition and the direct movement condition, and the activity of the right superior parietal cortex was confirmed to be a unique neural activity to avoid obstacles.

If obstacle avoidance is simply planning and executing collision-free trajectories, there should be no difference in the neural activity between obstacle avoidance condition and waypoint condition. However, we showed that the neural activity involved in obstacle avoidance was different from that of waypoint passing. Therefore, it was confirmed that avoidance of obstacles cannot be explained by simply planning and executing a collision-free trajectory.

Poster

311. Motor Control and Rehabilitation in Primates and Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 311.20/N9

Topic: E.04. Voluntary Movements

Support: NASA Grant #80NSSC17K0021
NSBRI SA02802

Title: Functional neural changes in response to head-down tilt bedrest with elevated CO$_2$ during a sensorimotor dual task

Authors: *A. D. MAHADEVAN$^1$, K. E. HUPFELD$^1$, J. K. LEE$^2$, Y. E. DE DIOS$^3$, I. S. KOFMAN$^3$, N. E. GADD$^3$, J. J. BLOOMBERG$^4$, A. P. MULAVARA$^3$, R. D. SEIDLER$^1$;
$^1$Dept. of Applied Physiol. and Kinesiology, Univ. of Florida, Gainesville, FL; $^2$DLR (Deutsches Zentrum für Luft- und Raumfahrt e.V, Cologne, Germany; $^3$KBRwyle, Houston, TX; $^4$NASA Johnson Space Ctr., Houston, TX

Abstract: Spaceflight and microgravity cause well-characterized changes in sensory weighting processes, central fluid distribution, and musculoskeletal structure and it is important to assess how these changes affect our ability to integrate sensorimotor information. Ground-based spaceflight analogues incorporating 6° head-down tilt bedrest (HDBR) are often used to simulate microgravity and its associated physiological changes but, recently, it has been hypothesized that combining HDBR with elevated carbon dioxide (CO$_2$) might better mimic the conditions of a closed environment like the International Space Station. Dual task cost is a sensitive indicator of change in central motor function, and one that is affected by HDBR. Here, we examine how a 30-day HDBR intervention, including 0.5% ambient CO$_2$, changes human performance and functional brain activity during single and dual task conditions. The task involved three phases in which participants i) counted the number of times an oddball stimulus was presented among distractor stimuli; ii) tapped one of two buttons in response to a visual cue at a rate of 1 Hz; and iii) performed both tasks concurrently. 11 participants (6 males) underwent functional MRI (fMRI) during the task: twice prior to HDBR+CO$_2$, twice during HDBR+CO$_2$, and twice following HDBR+CO$_2$, allowing us to assess changes with HDBR+CO$_2$ as well as subsequent recovery. Behavioral measures included reaction time and tapping accuracy during both the tapping and dual conditions, as well as counting accuracy during the counting and dual conditions. fMRI data were evaluated at an uncorrected $p<.001$. These exploratory analyses revealed areas following varying patterns of change and recovery, with some regions displaying only an initial change with the intervention while other regions displayed a dose-dependent effect. Across all task conditions, changes were found in the precentral, postcentral, and superior frontal gyri, all areas associated with sensorimotor or cognitive functions. Patterns of activation
change as they correlate with behavioral change differed by task condition. The tapping condition showed better accuracy/shorter reaction time with more activation, but the dual task condition showed a more compensatory pattern. When compared to our previous HDBR investigation, we found that the increased ambient CO₂ may have altered patterns of activation change seen throughout the brain. Together, these findings provide information about the additional and/or interactive effects of CO₂ administration with HDBR and about how spaceflight conditions may impact the brain’s ability to maintain function during sensorimotor interference.


**Poster**

**311. Motor Control and Rehabilitation in Primates and Humans**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 311.21/N10

**Topic:** E.04. Voluntary Movements

**Support:** DARPA FA8750-18-2-0259  
NSF 1630178  
EEC 1028725

**Title:** Spectral power fluctuations induced in electrocorticography during spontaneous upper-limb movements

**Authors:** *S. M. PETERSON, S. SINGH, R. P. N. RAO, B. W. BRUNTON;*  
Univ. of Washington, Seattle, WA

**Abstract:** Much of our knowledge of human motor movements have come from relatively short, controlled laboratory paradigms. However, it is unclear how well these results generalize to natural settings, where actions occur spontaneously and throughout the entire day. Therefore, we are motivated to study long-term electrocortical activity "in the wild," where movements are unconstrained by experimental paradigms. We analyzed electrocortical activity from 4 patients with intractable epilepsy using electrocorticography (ECoG) as they performed spontaneous wrist movements across 5 days of clinical recording. Wrist movements were identified by applying computer vision to continuous video recordings (Wang et al. 2018). To quantify movement-related electrocortical activity, we computed time-frequency power spectrograms at every contralateral surface electrode, averaged across over 500 spontaneous events. Based on previous ECoG findings performed in a controlled experimental setting (Miller et al. 2007), we expected movement events to decrease ECoG power at low-frequencies (8-32 Hz) and increase
power at high-frequencies (76-100 Hz), primarily in electrodes near sensorimotor cortex. Our results corroborated this expectation (Figure 1). In addition, high-frequency power fluctuations were localized to sensorimotor areas, while low-frequency power fluctuations were more widespread across the cortex. We found a consistent spectral power pattern that spatially overlapped between subjects and across days during spontaneous movements. Using this framework, we can explore how electrocortical power varies across days and in a greater number of subjects. Such an approach could enhance our understanding of how cortical activity relates to unconstrained, natural behavior outside of traditional experimental settings.

![Image of EEG spectrograms](image)

*Figure 1: Electrocorticography (ECoG) spectral power during spontaneous wrist movements for 2 of the 4 subjects, with an example spectrogram showing how we averaged spectral power across frequency bands.*

**Disclosures:**  S.M. Peterson: None. S. Singh: None. R.P.N. Rao: None. B.W. Brunton: None.

**Poster**

**311. Motor Control and Rehabilitation in Primates and Humans**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 311.22/N11

**Topic:** E.04. Voluntary Movements

**Support:** NSF Grant 1835317

**Title:** Mobile brain imaging in cooperative and competitive tennis play

**Authors:** *T. C. Hauge*¹, A. K. STUDNICKI², R. D. SEIDLER¹, D. P. FERRIS²;
¹Applied Physiol. & Kinesiology, ²Biomed. Engin., Univ. of Florida, Gainesville, FL

**Abstract:** Recent advances in EEG hardware and data analysis methods allow for examination of brain activity while participants freely move outside of the laboratory (e.g. Gramann et al., 2011; Nordin et al., 2019). Here, we leverage this technology to study the brain dynamics of cooperative and competitive interactions while participants play tennis. Previous functional MRI
work on the neural bases of cooperative versus competitive dyadic interactions show increased activation in the prefrontal and orbitofrontal cortex during cooperation as opposed to competition (Decety et al., 2004; Lee et al., 2018). Additional work in EEG shows increased mu suppression in sensorimotor regions in dyadic competitive interactions versus cooperative/perceptual interactions (Perry et al., 2011). We hypothesize that electrocortical activity will show increased theta and alpha spectral power in the medial prefrontal cortex and left medial orbitofrontal cortex during cooperative versus competitive play conditions. We also hypothesize that dorsolateral prefrontal cortex will exhibit increased synchronization during competition, and that there will be greater mu suppression at 8-13 Hz in the sensorimotor cortex during competition compared to cooperation. In this project, tennis participants are outfitted in mobile, high density dual-layer EEG equipment (BrainVision™), inertial measurement units (Cometa™) for upper limb kinematics during play, and instrumented insoles (novel™) to record force and stance data related to gameplay. All data streams are synchronized with a square wave pulse. Subjects play with a human player in both competitive and cooperative settings. We examine changes in power spectra across multiple frequency bands (theta, alpha, beta, and gamma) as well as differences in event related spectral perturbations time-locked to racquet-to-ball contact. The findings of this study contribute to the larger body of knowledge surrounding the link between the brain and the body during dynamic movements executed in a real-world environment. Moreover, results will explore the influence of dyadic interactions during real-time sport game play and how changing social contexts are expressed in neural dynamics during tennis.


Poster

311. Motor Control and Rehabilitation in Primates and Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 311.23/N12

Topic: E.04. Voluntary Movements

Support: Postdoctoral Fellowship from JSPS Grant 26-119

Title: Task-evoked and spontaneous brain activity characterizes individual differences in a function dictating frequency-rhythmicity relationship

Authors: *K. UEHARA¹², H. TOGO³, T. HANAKAWA³;
¹Div. of Neural Dynamics, Natl. Inst. for Physiological Sci., Okazaki, Japan; ²Integrative Brain Imaging Ctr., ³Dept. of Advanced Neuroimaging, Integrative Brain Imaging Ctr., Natl. Ctr. of Neurol. and Psychiatry, Kodaira-Shi, Japan
Abstract: When controlling a sequential movement such as keystrokes during piano playing, precise rhythmicity is required even though movement-frequency is increased. However, this ability has high individual variability that is likely to arise from neural systems. Yet, little is known about the neural substrates underlying individual differences in a frequency-rhythmicity (FR) function. To address this, we investigated relationships between task-evoked activity or spontaneous brain activity and an FR function during rhythmic movement. During task-fMRI, twenty-four healthy participants were asked to perform a finger tapping task with their right index finger at four different rates (0.25, 1, 2, 3 and 4 Hz) paced by auditory cues. Resting-state fMRI was acquired before the task-fMRI. As a measure of tapping rhythmicity, we calculated a coefficient of variation (CV) of the inter-tapping intervals in the 1-4 Hz conditions in each individual. To define individual differences in an FR function, CV values from 1 to 4 Hz were fitted by a linear regression model within each participant. The beta variable obtained from the linear regression model from each participant was treated as a parameter representing the FR function. After preprocessing of task-fMRI data, a first-level general liner model analysis included a parametric regressor modeling 5 different movement rates to capture brain activity correlated with the tapping rates. A correlation analysis between the FR function and task-related brain activity was then performed at the second level. The resting-state networks were examined based on regions of interests covered the whole brain. We then explored resting-state networks correlated with the FR function in a second-level. Task-related activity in the anterior cingulate cortex (ACC) and right middle frontal gyrus (MFG) was negatively correlated with the tapping frequencies, and, moreover, accounted for individual differences in the FR function. Individual differences in the FR function were also correlated with spontaneous activity fluctuation in the resting-state networks linking between the left ACC and right middle temporal gyrus (MTG) and between the right paracingulate gyrus and left MTG. Our findings suggest that individual differences in the FR function may be tagged with neural systems within task-evoked and spontaneous brain activities.

Disclosures: K. Uehara: None. H. Togo: None. T. Hanakawa: None.

Poster
311. Motor Control and Rehabilitation in Primates and Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 311.24/N13

Topic: E.04. Voluntary Movements

Title: The asymmetry of antagonistic muscle systems makes equilibrium joint postures dependent on muscle strength

Authors: *R. RAMADAN, G. SCHÖNER; Ruhr-Universität Bochum, Bochum, Germany
Abstract: Central to Equilibrium Point thinking is that movement commands are spatial variables, the muscle threshold lengths, $\lambda$ [Feldman, 1995]. When agonist and antagonist muscles are symmetrical, a joint reference configuration, $R$, can be computed from these threshold lengths. Realistic muscle systems, however, comprise muscles of different lengths, insertions, and strengths so that agonist and antagonist muscles are not symmetrical. The zero crossing of active joint torque then depends not only on muscle threshold lengths, but also on the levels of force generated by each muscle. Based on a neuro-muscular model of control, we simulated arm movements by obtaining time courses of muscle threshold lengths, $\lambda(t)$, that minimized their change while bringing about movements of a prescribed direction, extent, and duration. From the simulated movements, we computed at each point in time, the virtual attractor position to which the arm would converge in the absence of external forces [Hodgson, 2000]. We compared such virtual attractor joint trajectories to the time courses of the joint reference trajectories, $R(t)$. We found that virtual attractor trajectories were shifted from joint reference configurations such that stronger muscles were shorter. Significant structural differences between the two types of trajectories were consistent with threshold lengths, $\lambda(t)$, reflecting constraints at the level of muscle force, including interaction and inertial torques.

Disclosures: R. Ramadan: None. G. Schöner: None.

Poster

311. Motor Control and Rehabilitation in Primates and Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 311.25/N14

Topic: E.04. Voluntary Movements

Title: Inverting a model of neuro-muscular control to estimate central commands for fast reaching movements

Authors: C. HUMMERT, *G. SCHONER;
Inst. für Neuroinformatik, Ruhr-Universität Bochum, Bochum, Germany

Abstract: A longstanding debate surrounds the complexity of central commands for fast reaching movements. Early evidence for N-shaped virtual trajectories [2] suggested that central commands reflect biomechanical constraints. Simulations of a model that takes biomechanics, muscle dynamics, and neural reflex loops into account [1] provided evidence that monotonic ramp-like virtual trajectories may be sufficient central commands to overcome interaction torques and inertial coupling [1,3]. Direct estimation of the descending commands within such models may be a way to settle such questions. We developed a two-step inverse dynamics approach, in which joint torques are first estimated from kinematic data in the conventional way, and then descending commands are estimated by inverting the model of muscle force generation under reflex control [1] under a number of
simplifying assumptions. We applied the method to a data set that samples workspace and movement time. Participants performed planar movements involving the shoulder and elbow joint. Two long (0.4m) and six shorter (0.25m) movements sampled workspace. Fast (400ms duration) and slow (800ms duration) conditions sampled movement speed. The estimated commands approximated a monotonic ramp from initial to end position for the slow movements, while commands for fast movements had a non-monotonic form, first overshooting and then returning to the target.


Disclosures: C. Hummert: None. G. Schoner: None.

Poster

311. Motor Control and Rehabilitation in Primates and Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 311.26/N15

Topic: E.04. Voluntary Movements

Title: Reconstruction of threshold muscle lengths underlying reaching movements

Authors: *L. ZHANG, G. SCHÖNER;
Ruhr Univ. Bochum, Bochum, Germany

Abstract: According to the Equilibrium Point Hypothesis, arm reaching movements may result from changes in a referent arm configuration that specifies the threshold lengths at which muscles begin to be activated. Muscle activation and force generation is driven by the deviation of the actual from the referent configuration. This study aims to reconstruct commanded threshold lengths underlying single-joint reaching movements, based on a referent control model that integrates biomechanical muscle properties. We first performed unloading experiments at 4 levels of initial load. To keep commanded muscle thresholds invariant, subjects were instructed not to intervene after the unloading of the pre-loaded wrist muscles. We recorded voluntary wrist movements at different speeds (fast, medium and slow) and reconstructed the time course of threshold muscle lengths by fitting the model to experimental kinematics and EMG patterns. Reconstructed threshold muscle lengths were non-monotonic (N-shaped) during fast movements but monotonic and similar to the joint trajectories during slow movements. The N-shaped commanded threshold lengths may reflect dynamical properties of the neuromuscular system.
during fast movements. Possible influences of reciprocal inhibition and of other factors on the estimation of the time course of commanded threshold lengths are discussed.

**Disclosures:** L. Zhang: None. G. Schöner: None.

**Poster**

311. Motor Control and Rehabilitation in Primates and Humans

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 311.27/N16

**Topic:** E.04. Voluntary Movements

**Support:** NIH RNS096083A

**Title:** On the consistency of preferred movement speed

**Authors:** *R. J. COURTER, IV*¹, A. A. AHMED²;
¹Integrative Physiol., Univ. of Colorado Boulder, Boulder, CO; ²Mechanical Engin., Univ. of Colorado, Boulder, CO

**Abstract:** Understanding preferred movement speed is vital for characterizing overall health and for indicating underlying movement disorders. Preferred walking speed correlates with mortality in older adults, while diseases like Parkinson’s exhibit movement slowing [1], [2]. Though preferred locomotion speeds are relatively well understood, preferred reaching speeds have not yet been well described [3]. We sought to develop a robust protocol to elicit preferred reaching speeds in an objective manner by minimizing external or instructional biases. We tested whether subjects converged to a preferred speed, and whether this speed was conserved across individuals. Subjects were required to make a series of 10cm reaches from a central starting circle to one of 8 radial targets. Each subject performed reaches under 5 prescribed durations (400, 550, 700, 850, and 1000ms) in randomized order. Initially, subjects were required to move to the target within the prescribed duration while “held” within constraints of +/−25ms. The time constraints on the duration slowly loosened, or “released,” unbeknownst to the subject, allowing reaches to be performed slower or faster as desired. The speed at which a subject tended to converge was considered their preferred speed. These speeds were compared to pre and post unconstrained, baseline reaches. Using a linear mixed effects model, we found a main effect of time (p << 0.001) and condition (p << 0.001). There were no between subject differences in preferred reaching speeds (p > 0.05). There was a significant time-condition interaction (p << 0.001), indicating that subjects increased or decreased peak velocity at different rates dependent upon the prescribed duration. Preferred reach duration lies somewhere between 550 and 700ms. unconstrained reaches showed high variability in speeds within and between subjects, suggesting that unconstrained reaches may not be an adequate metric for preference. Results indicate that young adults have similar preferred speed when making 10cm reaches, around
0.276 m/s, and tend to exhibit elasticity in returning to this preference, supporting our initial hypotheses. Understanding the preferred reaching speeds is a vital metric for understanding how the CNS executes motor control, and how movements alter with age or disease.


Disclosures: R.J. Courter: None. A.A. Ahmed: None.

Poster

311. Motor Control and Rehabilitation in Primates and Humans

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Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 311.28/N17

Topic: E.04. Voluntary Movements

Support: NIH RNS096083A

Title: Effect of travel effort on movement vigor during foraging

Authors: *S. SUKUMAR*¹, R. SHADMEHR², A. A. AHMED³;
¹Dept. of Computer Sci., Univ. of Colorado Boulder, Boulder, CO; ²Dept Biomed. Eng, Johns Hopkins Univ. Dept. of Biomed. Engin., Baltimore, MD; ³Departments of Mechanical Engin. and Integrative Physiol., Univ. of Colorado, Boulder, CO

Abstract: During foraging, animals stay at a site and harvest reward, and then travel to the next reward site with a certain vigor. This behavior entails decision-making regarding how long to stay and harvest, and motor-control regarding how fast to travel to the next reward opportunity. Previous work has generally focused on the decision-making problem, producing the Marginal Value Theorem (MVT) [1]. However, the question of how the brain determines vigor of movements remains poorly understood. In a previous study [2], we extended MVT [1] to predict how travel vigor should vary as a function of the experience of the animal. That theory predicted that experience of low reward rates should result in slower movements between reward sites. Here, we tested some of the predictions of this theory in humans during a task that entailed reaching between reward patches.

We designed an arm reaching experiment that emulated a patch-foraging task. Seated subjects (n=6) performed horizontal planar arm reaches to travel between “patches” of reward. Once in a patch, reward was dispensed at a hyperbolically depleting rate. Subjects were free to leave a patch in favor of the newly replenished one at any time. Travel effort between patches was modulated by simulating added mass (m) via forces generated by the robot. Subjects performed foraging in two environments each with 200 trials, one with high travel effort (m = 3.5 kg) and one with a low travel effort (m = 0 kg). The order of the experienced environments was...
randomized across subjects. 40 trials in each environment were deemed ‘probe’ trials with intermediate travel effort (m = 2kg). Vigor of reaches was quantified via peak velocity and movement duration.

We observed that people reduced the vigor of their reaches in response to higher effort. Vigor was higher in the low effort trials (m=0kg) compared to probe trials (m=2kg, p<0.001), and was correspondingly lower in the high effort trials (m=3.5kg, p<0.001), as predicted by the MVT model. However, the theory additionally predicted that vigor associated with identical travel effort, would also be modulated by the travel effort in the environment. Contrary to model predictions, there was no significant difference in vigor between probe trials (m=2kg) in the two environments (p>0.1). Our findings therefore suggest that travel effort modulates the utility rate of the environment differently than assumed by the MVT framework.

REFERENCES

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Poster

312. Cortical Planning and Execution: Neurophysiology in Humans

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Topic: E.04. Voluntary Movements

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Title: Distinct neural signals in speech preparation differentially modulate auditory responses

Authors: *S. LI1,2, H. ZHU2,3, X. TIAN2,3,
1East China Normal Univ., Shanghai, China; 2NYU-ECNU Inst. of Brain and Cognitive Sci., Shanghai, China; 3New York Univ. Shanghai, Shanghai, China

Abstract: Actions influence sensory processing in a complex way to shape behavior. For example, it has been hypothesized that during action, a copy of motor signals -- termed corollary discharge or efference copy -- can be transmitted to sensory regions and modulate perception (motor-to-sensory transformation). Such mechanisms have been evident among animal species
and are extended to human speech production and control. Previous speech studies focus on the interaction between production and perception during articulation. However, the functional specificity of motor signals along the entire course of speaking, including intention, preparation, and execution, is far from clear. Theories have been proposed that corollary discharge and efference copy are two separate forms and generated at different stages of actions. Specific in speech, we hypothesized that the content in the motor signals available at distinct preparation stages determined the nature of signals (corollary discharge vs. efference copy) and constrained its modulatory functions on sensory processing. We tested this hypothesis in 3 experiments by recording electroencephalography (EEG) in a novel preparation-perception-delayed-vocalization paradigm. In Experiment 1, participants (total of 16, 5 males, mean age of 23.1) prepared to vocalize a syllable according to visual cues that were either symbols (general preparation, GP) or syllables in red (specific preparation, SP). When a syllable in green appeared, participants rapidly pronounced it. During each preparation stage, a 1k Hz pure tone or a syllable sound was presented to probe the modulatory function of preparatory signals. We found that SP enhanced the neural responses to the prepared syllables around 100ms after sound onset (presumably efference copy). In Experiment 2 (19 participants, 5 males, mean age of 23.9) where only GP condition was included and trials without auditory stimuli were added, we found neural suppression (presumably corollary discharge). In both experiments, the modulation effects were absent in the responses to tones that were hard to produce with human articulators and irrelevant to the task, suggesting the signals available during preparation and their functions are motor and action specific. In Experiment 3 (17 participants, 4 males, mean age of 23.9), the attentional modulation were different from motor preparatory modulation in the first experiment, and hence we ruled out the confound. These consistent results suggest that before action execution distinct motor signals can be generated in the motor-to-sensory transformation and integrated with sensory input to modulate perception.

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Poster

312. Cortical Planning and Execution: Neurophysiology in Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

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Topic: E.04. Voluntary Movements

Title: The effect of spatial and verbal working memory on different inputs to the corticospinal neuron

Authors: M. W. LENIZKY, *S. K. MEEHAN;
Kinesiology, Univ. of Waterloo, Waterloo, ON, Canada
Abstract: Volitional allocation of cognitive resources modulates afferent input projected on to corticospinal neurons. Short-latency afferent inhibition provides a method to probe the modulatory effects of somatosensory afference upon corticospinal output. Short-latency afferent inhibition involves preceding a monophasic transcranial magnetic stimulus with electrical stimulation of the corresponding peripheral nerve. Manipulating the monophasic current direction of the transcranial magnetic stimulus provides a method to probe the modulatory effect of somatosensory afference to specific corticospinal inputs. For example, we previously demonstrated that non-spatial attention selectively modulated somatosensory projections of corticospinal inputs sensitive to the anterior-posterior (AP) stimulating current whereas verbal working memory demands modulated somatosensory influence over inputs sensitive to both posterior-anterior (PA) and AP stimulating current. The present study determined whether spatial working memory load selectively influences somatosensory afference to PA- and AP-sensitive corticospinal inputs. Participants completed two similar sessions during which short-latency afferent inhibition was elicited, using PA or AP monophasic current, during the maintenance period of a modified Sternberg short-term memory task. Session 1 - Spatial memory task: Participants were required to encode a spatial display and maintain the spatial array in working memory to determine whether a probe matched or did not match the original display. The spatial array consisted of either two or six dots arrayed around a central fixation cross. Session 2 - Verbal memory task: Participants were required to encode and maintain a set of letters arranged in a concentric circle around a central fixation cross. The probe consistent of a single letter and participants indicated whether the probe was part of the initial set of letters. The verbal set consisted of either two or six letters. Preliminary results suggest that increasing spatial set size selectively reduced AP short-latency afferent inhibition, whereas increasing verbal working memory set size decreased both PA and AP short-latency afferent inhibition. The present results suggest that spatial and verbal working memory influence different inputs to the corticospinal neuron. The different inputs may provide distinct pathways by which declarative strategies can shape procedural motor control.

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Poster

312. Cortical Planning and Execution: Neurophysiology in Humans

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Topic: E.04. Voluntary Movements

Support: NIH R01 MH069456

Title: Intracranial evidence for retrieval of movement goals by the hippocampus
Abstract: Motor skills rely on the accurate selection of movement. For example, a baseball batter must produce the correct swing at just the right moment. The information indicating what movement is required is often encoded in arbitrary sensory cues (e.g., angle of pitcher’s arm, spin of ball laces), whose interpretation requires memory recall. There is ample evidence that neural activity in the neocortex, especially primary motor cortex (M1) and dorsal premotor cortex (PMd), during the pre-movement period represents information pertaining to the upcoming movement. How are pre-movement representations of movement goals instantiated in these cortical areas when elicited by sensory cues with no direct affordances? We hypothesized that the hippocampus facilitates preparation of movement in M1 and PMd through a predictive coding mechanism when the goals of that movement rely on recall of learned arbitrary visuomotor associations (VMAs). Prior studies have found that the hippocampus is necessary in order to learn arbitrary VMAs and that neural activity in the hippocampus changes as VMAs are learned. To test this hypothesis, we conducted a VMA task in eight human participants who were undergoing intracranial monitoring during pre-surgical evaluation for treatment of otherwise intractable epilepsy. Each participant had one or more depth electrodes implanted into their medial temporal lobe (MTL), with at least one contact in the hippocampus as well as in MTL cortical areas. Many also had electrodes on the cortical surface. The VMA task required participants to make reaching movements with their upper limb using a digitizing joystick to one of four possible target locations presented on a computer screen. Prior to some of these reaches, a cue indicating the location of the upcoming target was presented. Cues took the form of either the pre-presentation of the target itself or the presentation of an arbitrary symbol. Participants memorized the target location that each arbitrary symbol indicated prior to the experiment. Trials without any cue were also included. We found that the hippocampus responded selectively at the time of the cue when movement goal information was specified through a symbol requiring recall, compared to directly by a target or in cases without a cue. In comparison, at the time of the movement and the ultimate appearance of the target, the hippocampus responded similarly across conditions. Electrodes in the neocortex did not show this differential response to the presentation of the symbolic cue. These results are consistent with a model for the hippocampus as recalling movement goals signified by sensory cues, indicating a role for memory systems in motor skill.

**Abstract:** Developmental dyslexia (DD) is generally viewed as a disorder involving phonetics, with the underlying cause that of poorly processing sound. Many people with DD also exhibit difficulties with the temporal aspect of movement sequencing. It is well established that the pre-supplementary motor area (SMA) and SMA, involved in motor planning and execution of movement sequences, are also a part of the broad network for sound processing. One position is that the preSMA/SMA serves as part of a larger cortical-subcortical *temporal processing network* that underlies processing sound (Kotz et al., 2010, Schwartz et al., 2012; Schwartz et al., 2011). Therefore, when rhythmic movement (repetitive finger tapping) is performed in concert with rhythmic sound, the synchrony or desynchrony (variability) of this behavior and the activation pattern of the preSMA/SMA may provide insight into this temporal processing network in young adults with DD. **PURPOSE.** The purpose of this study was to examine EEG activity over the preSMA/SMA during finger tapping with and without a pacing tone in young adults with and without DD. It has been found that children with DD exhibit a dominance of theta band activity (4-7 Hz) at rest (Papagiannopoulou & Lagopoulos, 2016), which was interpreted as abnormal hypoarousal mechanisms, and sustained theta activity during linguistic tasks (Spironelli et al., 2008). We hypothesized that theta band activity would be greater in young adults with DD during rest and during finger tapping with or without a tone, and that theta would further increase in DD when tapping off beat. Furthermore, we hypothesized that intertap interval (ITI) variability would be greater in the DD group. **METHOD.** Participants were age- and gender-matched, and all were right-handed. They engaged in the following conditions: sitting quietly at rest with eyes closed (rest), sitting quietly at rest with eyes open (baseline), tapping with their right-hand index finger in synchrony with and without a tone at 70 bpm, doing the same at 140 bpm, and tapping off beat at 70 bpm with and without the tone. Analyses focused on EEG collected from electrode Cz (area over the preSMA/SMA). **RESULTS.** The power spectrum pattern of results supported our hypotheses. Theta activity at Cz was higher in those with DD during tapping at both rates, with or without the tone and tapping off the beat. Also, ITI variability was greater in the DD group. We interpret these data as support for the position that
movement sequencing is more difficult for young adults with DD and that the study of the preSMA/SMA is a productive approach to better understand linkage between movement sequencing and sound processing.


Poster

312. Cortical Planning and Execution: Neurophysiology in Humans

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Topic: E.04. Voluntary Movements

Support: NSF Grant 1753915
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Title: Realignment in visuo-proprioceptive estimates of hand position: Somatotopic specificity and the role of sensory cortices

Authors: *C. R. SEIGEL, J. L. MIRDAMADI, S. D. HUSCH, K. D. MORGAN, H. J. BLOCK; Indiana Univ. Bloomington, Bloomington, IN

Abstract: Spatial realignment of visual and proprioceptive hand position estimates can occur in response to a perturbation. E.g., viewing the hand underwater while washing dishes: water refracts light, shifting the visual estimate of the hand away from the proprioceptive estimate. The brain compensates for this misalignment by realigning visual and proprioceptive estimates of the hand. Such perceptual learning presumably affects the hand perception used in motor planning, raising the question of whether multisensory integration and motor control share a common sensorimotor map. Our recent experiments support this hypothesis. Using transcranial magnetic stimulation (TMS), we detected excitability changes in the primary motor cortex (M1) index finger representation after subjects experienced misaligned, but not veridical, visuo-proprioceptive information about the index finger (N=29). The effects were somatotopically focal: Strongly apparent in the index finger representation ($\beta=-0.99$ for proprioceptive and $0.42$ for visual realignment, $p<0.05$), weakly apparent in the pinky finger representation ($\beta=-0.96$ and $-0.19$, NS), and absent in the forearm and biceps representations. A behavioral experiment (N=13) further supports this interpretation: We asked subjects to indicate their proprioceptive estimate of the knuckle, wrist, and elbow, in addition to the misaligned fingertip. Proprioceptive realignment in the knuckle was similar to the fingertip, but realignment in the wrist and elbow estimates were significantly less ($p<0.05$). This suggests the effects of misaligned visual information at the fingertip are somatotopically focal.
Interestingly, subjects who realigned proprioception more than vision had decreased M1 excitability in the index finger representation, while subjects who realigned vision more than proprioception had increased M1 excitability. This suggests a modality-specific neural mechanism, such as modulation of somatosensory cortex or dorsal stream visual areas that impact M1. Indeed, short latency afferent inhibition (SAI) changes were significantly correlated with the magnitude of subjects’ proprioceptive realignment ($\beta=-1.84$, $p<0.01$), suggesting changes in somatosensory inputs to M1 were involved ($N=21$). We will next use a paired pulse TMS technique to assess whether changes in visual pathways to M1 are associated with subjects’ visual realignment. Taken together, these results suggest visuo-proprioceptive realignment is associated with somatotopically focal physiological changes in the sensory and motor systems, consistent with a common sensorimotor map for multisensory and motor control.


Poster

312. Cortical Planning and Execution: Neurophysiology in Humans

Location: Hall A

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Topic: E.04. Voluntary Movements

Support:   JSPS KAKENHI Grant Number JP18H03141
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Title: Non-invasive transvertebral magnetic stimulation discloses residual motor function of sublesional spinal circuitry in humans with spinal cord injury

Authors: *T. TAZOE$^1$, M. SUZUKI$^1$, M. KANESHIGE$^1$, K. IWATSUKI$^2$, Y. NISHIMURA$^1$; $^1$Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan; $^2$Senbokujinnai Hosp., Sakai city, Japan

Abstract: The interruption of supraspinal descending motor drives due to spinal cord injury (SCI) results in the paralysis of all muscles innerved by the sublesional spinal motoneurons. However, most of motoneurons spared from actual damages preserve their function. In humans, it is hard to examine residual function in sublesional spared motoneuronal circuitry. To address this issue, we used non-invasive magnetic stimulation delivering to the sublesional lumbar spinal cord in individuals with and without spinal cord injury. Single pulses transvertebral magnetic stimulation (TVMS) were given to 18 intervertebral pointes from T11 to L5 with three cascades. We mapped the peak-to-peak amplitude of stimulus-evoked responses in the bilateral leg muscles; iliopsoas, gluteus maxima, quadriceps, biceps femoris, tibialis anterior, soleus. In 13 intact participants, we acquired spinal motor maps with a rostro-caudal configuration in which proximo-distal leg muscles were represented. In 8 participants with SCI, the neurological level of
injury (NLI) was clinically diagnosed as C8 in 1 participant, T2-T12 in 6 participants, and L1 in another 1 participant. Six SCI participants exhibited that TVMS illustrated the preserved motor maps in the paralyzed leg muscles. In rest of 2 SCI participants, TVMS failed to elicit clear evoked-muscle responses in any leg muscles. The presence of TVMS-induced responses was not relevant with the completeness or level of injury. Our results indicated that the motor function was occasionally preserved in the sublesional spinal motoneuronal circuitry. TVMS is a useful diagnosis approach to discloses residual spinal motorneuronal circuitry.


Poster

312. Cortical Planning and Execution: Neurophysiology in Humans

Location: Hall A

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Topic: E.04. Voluntary Movements

Support: The Swedish Research Council 2017-00892
The Swedish Research Council for Sport Science P2017-0068

Title: Does fMRI reveal different brain response in persons with anterior cruciate ligament reconstruction compared to controls during a novel knee proprioception test?

Authors: *A. STRONG¹, H. GRIP², C.-J. BORAXBEKK³, C. K. HÄGER¹;
¹Dept. of Community Med. and Rehabilitation, Physiotherapy, ²Dept. of Radiation Sciences, Biomed. Engin., Umeå Univ., Umeå, Sweden; ³Danish Res. Ctr. for Magnetic Resonance, Copenhagen Univ. Hosp. Hvidovre, Copenhagen, Denmark

Abstract: Injury to the anterior cruciate ligament (ACL) of the knee is common, particularly during sporting activities, and causes damage to and/or loss of its proprioceptors. Knee proprioception, believed important in prevention of and recovery from injury to the joint, appears worse among those who have suffered an ACL injury compared to asymptomatic persons. Further, studies showing different brain response in persons post-ACL reconstruction during simple knee flexion/extension movements indicate neuroplasticity due to the injury and/or subsequent surgical reconstruction. However, brain response post-ACL reconstruction during knee proprioception tests has yet to be established, partly due to methodological constraints. We therefore aimed to develop a novel knee proprioception test suitable for simultaneous functional magnetic resonance imaging (fMRI) and implement the test in order to map proprioception-related brain response post-ACL reconstruction. 21 persons who had received ACL reconstruction (ACLR) 7 months to 5 years previously and 21 age-, sex-, and activity level-matched controls (CTRL) performed our novel knee joint position sense (JPS) test firstly in a
movement laboratory and secondly during simultaneous fMRI. Three-dimensional motion capture systems, also integrated in the MR environment, recorded knee angles to provide test instructions and knee JPS outcome measures. The order of leg and two target angles (40 and 65 degrees knee flexion) was randomised. Blood oxygen level-dependent (BOLD) contrast imaging was used to assess brain response during the knee JPS test. Preliminary analyses show overactivation in frontal and motor areas of ACLR compared to controls when attempting to reproduce the knee joint angles. Our study confirms the successful development and implementation of a novel knee proprioception test for simultaneous fMRI to investigate knee proprioception-related brain response. The preliminary results build on previous studies indicating neuroplasticity among persons with a reconstructed ACL and provide added evidence regarding specific brain regions utilised for knee proprioception. This understanding may help to inform targeted rehabilitation strategies for improved outcomes. Ongoing analyses will provide further information regarding brain response among our groups as well as assess the ability of both groups to reproduce the knee joint angles.

Disclosures:  A. Strong: None. H. Grip: None. C. Boraxbekk: None. C.K. Häger: None.

Poster

312. Cortical Planning and Execution: Neurophysiology in Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 312.08/N25

Topic: E.04. Voluntary Movements

Title: Neurophysiology of motor skill learning in chronic stroke

Authors: *R. MOONEY1,2, J. CIRILLO1,2, C. M. STINEAR3,2, W. D. BYBLOW1,2; 1Dept. of Exercise Sci., 2Ctr. for Brain Res., 3Dept. of Med., Univ. of Auckland, Auckland, New Zealand

Abstract: Despite early motor recovery, many stroke survivors are left with residual upper limb impairment at the chronic stage. Motor learning is relevant in chronic stroke for acquiring and improving the execution of compensatory strategies to motor control deficits. However, the neurophysiological mechanisms underlying motor skill acquisition with the affected upper limb after stroke have received little systematic investigation. The aim of this study was to assess the modulation of corticomotor excitability and inhibition within ipsilesional primary motor cortex (M1) during motor skill learning in chronic stroke. Ten people with chronic stroke and twelve neurologically healthy age-matched controls trained with their paretic and nondominant hand respectively on a sequential visual isometric wrist extension task. Each participant’s speed-accuracy function was determined to quantify skill before, immediately after, 24 hours 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 before and immediately after training. Both groups exhibited successful skill acquisition and retention, but absolute skill level was lower in chronic stroke participants compared with controls. In contrast to controls, ipsilesional corticomotor excitability was not modulated after skill acquisition, which may in part be attributed to heightened long-interval intracortical inhibition within ipsilesional M1. Short-interval intracortical inhibition within M1 decreased after training in both groups. Long-interval intracortical inhibition and short-interval intracortical facilitation were not modulated. Our findings indicate neurophysiological mechanisms within M1 relevant for motor learning after stroke, which could inform adjuvants aimed at augmenting motor recovery in neurorehabilitation contexts.


Poster
312. Cortical Planning and Execution: Neurophysiology in Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 312.09/N26

Topic: E.04. Voluntary Movements

Support: Fonds pour la formation à la recherche dans l'industrie et dans l'agriculture (FRIA)
Fonds national de la recherche scientifique (FNRS)

Title: Training-related modulation of preparatory activity in the motor system

Authors: *P. VASSILIADIS1,2, J. GRANDJEAN1, G. DEROSIERE1, J. DUQUE1;
1Univ. Catholique de Louvain, Brussels, Belgium; 2Ecole Polytechnique Fédérale de Lausanne, Geneva, Switzerland

Abstract: The preparation of movements, even the simplest ones, involves drastic changes of neural activity in the motor system well before action initiation (Gao et al., 2018). A recent behavioral study showed that training on a specific motor task can reduce reaction times (RT) for the trained movement, and that this reduction is due to faster motor processing during the preparatory period (Mawase et al., 2018). This finding suggests that motor training reduces RTs by modulating preparatory activity in the motor system. Here, we tested this hypothesis in humans by examining the neural correlates of this training-related reduction in RTs. To do so, we assessed preparatory activity in the motor cortex at different stages of training while subjects performed a RT task. 14 subjects performed 10 blocks of 40 trials of an instructed-delay choice RT task which required them to prepare left of right index finger movements according to a preparatory cue and to withhold their response until an imperative signal was displayed. Subject were told to respond as fast as possible to gain points while avoiding errors (e.g. responding too
early or too late or responding with the wrong hand) that would penalize their score. In order to probe corticospinal excitability, double-coil TMS was applied over the right and the left primary motor cortex to elicit near-simultaneous motor-evoked potentials (MEPs) in the left and the right hand, respectively (Vassiliadis et al., 2018). MEPs recorded during the preparatory period (i.e. between the preparatory cue and the imperative signal; MEP_{\text{Prep}}) were expressed in percentage of MEPs acquired at rest (i.e. between 2 trials; MEP_{\text{Baseline}}). We compared RTs, errors and MEPs from both hands in the early (Training_{\text{early}}) and late stages of training (Training_{\text{late}}). In a second-level analysis, we correlated MEPs to bins of 10 consecutive percentile windows of RT (i.e., 0 to 10th, 10th to 20th . . . 90th to 100th). At the behavioral level, RTs were reduced at Training_{\text{late}} compared to Training_{\text{early}} in both hands despite no significant changes in the number of errors. At the neural level, MEP_{\text{Prep}} were systematically suppressed compared to MEP_{\text{Baseline}}; a process that has been previously referred to as preparatory inhibition (Duque et al., 2017). Most importantly, this preparatory activity was modulated by the amount of training performed: MEP_{\text{Prep}} were more suppressed at Training_{\text{late}} than at Training_{\text{early}}. Moreover, we found that there was a strong correlation between MEP_{\text{Prep}} and the subsequent RT: the smaller the MEP_{\text{Prep}}, the faster the RT. Overall, our results suggest that training involves a modulation of preparatory activity in the motor system that subserves RT reduction.

**Disclosures:** P. Vassiliadis: None. J. Grandjean: None. G. Derosiere: None. J. Duque: None.

**Poster**

**312. Cortical Planning and Execution: Neurophysiology in Humans**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 312.10/N27

**Topic:** E.04. Voluntary Movements

**Support:** KAKENHI 25702033
KAKENHI 26560282
KAKENHI 18K10759
KAKENHI 26120002

**Title:** Motor imagery increments the cortico-motoneuronal excitation mediated via presumed cervical interneurons in humans

**Authors:** *S. IRIE*\(^1\), T. NAKAJIMA\(^1\), S. SUZUKI\(^1\)\(^2\), R. ARIYASU\(^1\), T. KOMIYAMA\(^3\), Y. OHKI\(^1\);
\(^1\)Dept. of Integrative Physiol., Kyorin Univ. Sch. of Medicine., Mitaka, Tokyo, Japan; \(^2\)Sch. of Rehabil. Sci., Hlth. Sci. Univ. of Hokkaido, Tobetsu, Hokkaido, Japan; \(^3\)Fac. of Educ., Chiba Univ., Chiba City, Japan
Abstract: Motor imagery is known to affect the reacquisition of motor functions after damages to the central nervous systems. However, it is unclear if motor imagery influences the corticospinal (CST) excitation mediated via cervical interneurons (INs), which may be important for functional motor recovery in animals and humans. To investigate this, we examined the spatial facilitation of motor-evoked potentials (MEPs) induced by combined stimulation (CS) of the pyramidal tract and peripheral nerves in humans. Twenty-nine healthy volunteers and three patients with spinal cord disorders (one incomplete spinal cord injury and two myelopathy patients) were included to record electromyograms (EMGs) from the right biceps brachii (BB). Transcranial magnetic stimulation (TMS) to the left motor cortex and electrical stimulation of the right ulnar nerve at wrist (NERVE) were delivered separately or in combination with inter-stimulus interval of 6-15 ms (NERVE ahead). As for motor imagery tasks, subjects were instructed to imagine elbow flexion with maximum effort during the stimulation without actual movements. During both motor imagery and control tasks, CS facilitated MEPs in the surface EMG compared with simple mathematical summation of responses with TMS alone and NERVE alone. Interestingly, the CS-induced facilitation was significantly increased by motor imagery, depending on timing of NERVE and TMS (7.5-12 ms of ISIs). Single motor unit recording also revealed increased facilitation during motor imagery, which was observed in peaks of the peri-stimulus time histogram 1-2 ms later than the onset latency. The present findings suggest that motor imagery facilitates indirect CST excitations to arm motoneurons, which are mediated by the cervical IN systems (i.e., di- or oligosynaptic contribution). As for the experiments with patients of spinal disorders, also, the facilitation was markedly enhanced during motor imagery tasks. Thus, motor imagery could be a useful tool for reacquisition of movement using the cervical IN circuitry.


Poster

312. Cortical Planning and Execution: Neurophysiology in Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 312.11/N28

Topic: E.04. Voluntary Movements

Title: The role of interhemispheric inhibition during partial cancellation of bimanual motor actions

Authors: *H. MACDONALD, C. LAKSANAPHUK, A. DAY, N. JENKINSON;
Univ. of Birmingham, Birmingham, United Kingdom

Abstract: The rapid and unexpected cancellation of action is vital for controlling motor behaviour. Sometimes a subset of a motor action must be cancelled while the remaining elements
continue. This partial cancellation is difficult and responding elements are substantially slowed. A specific temporal pattern of corticomotor excitability is seen in the delayed responding element that reflects the anticipation, non-selective inhibition and subsequent initiation of the required response. The neural mechanisms underlying this complex pattern of excitability are not yet understood. The present study examined whether interhemispheric inhibitory mechanisms play a role in modulating excitability in this context. In two experiments, healthy younger adults received transcranial magnetic stimulation to both primary motor cortices while performing a bimanual anticipatory response inhibition task. Participants performed bimanual index finger abduction to intercept two rising bars with an on-screen target (Go trials) and were cued to cancel the response in a single hand on 20% of trials (Unimanual Stop trials). During Go trials, a rise in corticomotor excitability (p < 0.001) and concomitant decrease in interhemispheric inhibition (IHI, p < 0.01) for both muscles preceded the prepared bimanual response. Following the stop signal on Unimanual Stop trials, there was a dissociation in IHI onto the responding versus cancelled hands; in experiment one, IHI increased onto the cancelled non-dominant hand, and was completely released for the responding dominant hand (p < 0.01). Experiment two confirmed an equivalent dissociation in IHI was also present, albeit at a later time point, when the non-dominant hand was the one responding (p < 0.05). This dissociation between hands indicates IHI enables uncoupling of the muscles comprising the anticipated bimanual response, and assists selective initiation of just the required unimanual element. Thus, IHI plays an integral role in partial cancellation of prepared bimanual action.

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Poster

312. Cortical Planning and Execution: Neurophysiology in Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 312.12/N29

Topic: E.04. Voluntary Movements

Support: MINECO/AEI/FEDER-UE (SAF2017-86246-R)

Title: Left-right asymmetry of motor cortex excitability and tsms-induced plasticity in essential tremor

Authors: C. AMMANN¹, D. URSO¹, J. A. PINEDA-PARDO¹, A. OLIVIERO², J. A. OBESO¹,³, *G. FOFFANI¹,²;
¹CINAC, Univ. Hosp. HM Puerta del Sur, Madrid, Spain; ²Hosp. Nacional de Parapléjicos, Toledo, Spain; ³CIBERNED, Inst. de Salud Carlos III, Madrid, Spain
Abstract: Essential tremor (ET) typically manifests with mild asymmetry between the left and right upper limbs. Early studies with accelerometric measures in clinic-based samples reported a predominance of right-hand tremor (Biary and Koller, Arch Neurol 1985), whereas later investigations with clinical measures in community-based samples reported an opposite predominance of left-hand tremor (Louis et al., Arch Neurol 1998). However, the pathophysiological significance of this asymmetry and the possible predominance of one side remain unclear. To address this issue, here we studied 30 patients with essential tremor. The amplitude and frequency of postural tremor quantified with accelerometry did not show any left/right hand predominance, whereas action tremor assessed with clinical scales displayed a clear left-hand predominance. Transcranial magnetic stimulation (TMS) in a subset of patients revealed significant left-right differences in intracortical excitability, with less inhibition and more facilitation in the right compared to the left hemisphere. Diffusion MRI also showed significantly lower mean diffusivity in the right compared to left motor cortex. Finally, a 30-min session of unilateral transcranial static magnetic field stimulation (tSMS) of the motor cortex (NCT03780426) significantly reduced contralateral hand postural tremor and bilateral hand action tremor, but only when tSMS was applied to the right cortex. These results suggest that asymmetries in motor cortex excitability and plasticity may lateralize the clinical manifestation and the response to non-invasive brain stimulation in essential tremor.

Disclosures: C. Ammann: None. D. Urso: None. J.A. Pineda-Pardo: None. A. Oliviero: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patents and stock ownership in the start-up company Neurek SL. J.A. Obeso: None. G. Foffani: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patents and stock ownership in the start-up company Neurek SL.

Poster

312. Cortical Planning and Execution: Neurophysiology in Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 312.13/N30

Topic: E.04. Voluntary Movements

Support: CBIR17PIL024

Title: Augmented attention during physical training improves brain connectivity and motor function

Abstract: Bimanual coordination deficit is common in individuals with sustained moderate to severe traumatic brain injury (TBI). Effective treatments require a large amount of physical training to recover normal motor function. Unfortunately, attention deficits and physical weakness limit the patients’ ability to engage in intensive physical training. In this study, physical and mental practice were combined to achieve high engagement and intensity during training without inducing fatigue. The study compares an intervention combining physical practice and motor imagery (MP group) versus physical practice and action observation (PP group). Ten participants were enrolled in the study after signing an informed consent approved by the IRB. 5 participants (1F, 4M, 35±14 yrs. old) were enrolled in the MP group and 5 (5M, 45±12 yrs. old) in the PP group. Participants were trained in 3 one-hour sessions per week for 4 weeks. Outcome measures included assessment of maximum voluntary contraction (MVC) strength in wrist flexion and extension for both hands at each session, and fMRI-based neurophysiological measures of brain connectivity at rest before and after training. Resting fMRI data were preprocessed using conventional methods and analyzed using seed-based analysis. Regions of interests used to study Sensorimotor (SM), Dorsal Attention Network (DAN), and Ventral Attention Network (VAN) were bilateral 1) primary motor, 2) premotor, and 3) ventral frontal cortices. Group level analysis showed a median increase in MVC of 6%±28% IQR after training in the MP group and 20%±26% IQR in the PP group, but it did not show significant difference between groups. Resting state fMRI data showed pre to post significant decrease (FWE, p<0.05) in the extent of connectivity within the SM, DAN, and VAN networks, indicating more focal networks after training, and no difference between groups. The findings suggest that high-level of attention during physical training, whether achieved through motor imagery or action observation, engages the sensorimotor and attention networks, and strengthens their connectivity that might translate into better motor function. The study is ongoing and a larger sample size and analysis of other outcomes would allow us to draw more surefire conclusions.


Poster

312. Cortical Planning and Execution: Neurophysiology in Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 312.14/N31

Topic: E.04. Voluntary Movements

Support: DFG KFO 247

Title: Oscillatory cortical dynamics predict successful motor preparation

Authors: B. POSTIGO-ALONSO¹, A. GALVAO-CARMONA¹, M. HOFMANN², A. KÜHN³, *W.-J. NEUMANN²;
Abstract: Background: Taking time to prepare voluntary movement can improve motor performance in dependence of required motor control capacity. In previous works, we have demonstrated that neuromodulation of supplementary motor cortex projections to STN decreases reaction times when movements were associated with higher cognitive demand. Aim: In the present study we aimed to identify cortical oscillatory correlates of successful motor preparation. Method: 20 right-handed healthy adults were assessed with 64-channel EEG while performing a visuomotor task consisting on navigating as fast as possible on a digitizing tablet to reach a target circle shown on the screen with a pen, whose axes representation was unperturbed (automatic mode) or inverted (controlled mode). Reaction time, movement time, trajectory error and peak velocity were decomposed using principal component analysis and a component associated with longer reaction- but shorter movement time and lower motor error was identified. Whole brain source extraction was conducted on a cortical mesh. Source activity was transferred to the frequency domain and spatio-temporal correlation maps with the motor control component were created. P-values were FDR corrected for multiple comparisons. Results: Correlation of oscillatory cortical activity peaked in the supplementary motor area ~500 ms before movement onset in the beta frequency range. Similarity of individual temporospatial activity patterns could significantly predict left out subjects through cross-validation. Conclusion: Our results confirm the implication of the supplementary motor area in preparation of motor commands under cognitive demand.

Abstract: Variability in motor responses influences the ability to perform everyday motor tasks. Primate work has shown that behavioral variability can be partly explained by the neural activity within primary motor cortex (M1) and premotor cortex. Our recent work has found contribution of human corticospinal system to the variability in the application of digit force. However, in humans, the specific sources at the central level that contribute to behavioral variability are not well understood. In this study, we determined whether a transient and reversible lesion of human M1 alters the variability in grip force applied to an object using a precision grip. Healthy young adults (n=7) performed an isometric grip force task before and after the delivery of continuous theta burst stimulation (cTBS) over contralateral M1 or vertex (a control site). During the isometric grip force task, target forces equivalent to 5, 15, or 30% of subject’s maximum grip force were displayed on a computer screen. During each trial, subjects were instructed to squeeze a grip device using index finger and thumb to reach a target force and then maintain the force for 8 sec. Following this, the screen was blocked, and subjects were instructed to continue to maintain the force for another 8 sec. We assessed neurophysiologic changes following cTBS over M1 by assessing corticospinal excitability (CSE), short interval intracortical inhibition (SICI), and intracortical facilitation (ICF). We found that cTBS over M1 increased the moment-to-moment variability in the grip force applied to the object for 15% (p=0.0006) and 30% (p=0.02) of force, but not for 5% of force. These effects were found when the task was performed under the visual feedback condition, but not when the feedback was blocked. However, we did not observe any change in the magnitude of CSE, SICI, and ICF following cTBS over M1. Furthermore, cTBS over vertex did not change grip force variability. These findings suggest a critical role of M1 in visually-driven control of force mainly at higher magnitudes.


Poster

312. Cortical Planning and Execution: Neurophysiology in Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:/Poster #: 312.16/N33

Topic: E.04. Voluntary Movements

Support: “Fonds Spéciaux de Recherche” (FSR)
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L’Oréal-UNESCO "For Women in Science"

Title: The subthalamic nucleus as a potential neural driver of motor inhibition during action preparation?
Abstract: By applying transcranial magnetic stimulation (TMS) over the primary motor cortex (M1) during reaction time (RT) tasks, many studies have revealed a suppression of motor-evoked potentials (MEPs) during action preparation (i.e., compared to a resting state) - a phenomenon referred to as preparatory inhibition. Intriguingly, little is known about the neural structures at the origin of this suppression. The subthalamic nucleus (STN) has a strong inhibitory influence on M1 and thus represents a plausible candidate for contributing to the generation of preparatory inhibition. Here, we investigated the functional contribution of the STN to preparatory inhibition by probing MEPs during action preparation in Parkinson’s disease (PD) patients treated with deep brain stimulation (DBS). Right-handed PD patients and matched healthy subjects participated in the study. They performed an instructed-delay choice RT task, in which they had to select either left or right index finger responses based on the position of a preparatory cue, but had to wait until the onset of an imperative signal to release their movement. TMS was applied over both M1 using a double coil method, eliciting concurrent MEPs in right and left index finger muscles. Pulses were applied either at rest (during the inter-trial interval) or during the preparatory period (just before the imperative signal). The patients realized the task either OFF- or ON-DBS on two consecutive days (randomized order), allowing us to probe preparatory inhibition in PD patients, and to examine the impact of the perturbation of STN activity (ON-DBS) on preparatory inhibition. In line with past research, healthy subjects exhibited a substantial suppression of MEPs during action preparation (i.e., compared to MEPs obtained at rest). This effect was evident for MEPs elicited in both the left and the right finger muscles and occurred regardless of whether the preparatory cue required a left or right hand response. In contrast, such an MEP suppression was less consistent in the PD patients. Intriguingly, in these patients, preparatory MEPs even displayed some facilitation in a number of conditions and this effect was stronger when the DBS was turned ON compared to when patients were tested in the OFF-DBS state. The latter observations suggest an alteration of preparatory inhibition in PD patients, especially when DBS perturbs the STN, probably releasing M1 from its inhibitory tone.

Disclosures: E. Wilhelm: None. G. Derosiere: None. C. Quoilin: None. A. Jeanjean: None. J. Duque: None.
Title: The neural correlates of combining submaximal contraction with motor imagery in breast cancer survivors


Abstract: Cancer and its treatment is often associated with long term side-effects of fatigue and physical weakness. Engaging in low intensity strength training has found to be beneficial in lessening the side effects. Similarly, motor imagery (MI) which engages the same motor network than the one controlling the actual motor task, can on its own improve motor performance and physical strength. This study examined the potential added benefit of combining MI with low intensity exercise in breast cancer survivors with physical weakness by investigating its neural correlates using an EEG source-space time-frequency analysis. 10 right handed breast cancer survivors performed a series of 30 mixed trials of the following three handgrip conditions held for 10s: (1) kinesthetic MI where they imagined performing handgrip at maximal voluntary force (MVF), (2) submaximal handgrip contractions (SC) at 20% MVF, and (3) SC at 20% MVC combined with kinesthetic motor imagery (MI) of handgrip contraction at MVF (SC+MI). EEG data were collected during the task using a 64 channel actiCAP EEG system (Brain Products) and all data were processed using EEGLAB toolbox. After standard preprocessing, Infomax ICA source separation was applied to the concatenated data of all participants and conditions to obtain group-level sources. This led to the selection of 13 artefact-free brain sources over the prefrontal cortex (PFC), Supplementary Motor Area (SMA), primary motor (M1) and sensory (S1) cortices, posterior cingulate cortex (PCC) and parietal cortex (PC). Time-frequency analysis was then performed to compare conditions using permutation-based statistics. SC+MI and SC showed significantly greater desynchronization (ERD) in the alpha (8-12Hz) and beta (12-25Hz) frequency bands than MI, consistently across all sources. Significantly greater ERD for SC+MI was only observed in the left PFC source in the alpha and theta (5-8Hz) band. Left PFC source showed also greater gamma (30-50Hz) band synchronization in MI vs SC+MI and SC. A single session of SC+MI only marginally increased brain activation compared to SC alone. The greater alpha and theta band activation for the left PFC source suggests increased attention to perform MI during handgrip contraction. Study participants were randomly assigned to either receive twenty 45-min sessions of SC or SC+MI handgrip strength training over 4 weeks. We are in the process of analyzing and investigating pre to post training strength gain and pre to post neuroplasticity changes using time-frequency and brain connectivity analysis.

Title: Modeling neural population dynamics informs and improves decoding of electrocorticographic signals

Authors: *K. Li*\(^1\), R. Flint\(^2\), M. W. Slutsky\(^2\), C. Pandarinath\(^{1,3}\);
\(^1\)The Wallace H. Coulter Dept. of Biomed. Engin., Emory University/Georgia Tech., Atlanta, GA; \(^2\)Dept. of Neurology, Feinberg Sch. of Med., Northwestern Univ., Evanston, IL; \(^3\)Dept. of Neurosurg., Emory Univ., Atlanta, GA

Abstract: Brain-machine Interfaces (BMIs) aim to assist people with paralysis due to injuries or neurodegenerative diseases by creating a connection between the brain and external assistive devices. One commonly-used interface for BMIs is electrocorticography (ECoG), which measures cortical surface potentials and provides substantial information about movement intention. ECoG covers large areas of the brain, thus providing access to broad cortical networks, but generally has lower signal quality than interfaces such as intracortical microelectrode arrays. A potential avenue to improve its performance comes from deep learning methods such as Latent Factor Analysis via Dynamical Systems (LFADS), which uncover structure from neural population activity that is consistent with a low-dimensional dynamical system. In previous applications to intracortical recordings from primary motor cortex, LFADS increased decoding accuracy by uncovering estimates of neural population dynamics on a single-trial, moment-by-moment basis. However, it was unclear whether it could similarly uncover structure and “de-noise” recordings with lower spatial resolution that sample from broad cortical networks, such as ECoG. In this study, we tested whether modeling neural population dynamics could uncover signatures of behavior from broad cortical activity and improve ECoG decoding performance. We applied LFADS to ECoG from seven human subjects who were being monitored as part of surgical treatment for epilepsy or brain tumors. Subjects performed a one-finger flexion task that incorporated movement and isometric force production. We compared our ability to decode
behavioral states (i.e. pre-movement, movement or force) before and after application of LFADS. The latent factors that described the underlying dynamical system showed clear differences between states, which improved the accuracy of a discrete classifier in correctly identifying states. Across subjects, median (+/-IQR) prediction accuracy prior to LFADS was 84%±8%, while prediction accuracy following LFADS increased to 90%±6%, a significant improvement (p=5x10^-14; kruskal-wallis test). Our results show that lower-dimensional representations can parsimoniously describe the combined activity of widespread cortical networks spanning multiple regions. They also represent a new path for improving the performance of ECoG-based BMIs. Future work will test whether using “dynamic neural stitching” to combine data from multiple sessions could further improve ECoG decoding performance, and whether the improvements translate to more complicated behavioral tasks.

Disclosures:  K. Li: None. R. Flint: None. M.W. Slutzky: None. C. Pandarinath: None.

Poster

312. Cortical Planning and Execution: Neurophysiology in Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 312.19/N36

Topic: E.04. Voluntary Movements

Title: Cortical silent period in task-irrelevant muscles is shortened during motor response preparation

Authors: *I. GOMEZ, I. GREENHOUSE;
Human Physiol., Univ. of Oregon, Eugene, OR

Abstract: Several transcranial magnetic stimulation (TMS) studies have observed decreased excitability during the preparation of movements that spreads to task-irrelevant muscles. Whether widespread changes in preparatory corticospinal excitability reflect the involvement of intracortical, subcortical, and/or spinal mechanisms remains an unanswered question. The duration of the cortical silent period (CSP) in the electromyogram (EMG) of a tonically active muscle following a single TMS pulse is believed to reflect GABA-b dependent intracortical inhibition. Here, we test the hypothesis that widespread changes in corticospinal excitability depend on GABA-b intracortical mechanisms by measuring the CSP in a task-irrelevant muscle during the preparation of movements. Seven healthy, right-handed subjects (2 females, 26 ± 5 y.o.) completed a unimanual delayed response task while maintaining a tonic contraction with the non-responding hand. Participants performed fifty trials using each hand with the hand order counterbalanced to account for laterality differences. EMG was recorded from both first dorsal interosseum. Participants squeezed a ball between the index and thumb of the non-responding hand for the duration of the task and were trained to hold an isometric contraction at 25% of the maximum voluntary. Each trial consisted of a baseline fixation cue (200ms), a preparatory cue
(900ms) and an imperative stimulus (500ms). Participants made lateral index finger button presses in response to the imperative stimuli. Six catch trials, during which no imperative stimulus appeared, were included for each hand to discourage premature responding. TMS was administered at 115% resting motor threshold over M1 contralateral to the non-responding hand. Motor-evoked potentials (MEP) and CSP’s were elicited in the contracted hand at one of two time points per trial: either at fixation onset (baseline) or 100ms before the imperative cue (delay). CSP duration was significantly shorter during the preparatory period compared to baseline for both left-hand (baseline = 82 ± 9.6ms, delay = 63 ± 9.2ms, p = .004) and right-hand (baseline = 117 ± 6.2ms, delay = 103 ± 2.2ms, p = .001) responses. MEP amplitudes did not differ between the preparatory period and baseline for either the left-hand (baseline = 6.32 ± 1.6mV, delay = 6.38 ± 1.4mV, p = .75) or right-hand (baseline = 7.66 ± 0.8mV, delay = 7.63 ± 0.8mV, p = .9) responses. Reaction times were similar between hands (left = 329 ± 40ms, right = 304 ± 27ms, p = .12). The significant reduction in CSP duration during the delay period may reflect a release of inhibition in ipsilateral motor cortex to facilitate execution of goal-oriented movement.


Poster

312. Cortical Planning and Execution: Neurophysiology in Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 312.20/N37

Topic: E.04. Voluntary Movements

Support: NIH Grant NS097480

Title: Continuous kinematic encoding model of ECoG activity in the contralateral and ipsilateral hemisphere during a reaching task

Authors: *C. M. MERRICK¹, T. C. DIXON², A. BRESKA¹, J. LIN³, E. F. CHANG⁴, J. M. CARMENA⁵, R. T. KNIGHT¹, R. IVRY¹;
¹Psychology, Univ. of California, Berkeley, Berkeley, CA; ²Bioengineering, Univ. Of California Berkeley, Berkeley, CA; ³Dept. of Neurol., Univ. of California, Irvine, Irvine, CA; ⁴Neurosurg., UCSF, San Francisco, CA; ⁵Electrical engineering and computer science, UC Berkeley, Berkeley, CA

Abstract: Upper limb movements are primarily controlled by the motor cortex in the contralateral hemisphere. However, the decoding success of unilateral movement from electrocorticography (ECoG) or single unit activity is surprisingly similar for contralateral and ipsilateral motor cortex (Ganguly, 2009). While this observation offers a promising neuroprosthetic alternative approach for patients with hemiparesis, the functional relevance of
ipsilateral motor representations remains unclear. Decoding motor kinematics from ECoG often entails multiple neural features from electrodes that span several centimeters of cortex comprising different cortical areas. The goal of this study is to gain a better understanding of how and where kinematics are encoded, and how these neural representations compare in the contralateral and ipsilateral hemisphere. We collected data from four individuals undergoing intracranial monitoring, who performed an instructed-delay reaching task using either the hand ipsilateral or contralateral to the ECoG electrodes. We built a cross-validated encoding model to predict ECoG data from continuous kinematic features of the patient’s movements while they performed the task. Specifically we used continuous kinematic features to predict the activity of eight different neural features: 1) the local motor potential (LMP) and 2) the analytic amplitude of seven time-frequency bands (delta (1-4Hz), theta (4-8Hz), mu (8-12Hz), low beta (12-20Hz), high beta (20-30Hz), gamma (30-60) and high frequency broadband (HFB; 65-150Hz)). We used two different kinematic feature sets to predict the neural activity. The first represents the patient’s movements in a cartesian coordinate space (PositionX, PositionY, PositionZ) and the second in a spherical coordinate space (Magnitude, azimuthal angle, bipolar angle). We found that kinematic features represented in a spherical coordinate frame better predicts held-out ECoG activity across electrodes. The neural features that were best predicted by the spherical kinematic model were the LMP and high frequency bands. To better understand the role of the ipsilateral hemisphere in motor control we trained the spherical kinematic model from reaches when the hand ipsilateral to the ECoG electrodes was moving and predicted the neural activity when the hand contralateral to the ECoG electrodes was moving. We find neural features that can generalize across the two hands and neural features that cannot generalize across the two hands, suggesting that some neural features are encoding information that is effector independent and some neural features are encoding information that is effector dependent.


Poster

312. Cortical Planning and Execution: Neurophysiology in Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 312.21/N38

Topic: E.04. Voluntary Movements

Support: NSERC 418589

Title: Evidence for sequential recruitment of contralateral PPC and motor regions for rapid online control
Authors: *P.-M. BERNIER*¹, D. GAUDREault¹, F. R. SARLEGNA²; 
¹Univ. De Sherbrooke, Sherbrooke, QC, Canada; ²CNRS and Aix-Marseille Univ., Marseille, France

Abstract: Upon a perturbation of hand visual feedback during movement, humans are able to rapidly correct their movement during its execution. Regions of the parieto-frontal network, including the posterior parietal cortex (PPC), premotor cortex (PMC) and primary motor cortex (M1) are involved in this process (Mulliken et al., 2008, Shadmehr and Krakauer, 2008, Archambault et al., 2011). However, it is still unclear how these regions coordinate their activities during online control. Here, we study the time-course of recruitment of these regions and ask whether PPC activity precedes PMC/M1 activity during rapid online corrections. Electroencephalography (EEG) was recorded while participants (n=19) performed a rapid arm reaching task with their right hand toward visual targets (MT ~ 600ms). Hand visual feedback was provided during the movement (only between 100ms and 200ms) with a cursor that could be either spatially congruent with the hand position (Congruent condition; CONG), shifted left or right from the hand position by 1.5cm (Incongruent 1.5 condition; INC-1.5), or shifted left or right by 3cm (Incongruent 3 condition; INC-3). Kinematic analyses revealed that subjects did compensate for the cursor shifts during movement, as evidenced by significant differences in X-accuracy at movement endpoint across conditions (p<0.01). The onset of correction, defined as the first time-point where the trajectory in each incongruent condition went beyond the 99% confidence interval of the congruent trials, revealed that participants were slightly but significantly faster to initiate online corrections in the INC-3 condition (300ms) than in the in the INC-1.5 condition (320ms) (p<0.05). To track the time-course of online corrections within the parieto-frontal network, the ERPs associated with the incongruent conditions were compared to those of the congruent condition, which served as a baseline. Data were time-locked to the onset of the movement. Cluster-based permutation tests were used. For the Inc-3 vs Cong contrast, results revealed an early significant cluster (p<0.01) over the left-central parietal regions from 203 to 243ms (peak differential activity at 237ms). Slightly later, another significant cluster (p<0.01) was observed over the left motor regions between 270 and 323ms (peak differential activity at 290ms). No significant cluster was found in the Inc-1.5 vs Cong contrast. We suggest that the sequential recruitment of the PPC and motor regions reflects two processes: the PPC would first be involved in updating the state of the limb in real time, while motor regions would then implement a corrective motor command.


Poster

312. Cortical Planning and Execution: Neurophysiology in Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 312.22/N39
Abstract: Making motor decisions often requires balancing the desire to take time to choose accurately with the urge to act. During a speeded decision, the urge to act increases as time passes but the overall level of urgency also varies depending on the context. Recent work suggests that urgency operates as a gain modulator of task-related activity: when decisions between reaching movements are made under time pressure, activity in motor areas involved in arm movements is amplified. An open question relates to the generalization of this gain modulation in the motor system. Here, we investigated the impact of urgency on corticospinal excitability in different task-related and task-unrelated motor representations in humans, by applying transcranial magnetic stimulation (TMS) over the primary motor cortex. Subjects performed a decision-making task. In each trial, 15 tokens jumped one-by-one every 200 ms from a central circle to one of two lateral target circles; participants had to guess which target circle would ultimately receive the majority of the tokens, and to report their decision on a keyboard with either the left or the right index finger. Importantly, the reward provided for correct choices was proportional to the number of tokens remaining in the central circle at the time of the response. Because this number decreased over time, the urge to act grew accordingly. Further, we manipulated the overall level of urgency in two contexts, by providing a different penalty for incorrect choices in separate blocks. The use of a low penalty encouraged the subjects to make hasty choices, thus ensuring a high urgency (Urgency\textsubscript{High} context), while a high penalty promoted accurate choices and ensured a low urgency (Urgency\textsubscript{Low}). We exploited TMS to elicit motor evoked potentials (MEPs) at different times during the token jumps in each context, in muscles that were either involved in the task (i.e., index finger “task-related” muscles) or not (i.e., thumb, pinky and leg “task-unrelated” muscles). MEP amplitudes obtained from these muscles provided us with a muscle-specific assay of corticospinal excitability at the time of stimulation. MEP amplitudes increased over time in all the investigated muscles (including in the leg) independently of the context, putatively reflecting a global time-dependent gain effect of urgency on corticospinal excitability. Further, amplitudes were higher in the Urgency\textsubscript{High} than in the Urgency\textsubscript{Low} context for task-related muscles, specifically when the MEPs were measured in the hand that was selected for the forthcoming action. The latter finding suggests the presence of a more specific context-dependent effect of urgency on corticospinal excitability.
**Title:** Between hand coupling during response inhibition

**Authors:** C. G. WADLSEY, J. CIRILLO, *W. D. BYBLOW; Dept. of Exercise Sci., Univ. of Auckland, Auckland, New Zealand

**Abstract:** Response inhibition reflects the process of terminating inappropriate pre-planned or ongoing movements. When one hand is cued to stop after preparing a bimanual response (Partial trial) there is a substantial delay on the responding side. This delay is termed the interference effect and identifies a constraint that limits selective response inhibition. Gamma-aminobutyric acid (GABA)-mediated networks within primary motor cortex (M1) may have distinct roles during response inhibition. In this study we examined whether the interference effect is the consequence of between-hand “coupling” into a unitary response, and whether this is reflected in GABAergic intracortical inhibition within M1. Eighteen healthy right-handed participants performed a bimanual synchronous (easy) and asynchronous (hard) anticipatory response inhibition task. Electromyographic recordings were obtained from the first dorsal interosseous muscle bilaterally. Motor evoked potentials were elicited by single- and paired-pulse transcranial magnetic stimulation over right M1. As expected, performance was better and paradoxically response delays were worse, with the easier versus the harder version of the task. Although task difficulty did not modulate GABAergic intracortical inhibition, there was a trend for between-hand coupling to be associated with greater inhibitory tone and lower synaptic inhibition. The novel findings indicate that the interference effect is in part a consequence of between-hand coupling into a unitary response. The ability to respond independently with the two hands may rely on modulation of distinct inhibitory processes.

**Disclosures:** C.G. Wadlsey: None. J. Cirillo: None. W.D. Byblow: None.
Abstract: Modulation of GABA-mediated inhibition in primary motor cortex is important for the induction of training-induced plasticity. The downregulation of inhibition during acquisition may promote cortical reorganisation, whereas an upregulation once performance has plateaued may promote consolidation of the newly acquired skill. GABA related inhibition in human primary motor cortex is routinely assessed using the paired-pulse transcranial magnetic stimulation paradigm of short-interval intracortical inhibition (SICI). However, modulation of SICI with motor skill learning is not a consistent finding and may be influenced by transcranial magnetic stimulation parameters. The aim of this study was to compare the modulation of SICI by motor skill learning between conventional and adaptive threshold-hunting techniques with an anterior-posterior (AP) and posterior-anterior (PA) induced current. Sixteen participants (21 - 33 years) trained with their dominant (right) hand on a sequential visual isometric pinch task. Electromyographic recordings were obtained from the right first dorsal interosseous muscle. Corticomotor excitability and SICI were examined before and immediately after 12 blocks of training. Skill increased throughout the training, with performance plateauing before the completion. Corticomotor excitability increased after motor training for both AP and PA induced current. The amount of SICI was greater with AP stimulation than PA for both conventional and adaptive threshold-hunting techniques. SICI increased after motor training, but this was only evident for adaptive threshold-hunting with AP induced current. These findings indicate that increased GABA-mediated inhibition after motor skill learning may promote consolidation of the newly acquired skill. Our findings also highlight the importance of selecting appropriate transcranial magnetic stimulation parameters, supporting the notion that adaptive threshold-hunting SICI using an anterior-posterior current provides a robust and sensitive assessment in interventional studies.
**Title:** Directed connectivity markers for transcallosal interactions: A finger movement study

**Authors:** *H. WEI*\(^{1,2}\), A. FRANCOIS-NIENABER\(^2\), J. MELTZER\(^{2,1}\);

\(^1\)Psychology, Univ. of Toronto, Toronto, ON, Canada; \(^2\)Baycrest Hosp., Rotman Res. Inst., Toronto, ON, Canada

**Abstract:** Stroke patients frequently exhibit increased task-induced activation of the contralesional hemisphere (cH). This may be either beneficial or detrimental to functional recovery, an ongoing debate with important implications for treatment, especially using transcranial magnetic stimulation (TMS). It is theorized that the cH exerts an inhibitory influence on the lesioned hemisphere, impairing recovery. Transcallosal inhibition (TCI) between motor cortices may be directly measured using TMS to elicit motor-evoked potentials (MEPs), which may be reduced in the presence of TCI. However, this measure does not generalize easily to other cognitive domains and regions beyond the motor cortex. Thus, we aimed to quantify TCI directly from magnetoencephalography (MEG) data, using finger movement as a starting point. 29 right-handed young healthy performed a finger movement task during MEG, executing visually cued left or right index finger movements in different blocks. We implemented frequency-domain Granger Causality analysis to measure directed connectivity between virtual channels localized to motor cortices (M1s) and visual cortices (V1s). A high-frequency (10-100 Hz) connectivity signal appears 500 ms after movement onset from moving to non-moving M1, whereas a similar signal from non-moving to moving M1 is elevated at baseline but drops off upon movement onset. The signal is overall stronger inter-hemispherically between M1s than intra-hemispherically from M1 to V1 (Figure 1). Moreover, post-movement connectivity exhibits a strong asymmetry with greater left-to-right influence, presumably related to right-handedness. These findings suggest that high-frequency directed connectivity may index TCI. Ultimately, the establishment of electrophysiological markers of TCI can guide post-stroke intervention in the optimal direction.
Disclosures: H. Wei: None. A. Francois-Nienaber: None. J. Meltzer: None.

Poster

312. Cortical Planning and Execution: Neurophysiology in Humans

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 312.26/N43

Topic: E.04. Voluntary Movements

Support: NIH grant ZIA MH002920-09

Title: Layer specific contributions to imagined and executed hand movements in primary motor cortex

Authors: *A. S. PERSICHETTI, J. A. AVERY, L. HUBER, A. LIU, E. P. MERRIAM, P. BANDETTINI, A. MARTIN;
Lab. of Brain and Cognition, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: The extent to which representations of imagined and executed movements overlap in primary motor cortex (M1) is an old and important question in cognitive science. Until now, it has been difficult to answer this question due, in part, to the poor spatial specificity of neuroimaging techniques. We used a 7-Tesla MRI scanner to measure the cerebral blood volume (CBV) in M1 using a cutting-edge method called vascular space occupancy (VASO). Using VASO to measure changes in CBV, instead of using traditional functional MRI methods that
measure changes in blood oxygenation levels (i.e., BOLD signal), we achieved sub-millimeter spatial specificity that allowed us to measure changes across cortical layers of the hand-selective region of M1. Since previous studies have shown that cortico-cortical connections with M1 terminate predominately in superficial layers (II/III), while cortico-spinal output from M1 originates predominantly in the deep layers of cortex (Vb/VI), we hypothesized that imagined hand movements would activate neurons in the superficial layers of M1 only, while executed hand movements would activate both superficial and deep layers. As predicted, our data show an increase in CBV when participants imagined tapping their thumb and index fingers together in the superficial layer only, but we see increased CBV when participants were actually tapping their fingers together in both superficial and deep layers of M1. Finally, a motion detecting glove was used to confirm that any neural activation found while participants imagined tapping their fingers together was not due to actual motion. These data suggest that imagined and executed movements are differentially represented across layers in M1. Furthermore, these data are evidence of a mechanism responsible for simulating motor performance without actually executing the movements.

**Disclosures:**  

**Poster**

312. Cortical Planning and Execution: Neurophysiology in Humans

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 312.27/N44

**Topic:** E.04. Voluntary Movements

**Support:** BOF UHasselt

**Title:** Dissociating the causal role of the dorsolateral prefrontal cortex and the dorsal premotor cortex in preparing and executing a bimanual coordination task - A neuro-navigated rTMS study

**Authors:**  
*R. L. J. MEESEN*¹, S. VERSTRAELEN*¹, S. DEPESTELE¹, K. VAN DUN¹, J. DUQUE², H. FUJIYAMA³, S. P. SWINNEN⁴, O. LEVIN⁴, M. A. NITSCHÉ⁵, E. GHASEMIAN⁵, K. CUYPERS⁴;  
¹Univ. Hasselt, Hasselt, Belgium; ²Univ. Catholique Louv, Brussels, Belgium; ³Murdoch, Murdoch, Australia; ⁴K.U.Leuven, Leuven, Belgium; ⁵Leibniz Res. Ctr. for Working Env., Dortmund, Germany

**Abstract:**  
**Background:** Previous neural imaging studies have shown that the dorsolateral prefrontal cortex (DLPFC) and dorsal premotor cortex (PMd) are active during complex bimanual tasks.  
**Objective:** This study used disruptive repetitive transcranial magnetic stimulation (rTMS) to
further explore (1) if respective correlational evidence is a representation of a causal relationship and (2) if DLPFC and PMd show a differential causal contribution to specific task outcome measures.

**Methodology:** A total of 41 young healthy adults (mean age: 22.20 years ± 2.95 SD) were enrolled in this study. We applied disruptive short-train rTMS (5 pulses, 10 Hz) on either DLPFC or PMd during either the preparation or execution of a complex bimanual tracking task (BTT). Specifically, we examined the effect of disruption on two BTT performance measures: task accuracy (TA), and movement stability (MS). While TA is assumed to measure the conscious monitoring of the task, MS measures only the stability (smoothness) of the bimanual movement.

**Results:** DLPFC disruption during motor preparation had a short detrimental effect on TA, while MS was unaffected. There was no effect of DLPFC disruption during motor execution on either TA or MS. In contrast, PMd disruption during motor preparation and execution had a short detrimental effect on MS, while TA was unaffected.

**Conclusion:** The current findings revealed a differential causal involvement of DLPFC and PMd in preparing and executing a complex bimanual coordination task. More specifically, DLPFC is suggested to be more involved in the conscious monitoring of the task and these cognitive operations of movement control were most crucial during motor preparation. In contrast, the role of PMd is restricted to the integration of two unimanual movements into a single control structure for both hands during both motor preparation and execution.

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**Poster**

**312. Cortical Planning and Execution: Neurophysiology in Humans**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 312.28/N45

**Topic:** E.04. Voluntary Movements

**Title:** Cerebellar transcranial direct current stimulation (ctDCS) to modulate cerebello-cerebral networks in bimanual coordination in young and older adults

**Authors:** *K. VAN DUN*¹, S. VERSTRAELEN², S. DEPESTELE³, F. MICHIELS³, R. MEESEN⁴;  
¹Uhasselt, Hasselt, Belgium; ²REVAL Rehabil. Res. Centre, Biomed. Res. Institute, Fac. of, Univ. of Hasselt, Diepenbeek, Belgium; ³UHasselt, Hasselt, Belgium; ⁴Univ. Hasselt REVAL, Diepenbeek, Belgium
**Abstract: Background:** Complex bimanual coordination requires a good coordination between different neurological networks. Despite a crucial role of the cerebellum in motor coordination and motor learning, little is known about the cerebellar role in bimanual coordination, especially in aging. In young people the cerebellum is consistently activated during finger and hand movements, but it appears to be a truly crucial structure during development and in older adults, in whom it is the strongest predictor of bimanual coordination performance. By measuring the neurophysiological changes after cerebellar transcranial direct current stimulation (ctDCS), we have investigated the crucially implicated cerebello-cerebral networks in bimanual coordination.

**Study design:** An ALE meta-analysis of functional MRI studies was performed to identify the cerebellar areas that are involved in bimanual coordination. 14 young (18-30 years old) and 14 older adults (65-77 years old) performed a bimanual tracking task (BTT) while being stimulated with ctDCS (real or sham). EEG was recorded before and after to measure neurophysiological changes. **Results:** Seven clusters were identified in total with a large cluster in the right anterior cerebellum (antCB). Contrasted with unimanual coordination, the right globus pallidus externus (GPeX) was the most significant one. Behavioral and EEG data will be analysed to identify behavioural and neurophysiological changes during bimanual coordination after ctDCS in young and older adults. **Discussion:** Bimanual coordination is mainly controlled by the dominant hemisphere explaining the involvement of the left precentral gyrus and right antCB. In addition, the GPeX plays a crucial role in the control of voluntary movement by suppressing unwanted movements. By stimulating the cerebellum, we expect to modulate cerebello-cerebral networks involved in bimanual coordination. By linking behavioural performance to neurophysiological changes, we will be able to identify the networks that are crucially involved and controlled by the cerebellum.

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**Poster**

**312. Cortical Planning and Execution: Neurophysiology in Humans**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 312.29/N46

**Topic:** E.04. Voluntary Movements

**Title:** The impact of cognitive functioning on driving performance of older persons: A systematic review

**Authors:** *S. DEPESTELE, T. BRIJS, V. ROSS, K. VAN DUN, S. VERSTRAELEN, R. MEESSEN; Hasselt Univ., Hasselt, Belgium
Abstract: BACKGROUND: The proportion of persons aged 65 and over is steadily increasing worldwide. Thus, the number of older adults in the possession of a valid driver’s license has been growing. As mobility is considered a fundamental need, driving cessation at a higher age could negatively impact quality of life. However, the driving performance of healthy older persons can decrease due to normal aging processes. For instance, cognitive capabilities can deteriorate, possibly leading to higher risk of crash involvement in healthy older persons.

OBJECTIVE/HYPOTHESIS: To investigate the association between cognitive functioning and driving performance in a simulator in an older population. We hypothesize that driving ability of older persons is negatively influenced by a deterioration of cognitive function (such as dual-tasking, reaction time, attention strategies, executive function). METHODS: A systematic literature search was conducted on the PubMed, Web of Science, Scopus and Cochrane electronic databases. Articles of the last ten years that examined the differences in cognitive functioning between older and younger persons, using either behavioral assessment or neuroimaging techniques, and its association to driving ability in a driving simulator were identified. Methodological quality was assessed using an adapted checklist based on the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines for observational studies. The findings are reported according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. RESULTS AND CONCLUSIONS: A total of 1933 articles were identified. Twenty-nine articles met the inclusion criteria. Cognitive functioning was assessed using neuroimaging techniques (EEG, fMRI) in 4 studies. Other studies employed a variety of neuropsychological tests or behavioral tests to assess different aspects of cognitive functioning (attention, reaction time, mental workload, dual-tasking, executive function, intelligence). In general, cognitive functioning was found to be worse in older persons and this was associated to a decrease in driving ability. A more in-depth analysis is still in progress. As cognitive functioning in relation to driving is rarely studied using neuroimaging techniques, future studies should employ this more fundamental approach to identify the underlying neurological mechanisms that impact driving performance of older persons.

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Poster

313. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 313.01/O1

Topic: E.04. Voluntary Movements

Support: Whitaker International Scholars Program
Swiss NSF Ambizione
Title: A communication subspace isolates population level interactions between motor and somatosensory cortex

Authors: *M. G. PERICH1, S. CONTI3, B. BARRA4, S. M. WURTH7, M. KAESER5, J. BLOCH8, G. COURTINE9, S. MICERA10, M. CAPOGROSSO6, T. MILEKOVIC2;

1Univ. of Geneva, Geneva, Switzerland; 2Fac. of Medicine, Dept. of Basic Neurosci., Univ. of Geneva, Geneve, Switzerland; 3Inst. di BioRobotica, Scuola Superiore Sant'Anna, Pontedera, Italy; 4Dept. of Neurosciences and Movement Sciences, Section of Med., 6Dept. of Neurosci., 5Univ. of Fribourg, Fribourg, Switzerland; 7Bertarelli Fndn. Chair in Translational Neuroengineering, EPFL - Campus Biotech B3.04, Geneve, Switzerland; 8CHUV, Paudex, Switzerland; 9EPFL, Geneve, Switzerland; 10Ecole Polytechnique Federale De Lausanne, Lausanne, Switzerland

Abstract: During the control of reaching, motor commands are integrated with sensory feedback to enable robust movements. Yet, it remains unclear how sensorimotor cortical areas achieve this integration. Isolating cortical activity related to ongoing motor commands from the sensory-related signals will help us to understand the neural control of movement. Recent advances have shown how “subspaces” that capture population-wide patterns of neural covariation can mediate specific functions, such as motor planning or adaptation. We hypothesized that interactions between primary motor (M1) and somatosensory (S1) cortex - such as efference copy to S1 or reafference to M1 - are captured at a population level within a “communication subspace” that is isolated from the other inputs to and outputs from these regions.

We trained two monkeys to reach for, grasp and pull an object while we recorded limb kinematics, pulling force, and the activity of neural populations in M1 and area 2 of S1. Using Principal Components Analysis, we identified a low-dimensional manifold within each region capturing the majority of neural variance. Since the interactions between these regions is only a subset of the functions performed by each, we expected the communication between them to be lower-dimensional than the full population activity. We also expected that such communication should be separable from activity directly related to the inputs and outputs of each cortical area. We developed an optimization framework that identified two orthogonal subspaces within these M1 and S1 manifolds. The communication subspace robustly linked M1 and S1, while the “behavior subspace” was strongly correlated with limb movement.

We then explored the roles of these subspaces during movement and the separability of the information and cortical dynamics within each. We first used linear regression to decode limb kinematics from the activity of the communication or behavior subspaces. We found that the behavior subspaces were the superior predictor. We then trained Generalized Linear Models that used the communication or behavior subspaces within one area to predict single neuron spiking in the other area. As predicted, the communication subspaces contained the most information about cortical spiking. These results indicate that population level interactions between M1 and S1 can be isolated from other features of the population, which may include inputs from, or
outputs to, spinal circuits. This method could help us to understand rapid sensorimotor integration for feedback control, and enable robust decoding for brain computer interfaces.


Poster

313. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 313.02/O2

Topic: E.04. Voluntary Movements

Support: HHMI
NIH
DARPA
Simons Foundation

Title: Causal role of motor preparation during error-driven learning

Authors: *S. VYAS\textsuperscript{1}, D. J. O'SHEA\textsuperscript{1}, S. RYU\textsuperscript{2}, K. V. SHENOY\textsuperscript{3};
\textsuperscript{1}Stanford Univ., Stanford, CA; \textsuperscript{2}Dept Neurosurg, Palo Alto Med. Fndn., Palo Alto, CA; \textsuperscript{3}EE, BioE & Neurobio., Howard Hughes Med. Inst. - Stanford Univers, Stanford, CA

Abstract: Current theories suggest that an error-driven learning process updates trial-by-trial to facilitate motor adaptation. The mechanism by which motor cortical preparatory activity interacts with this process or drives learning remains unknown. We previously found that visuomotor learning is correlated with systematic changes in preparatory activity on single trials (Vyas et al., Neuron 2018). The goal of this study was to investigate if there exists a causal relationship between motor preparation and learning. We began by correlating preparation time and learning as a Rhesus macaque adapted to a visuomotor rotation. We found preparation time was inversely correlated to both variance of errors on current trials (p < 0.01), and mean error on subsequent trials (p < 0.01). Next, we performed intracortical microstimulation (ICMS) in premotor (PMd) and primary motor (M1) cortex. We found no statistically significant difference, including between the error distributions of ICMS and non-ICMS trials, except for the previously reported reaction time penalty (Churchland et al., JNP 2006). Surprisingly, we found a disruption to the “expression of learning,” i.e., trials following ICMS showed a significant disruption compared to trials following no-ICMS (p < 1e-3). This suggests that ICMS of preparatory activity engages an error-driven learning process, and in particular disrupts its update computation, thus manifesting as a disruption on subsequent trials instead of current trials. In separate experiments we varied the time of ICMS during preparation and found that disruption to expression of learning was
only present when ICMS was performed near the go-cue. We also observed a “dose-dependent” effect to the disruption by manipulating brain region (PMd vs. M1; where M1 has less preparatory activity), and amount of preparation time prior to ICMS (p < 0.05). State-space models revealed that disruption to expression of learning was consistent with a gain-control mechanism whereby the error-driven learning process reduces the gain associated with its update when faced with unexpected perturbation. Consistent with the data, the model predicted that gain returns to baseline with a slow time constant, compared to the original decrease, but only when stimulation ceases. With additional stimulation, gain no longer changes, but stays at a decreased, albeit saturated level (p < 1e-3). To our knowledge, this constitutes the first causal evidence for the role of preparatory activity during learning. This study also establishes a link between motor preparation and the error-driven learning system, which may provide a new lens into investigating trial-by-trial adaptation.

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**Poster**

**313. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 313.03/O3

**Topic:** E.04. Voluntary Movements

**Support:** Brain Research Trust
Wellcome Trust
BBSRC

**Title:** Time-dependent modulation of spinal excitability during action observation in the macaque monkey

**Authors:** *S. J. JERJIAN*¹, R. N. LEMON², A. KRASKOV¹;
¹Dept. of Clin. and Movement Neurosciences, ²UCL Queen Square Inst. of Neurol., London, United Kingdom

**Abstract:** Neurons in the primate motor cortices, including identified pyramidal tract neurons (PTNs) which project directly to the spinal cord, respond to the observation of others’ actions, yet these responses do not give rise to any movement in the observer. Human studies, using transcranial magnetic stimulation (TMS) or H-reflex responses, have reported motor ‘resonance’ between observed and executed actions in the corticospinal system. Here, we investigated time-dependent modulation in the level of spinal excitability by monitoring electromyographic (EMG) responses produced by single shocks delivered directly to
the pyramidal tract (PT) of two adult rhesus macaque monkeys. It is well known that H-reflex is very difficult, if impossible, to record in intrinsic hand muscles. TMS evoked response represents a sum of multiple indirect volleys (I-waves) whereas direct PT stimulation evokes a clear short-latency descending volley in corticospinal axons and therefore allows to investigate spinal excitability without influence of cortico-cortical interactions. We recorded and analysed short-latency PT evoked responses in hand and digit muscles while monkeys performed, observed or withheld reach-to-grasp and hold actions. On some observation trials, we also altered the visibility of the observed action and the information available regarding the upcoming grasp, in order to test the dependence of any spinal modulation on the visual information available to the monkey.

We found modest grasp-specific facilitation of hand muscle responses around the time of grasp observation, which persisted when the grasp was predictable but obscured from the monkey’s vision. We also found evidence of a more general inhibition of movement before the grasp. Interestingly, MEPs during initial part of the observation trial were comparable in amplitude to MEPs after cued with a NoGo signal when monkeys explicitly suppressed their movement.

In summary, we confirm that the spinal circuitry controlling hand muscles is modulated during action observation, and this may be driven by internal representations of actions. The relatively modest changes in spinal excitability during observation suggest that the corticospinal activity present exerts only minor, sub-threshold changes on hand motoneuron pools, thereby preventing any overflow of mirror activity into overt movement.

Disclosures: S.J. Jerjian: None. R.N. Lemon: None. A. Kraskov: None.

Poster

313. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 313.04/O4

Topic: E.04. Voluntary Movements

Support: Swiss National Science Foundation fellowship P2FRP3_168460
Swiss National Science Foundation fellowship P400PB_180818

Title: Toward electrophysiological and behavioral effects of causal perturbations in the hand grasping network of rhesus monkeys

Authors: *A.-D. GINDRAT¹, H. SCHERBERGER¹,²;
¹Deutsches Primatenzentrum GmbH, Goettingen, Germany; ²Fac. of Biol. and Psychology, Univ. of Goettingen, Goettingen, Germany

Abstract: Voluntarily grasping objects with the hand is crucial for primates and constitutes a highly complex sensorimotor process. In macaque monkeys, the fronto-parietal hand grasping
network includes the anterior intraparietal area (AIP), the hand area of the ventral premotor area (F5 or PMvr) and the hand area of primary motor cortex (M1). Important insights into the functioning of this network were obtained using techniques that perturb brain activity in monkeys. For example, while electrical intracortical microstimulation (ICMS) in F5 could not elicit any hand movements, it was shown to exert a facilitatory effect on M1 outputs to upper limb motoneurons. Nevertheless, the specific functional contributions of each area of this network to hand grasping remain elusive.

This study is part of a larger project aiming at causally investigating the primate hand grasping network using the newly established and highly innovative technique of neuro-optogenetics. We aim to compare the effects of optogenetic stimulation in F5 on the electrophysiological activity in the grasping network and on hand grasping behavior, with those induced by well-established interference methods, here ICMS.

We trained two adult female rhesus monkeys (Macaca mulatta, age: 15 years) to perform a visually-instructed delayed grasping task, in which they had to lift different objects presented on a turntable in random order. During this task, we recorded spiking activity in areas F5, M1 and AIP with single movable microelectrodes, and we simultaneously measured hand kinematics using a custom-made instrumented glove able to record 32 degrees of freedom of the fingers, hand and arm. Recordings confirmed previous findings of the grasp-related activity of these areas and their associated hand kinematics.

Using this setup and paradigm, we will deliver ICMS in F5 in 50% of all trials, randomly interleaved with no-stimulation trials. We expect that ICMS in F5 per se will not elicit overt hand movements (Umilta et al., 2007), but induce a reduction in movement time, indicating a facilitatory effect of F5 on M1 (Prabhu et al., 2009). Furthermore, the instrumented glove will allow us to track detailed alterations of hand kinematics.

Disclosures: A. Gindrat: None. H. Scherberger: None.
Abstract: In order to perform real-world tasks like grasping efficiently, the primate brain has to process visual object information so that the grip aperture can be adjusted before contact with the object is made. Electrical microstimulation of 3D shape-selective clusters in AIP during fMRI activates areas F5a and 45B (Premereur E et al., 2015, PLoS Biology 13: e1002072), indicating that these frontal areas represent important downstream areas for object processing during grasping, but the role of area 45B in grasping is unknown. To assess the causal role in the grasping network of these frontal areas located in the arcuate sulcus, we reversibly inactivated 45B, F5a and F5p during a visually guided grasping task in macaque monkeys. Using a robot, we presented two different spheres (large and small – 3 and 1.5 cm diameter), within reaching distance (36 cm) at chest level. First, we recorded single neuron activity in 45B, F5a and F5p to functionally identify the center of object selectivity during grasping. Then, we injected muscimol (three sessions per area) or saline (one session per area) to measure the grasping deficit induced by the temporary disruption of these three nodes in the grasping network. Behavioral data were collected before and after muscimol injection and were compared to saline sessions. The inactivation of all three areas resulted in a significant increase in the total grasping time (i.e. the time between the go-signal and the pull of the object, t-test p<0.01). However, we observed a different pattern of deficits across sessions: while inactivation of area F5p resulted in a very strong deficit only during the first session, inactivation of area 45B produced more stable deficits in grasping across sessions. Finally, the pattern of deficits after inactivation of area F5a was intermediate between the two previous neighboring areas. Overall, these results not only confirm a clear involvement of F5p, in line with previous studies, but also suggest a causal contribution of area F5a to grasping, and an unexpected role for area 45B in object grasping, even though previous studies suggested an involvement in oculomotor control.

Disclosures: I. Caprara: None. P. Janssen: None.

Poster

313. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

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Topic: E.04. Voluntary Movements

Support: European Research Council grant WIRELESS (678307)

Title: The neuroethoroom: A new tool for neurobehavioral studies in freely behaving monkeys

Authors: *F. LANZARINI, M. LANZILOTTO, L. BONINI;
Dept. of Med. and Surgery, Univ. of Parma, Parma, Italy

Abstract: Neurophysiological studies in non-human primates (NHP) typically require the use of primate chairs and constrained laboratory settings. This is necessary to make the recording of
neuronal activity technically possible and to achieve a sufficient behavioral control, but at the same time strongly limits the possibility to understand brain functioning underlying unconstrained, natural behavior. In recent years, thanks to newly developed technologies, untethered neural recording from unrestrained animals is becoming more and more feasible (Mimica et al., 2018; Omer et al., 2018), and video behavioral tracking is improving dramatically, making possible to apply these technologies to large animals, such as NHP. Here, we present a new setup and methodology to simultaneously study NHP behavior and brain activity during unconstrained situations. To this purpose, we designed and built a transparent plexiglass enclosure (WxHxD, 208x205x181 cm) in which the space can be easily manipulated and/or flexibly filled with different devices, such as food and non-food items, ropes and wooden structures, in order to elicit a wide variety of NHP ethologically relevant behaviors (NeuroEthoRoom, NER). A system of 8 IR-sensitive cameras was set around the NER, to record NHP behavior at a frequency up to 100 Hz from multiple viewpoints. Two macaque monkeys, housed in pair (one dominant and one subordinate), were individually trained to enter in the NER. We could automatically track the monkey’s position during natural behaviors through a customized head-post cover equipped with one or up to 4 retroreflective markers (RMs). Each monkey performed multiple sessions in the NER, where it was free to climb and walk over a wooden structure to reach food rewards and to manipulate a dangling plastic ball filled with fruit pieces. We automatically tracked the RM position by means of SIMI technologies software (SIMI Motion). We could then calculate, off-line (MATLAB), the exact position of the monkey’s head relative to the enclosure bounding walls, the wooden structure and the plastic ball, as well as kinematics parameters regarding monkey’s head motion, such as speed and acceleration. By using 4 RMs we could also extract information about head orientation, roll and pitch. This new setup makes it possible to study a large variety of ethologically relevant behaviors in the NHP repertoire, which were impossible to investigate with conventional methods. By integrating this technology with wireless neural recordings synchronized with the multicamera system, the NER will allow to correlate neural activity with NHP natural behavior.

Disclosures: F. Lanzarini: None. M. Lanzilotto: None. L. Bonini: None.

Poster

313. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates I

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Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 313.07/O7

Topic: E.04. Voluntary Movements

Support: The Center for Sensorimotor Neural Engineering
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Title: Calibrating temporal expectations through flexible tuning of neural dynamics

Authors: *N. MEIRHAEGHE*¹, H. SOHN², M. JAZAYERI³;

Abstract: Humans continuously form expectations about the world based on prior experience, and combine this information with incoming sensory inputs to refine their behavior. This process critically relies on the ability of the nervous system to predict and simulate events based on environmental cues and statistical regularities. Moreover, in a dynamic environment, predictions must be continuously adjusted to match the ever-changing behavioral demands. How regularities in the environment constrain neural activity to afford predictive capacities, and how neurons change their patterns of activity to update predictions is not known.

To tackle these questions, we trained monkeys on a time reproduction task in which we could readily manipulate statistical regularities. Animals had to measure a sample interval between the first two beats of a rhythm, and initiate a saccade on the third omitted beat. When exposed to various distributions, animals’ responses were biased toward the mean of the learned distributions. These biases were accompanied by an adjustment of the speed of neural activity during the measurement epoch within dorsomedial frontal cortex. Specifically, the speed of population dynamics was adjusted according to the mean of the distribution. We hypothesize that this population tuning reflects the ongoing temporal expectations of animals throughout learning.

We tested this hypothesis using a two-stage computational model. The model consists of two serially coupled drift-diffusion processes (DDP)—an unbounded DDP for the measurement epoch (DDPm), followed by a bounded DDP for production (DDPp). DDPm uses a fixed drift rate to track elapsed time between the first two beats. When the second beat comes, the deviation of DDPm from a fixed threshold serves as sensory prediction error, and is used to adjust the drift of DDPp controlling movement initiation. Critically, the drift of DDPm is adjusted to reduce prediction error in subsequent trials, allowing the model to tune to the interval distribution. The model captured the history dependence of behavioral biases and provided a readout of animals’ ever-changing estimate of the distribution mean. Ongoing work seeks to validate our model by exposing animals to new distributions while tracking changes in cortical activity.

Disclosures: N. Meirhaeghe: None. H. Sohn: None. M. Jazayeri: None.
Rapid feedback responses in primary motor cortex are sensitive to goal redundancy

**Authors:** *K. P. CROSS*¹, D. J. COOK², S. H. SCOTT³;
²Neurosurg., ³Dept Biomed. and Mol. Sci., ¹Queen's Univ., Kingston, ON, Canada

**Abstract:** A hallmark of our motor system is the ability to flexibly alter motor actions to attain a behavioural goal. Recently, we had human participants reach for either a narrow or wide target while we unexpectedly applied a mechanical load that displaced the hand along the redundant spatial dimension of the wide target. We found subjects corrected less when reaching for the wide target and found muscle responses reflecting target shape started in <100ms after the mechanical disturbance. We hypothesized that this rapid muscle response was generated by transcortical feedback involving primary motor cortex (M1). We tested this hypothesis by training a monkey (macaque mulatta) to make goal-directed reaches to targets that primarily required elbow extension. The goal target could either be a narrow (width=3cm) or a wide goal resembling a chevron (width=28cm), and target shape was changed randomly on a trial-by-trial basis. On random trials a mechanical load was applied that deviated the animal’s hand lateral to the target. When no loads were applied, monkeys tended to reach towards the center of both targets. However, reach endpoints were far more variable for the wider target as the endpoint ellipses were ~4x larger than the ellipses for the narrow target (paired t-test t(7)=7.3, p<0.001). When loads were applied, monkeys made large corrections for the narrow target. In contrast, monkeys corrected less for the wide target exploiting that a small correction could still attain the goal. Kinematic changes due to target shape could be detected in the lateral velocity starting at ~210ms. We recorded from M1 using a 96-channel floating micro-electrode array. We examined the population response following mechanical disturbances across all 96 channels and across multiple days. We found changes in the population firing rate started ~50ms after the applied load. The initial response (50-75ms) was similar regardless of target shape (paired t-test p>0.1). Critically, in the 75-100ms epoch we observed a significant increase in firing rate for the narrow as compared to the wide target (t-test p<0.01). Our data suggests that a transcortical feedback pathway through M1 is involved in generating flexible, target-dependent feedback responses during reaching.
**Disclosures:** K.P. Cross: None. D.J. Cook: None. S.H. Scott: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BKIN Technologies Ltd.

**Poster**

313. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates I

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 313.09/O9

**Topic:** E.04. Voluntary Movements

**Support:** NIH R01NS045853

**Title:** Encoding spaces for multiplexing movement representations across timescales in motor cortex

**Authors:** *P. MALONIS*¹, N. G. HATSOPOULOS², J. N. MACLEAN³;
¹Committee on Computat. Neurosci. Grad. Program, ²Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL; ³Neurobio., The Univ. of Chicago, Chicago, IL

**Abstract:** Primary motor cortex (M1) has been reported to exhibit activity that encodes current movement as well as planning for future movements. These distinct signals have, for the most part, been studied using behavioral tasks in which motor planning and execution occur during distinct epochs of the task. However, in the case of natural behavior, it is often necessary to plan future movements while simultaneously executing ongoing movement. Here, we record from ensembles of neurons in motor cortex during continuous movement, which allows us to determine the extent to which ensembles exhibit planning signals for future movements simultaneously with representations of ongoing movements in motor cortex. By training decoders that use neural activity to predict movement at different points of time relative to the activity and comparing the decoders’ parameters, we can compare the subspace of ensemble activity that encodes movement at each time point. Comparison of these “encoding spaces,” provides insight into the degree to which representations of movement at different timescales are multiplexed in the same cortical area. In M1 of macaque monkeys, we find a component of the encoding space for arm movements at specific time points in the near-term (~150ms) that is orthogonal to the encoding space for arm movements further into the future (~600ms), but not to encoding spaces for movements at other time points. This result suggests that movement representations for these two timescales could to some extent be linearly distinguished in a multiplexed code.

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**Poster**

**313. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates I**

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**Program #/Poster #:** 313.10/O10

**Topic:** E.04. Voluntary Movements

**Support:** NIH Grant R01NS092626

**Title:** Neural activity of units not controlling BCI directly during a BCI task

**Authors:** *Z. LIU*¹, *M. H. SCHIEBER*²;

¹Biomed. Engin., Univ. of Rochester, Rochester, NY, NY; ²Neurol. and Neurosci., Univ. of Rochester, Rochester, NY

**Abstract:** The development of brain-computer interfaces (BCI) that use spike recordings for input has primarily used neural recordings from the primary motor cortex (M1). Nevertheless, the firing rates of many M1 units not directly involved in controlling the BCI (non-BCI units) has shown varied in relation to the BCI task. We investigated non-BCI units among multiple cortical areas while a BCI is controlled with a small number of M1 units.

We trained two monkeys (*Macaca mulatta*) to perform an 8-target center-out task using a BCI with four M1 units. We recorded the spike activity of non-BCI units simultaneously in 6 cortical areas—M1, dorsal premotor cortex (PMd), ventral premotor cortex (PMv), the primary somatosensory cortex (S1), the anterior intraparietal area (AIP), and dorsal posterior parietal cortex (dPPC).

For units that are not controlling the BCI directly (non-BCI units) in Monkey P, over 60% of the units in each of the six cortical areas are modulated with the BCI task (*p < 0.05*). For Monkey Q, more than 75% of the units in each of the six cortical areas are modulated with the BCI task (*p < 0.05*). For the population neural activity in each cortical area, we partitioned the variance of firing rates that were related to 1) the execution of a movement in general (condition-independent or general task), 2) the dependent variables of the behavioral task—eight target locations (condition-dependent), and 3) the remaining noise. Condition-dependent variance only starts after Go Cue, and peaks at around 500ms after Go Cue, with BCI M1 units having significantly greater variance than any other areas. The condition-independent variance peaks at about 200ms earlier than the condition-dependent variance. BCI units, Non-BCI M1 units and PMd units have larger condition-dependent variance than the condition-independent variance, while AIP units have larger condition-independent variance than the condition-dependent variance. We further decode the task variable (instructed targets) with the firing rates of neurons. We started with using all available units in each cortical area, and the decoding accuracy with non-BCI M1, PMd, and PMv units is higher than that with BCI units. However, if we perform the decoding using four-unit combinations, the average decoding accuracy with BCI units is
higher than that with units in any other cortical area. Our findings demonstrate that non-BCI units in many cortical areas are modulated significantly while M1 units control a BCI, with the condition-dependent and condition-independent variance varying among areas. We suggest that control of a BCI using M1 neurons may require a network extending well beyond M1.

**Disclosures:**  
Z. Liu: None. M.H. Schieber: None.

**Poster**

313. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates I

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

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**Topic:** E.04. Voluntary Movements

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Department of Veterans Affairs Grant I01 RX002835-01A1  
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**Title:** Primary motor cortex employs similar yet distinct population dynamics during locomotion and obstacle avoidance

**Authors:** *D. Y. XING1, W. TRUCCOLO2, D. A. BORTON1;  
1Sch. of Engin., 2Dept. of Neurosci., Brown Univ., Providence, RI

**Abstract:** Primary motor cortex (M1) is necessary in primates for generating precise, voluntary actions. However, the involvement of M1 during locomotion, which may not require attention or even online control, as well as how M1 integrates voluntary actions onto ongoing locomotor rhythms, such as obstacle avoidance, is not well understood. It has been postulated that voluntary limb movements like reaching and grasping may have evolved out of precise gait modifications during locomotion, in which case, we would expect to see similar neural activity patterns during these motor behaviors. To investigate the relationship between M1 activity during locomotion and the activity during directed, volitional actions, we employed an obstacle avoidance paradigm which requires the generation of directed limb movements both from rest as well as during locomotion. We recorded leg-M1 and arm-M1 activity with implanted microelectrode arrays from nonhuman primates (Rhesus Macaque) while the animals performed basic walking on a treadmill at 2.2 km/h (“autonomous” locomotion), stepping over an obstacle moving towards them at 2.2 km/h while stationary (volitional movements), and stepping over the obstacle while walking on the treadmill (integrated movement and transition of states). In arm area, we found that 42/53 neurons changed their depth of modulation and 26/53 neurons changed their gait cycle phase tuning between stationary obstacle avoidance steps and locomotion steps (unpaired t-test,
n=39 obstacle trials, n=73 walking trials, Benjamini-Hochberg correction with FDR=10%, suggesting that voluntary movements and basic locomotion are encoded differently in M1. To compare the cortical activity on a population level, we utilized a Poisson linear dynamical system (PLDS) model to explicitly extract the neural dynamics from recorded neurons. Low-dimensional trajectories in the latent state-space revealed similar rotational structure for both basic locomotion and stationary obstacle avoidance. However, we also found a single dimension, using linear discriminant analysis (LDA), that separated the neural trajectories during the diverse movements. During integrated movements, e.g. obstacle avoidance while walking, the neural latent state smoothly transitioned from the putative locomotion region to the putative volitional movement region in the LDA dimension while maintaining its rotational structure. These findings suggest that for voluntary movements and autonomous locomotion, rotational dynamics are conserved in motor cortex, but additional dimensions distinguishes the population activity between the two movement modalities.


Poster

313. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 313.12/O12

Topic: E.04. Voluntary Movements

Support: MRC grant RES/0165/7611/097

Title: Activity in macaque primary motor cortex during a motor inhibition task

Authors: *T. TOHYAMA, S. N. BAKER;
Inst. of Neuroscience, Newcastle Univ., Newcastle upon Tyne, United Kingdom

Abstract: Voluntary motor control requires not only execution of desired movements, but also inhibition of inappropriate responses. Such motor inhibition has received less attention, especially in experiments performing direct neural recordings in non-human primates. Studies with the stop-signal paradigm have shown that suppressing a prepared response depends primarily on the prefrontal cortex, however it is unclear what downstream structures enact the stop command. The primary motor cortex (M1) is a key center for motor execution. It is unclear whether M1 activity changes to stop the movement, or whether downstream inhibition prevents the execution of the unaltered go command from M1. We therefore investigated how M1 activity modulates during motor inhibition. A macaque monkey was trained to perform a stop-signal task. Trials were initiated by grasping a handle. On 75% of trials, a green LED illuminated and the handle had to be released to earn a reward (a go trial). On 25% of trials, the green LED was followed by illumination of a red LED after a delay (a stop trial); a reward was then only earnt if
the monkey did not release the handle. Using a race model[1], the stop-signal reaction time (SSRT, an estimate of the latency of the stop process), could be calculated. Single cell recordings from the contralateral hand area of M1 were made during task performance; pyramidal tract neurons (PTNs) were identified by antidromic activation from a chronically implanted PT stimulating electrode and collision test. The estimated SSRT was 221 ms (the 95% confidence interval: 218-223 ms; n=97 sessions). PTNs were classified according to whether they fired significantly more in the period 0.3-1 s after the go cue during go (81/139 cells) or stop trials (37/139 cells). Average firing rates compiled selectively over these two PTN populations revealed a change in rate between stop and go trials prior to the end of the SSRT. The response of corticospinal cells to the stop-signal occurs prior to completion of the stop process, suggesting that the descending command from M1 is modified as part of the response to prevent movement. 1. Logan, G.D. and Cowan, W.B., Psychological Review, 1984. 91: p. 295-327.

Disclosures: T. Tohyama: None. S.N. Baker: None.

Poster

313. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates I

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Topic: E.04. Voluntary Movements

Support: Ku Leuven Grant C14/18/100
Odysseus Grant G.0007.12

Title: Simultaneous multichannel recordings in dorsal and ventral premotor cortex during a reach-to-grasp task

Authors: *S. DE SCHRIJVER, E. PREMEREUR, T. DECRAMER, P. JANSSEN; Kuleuven, Leuven, Belgium

Abstract: The premotor cortex is involved in visually guided motor behavior with the dorsal premotor cortex (PMd) more implicated in reaching and the ventral premotor cortex (PMv) more involved in grasping. To investigate the potential of both areas for novel brain-machine interfaces (BMIs) based on visuomotor activity, we implanted 96-channel Utah arrays in dorsal premotor area F2 and in ventral premotor area F5c. Single unit (SUA) and multiunit activity (MUA) were recorded while the monkey performed a reach-to-grasp task towards 3 identical objects at different locations in space, hence requiring different reach directions. In 19 sessions, we recorded the activity of 2097 MUA sites (PMd: 1054; PMv: 1043) and 1005 single neurons (PMd: 571; PMv: 434) with significant task-related activity. On average, neuronal activity in PMd showed a strong increase in spike rate at object onset and remained elevated throughout the trial until the grasp movement. In contrast, the average activity of PMv neurons decreased below
baseline level after object onset and only sharply increased after the onset of the hand movement. A visuograsp index (comparing the activity after object onset with the activity around pull of the object) differed significantly between dorsal (MUA: -0.0202, SUA: 0.0256) and ventral premotor cortex (MUA: -0.0300, SUA: -0.3112; t-test MUA: p<0.01, SUA: p<0.001). Furthermore, the activity during the reaching phase was higher in PMd than in PMv, whereas the opposite was true during the grasping phase (reach-grasp index comparing the activity after the lift of the hand with the activity around the pull of the object, PMd MUA: 0.0183, SUA: 0.0137; PMv MUA: -0.0209, SUA: -0.1992; t-test MUA: p< 0.001, SUA: p<0.001). Finally, both areas showed significant direction selectivity (selectivity indices comparing the activity for the best direction versus the worst direction, PMd: >0.0364, t-test: p<0.001; PMv: >0.0299, t-test: p<0.001), which was significantly higher for SUA than for MUA (anova, p<0.001). Taken together, we can conclude that the premotor cortex is functionally heterogeneous, with high visual responses in PMd and more movement-related responses in F5c. Furthermore, PMd neurons show higher activity for reaching, whereas PMv neurons show higher activity during grasping, although both areas encode reaching and grasping. Finally, our data indicate that SUA differentiates more between reach directions than MUA, which has strong implications for the development of novel BMIs based on visuomotor activity.

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Poster

313. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates I

Location: Hall A

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Program #:Poster #: 313.14/O14

Topic: E.04. Voluntary Movements

Title: Optogenetic inhibition of premotor-to-parietal projections reveals a top-down modulation in rule-based sensorimotor transformations in rhesus monkeys

Authors: *H. GUO1,2, M. FORTUNA1, J. HUEER1, J. GRUBER1, S. TREUE1,2,3, H. SCHERBERGER1,2,3, A. GAIL1,2,3;
1German Primate Ctr., Goettingen, Germany; 2Univ. of Goettingen, Goettingen, Germany; 3Bernstein Ctr. for Computat. Neurosci. Goettingen, Goettingen, Germany

Abstract: Context-dependent sensorimotor transformations have been associated with the frontoparietal network in the cerebral cortex of primates. It has been hypothesized that the mutually connected parietal reach region (PRR) and the dorsal premotor cortex (PMd) coordinate their activity for context-dependent motor goal selection, particularly, that parietal motor goal encoding might be contingent upon PMd input in non-standard visuomotor mappings. Yet, such causal functional link has not been shown so far.
We used pathway-selective optogenetic reversible inhibition (AAV2/5-CaMKIIα-eArchT3.0-eYFP) of the neural projections from PMd to PRR while the monkey performed a memory-guided center-out anti-reach task with ocular fixation. Continuous laser stimulation (532 nm, 330 ms) of the neuropil was applied simultaneously with the visual cues which were presented to the animal prior to an instructed delay period and which instructed the reach goal. Stimulation trials were randomly interleaved with no-stimulation trials. Stimulation was applied either in the transfected PMd or in PRR. Single-unit microelectrode recordings were performed simultaneously in the light-stimulated neuropil, with an inter-tip linear distance between electrode and optical fiber of 500-950 μm. Laser stimulation in PMd resulted in reliable silencing of nearby PMd neurons. Light-induced inhibition of projections from PMd within PRR resulted in neural response modulations in nearby neurons during the movement planning following light stimulation. In both pro- and anti-reach trials, directional selectivity of individual neurons could be preserved, erased or evoked. At the population level, motor goal encoding in PRR was delayed after laser-stimulation exclusively in anti-reach trials. These results support the hypothesis that dynamic reorganization in PRR, as it is selectively needed for spatial remapping in anti- but not pro-reach trials, is contingent on functional input from PMd. Our findings provide causal evidence that encoding of rule-based reach goals in parietal cortex partly builds on frontal-to-parietal functional interactions.


Poster

313. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates I

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Title: Constraints on the time course of neural population activity

Authors: *A. D. DEGENHART¹,³, E. M. GRIGSBY¹,³, N. T. MCCLAIN¹,³, E. R. OBY¹,³, A. P. BATISTA²,³, B. M. YU⁴,³;
Abstract: Across different tasks and brain areas, the time course of neural population activity has been suggestive of network-level mechanisms, such as point attractors and line attractors, underlying sensorimotor behavior. These mechanisms, if present, specify particular sequences of population activity patterns that are naturally produced, given the underlying network constraints. A key prediction suggested by these constraints is that it is difficult for the brain to produce time-reversed sequences of the naturally-produced activity. To test this prediction, we leveraged a brain-computer interface (BCI) to probe the ability of Rhesus monkeys to volitionally produce time-reversed sequences of population activity. The key advantage of using a BCI in this context is that it allows us to place task requirements on the generation of specific sequences of neural activity. Animals performed a BCI task where they moved a cursor between two targets in a virtual environment. This task design required animals to move between the same two targets (A and B), but in time-reversed sequences (A->B and B->A). Neural activity, recorded using a 96-electrode “Utah” array, was mapped onto the 2D position of the BCI cursor. Animals first performed the two-target task using an “intuitive” BCI mapping calibrated to yield proficient control. We observed that even though the paths traversed by the cursor were highly overlapping in the animal’s workspace, high-dimensional neural population trajectories exhibited distinct paths for each target. We then sought to determine the extent to which animals could violate these observed sequences of neural activity. We used neural activity observed during intuitive BCI control to define a “rotated” mapping from the high-dimensional neural activity space to the 2D cursor workspace where the A->B and B->A neural trajectories were most different. Using this rotated mapping, animals were then required to generate cursor trajectories which were inconsistent with those predicted from intuitive BCI control. We found that it was difficult for animals to modify their cursor trajectories in the rotated projection when task success was contingent upon doing so, indicating that they were incapable of producing time-reversed sequences of neural activity. These results provide evidence that it is difficult to violate the naturally-occurring sequences of neural population activity. This implies that the underlying network imposes strong constraints on the time course of population activity and, therefore, appears capable of implementing widely-hypothesized network-level mechanisms that specify particular sequences of population activity patterns.


Poster

313. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 313.16/O16
**Topic:** E.04. Voluntary Movements

**Support:**
- NIH R01 104898-01
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- Tarson Fund

**Title:** Chronic wireless neural population recordings in freely behaving marmosets

**Authors:** *J. D. WALKER¹, D. MOORE², M. SUNDIANG⁴, J. N. MACLEAN³, N. G. HATSOPoulos¹;

¹Univ. of Chicago, Chicago, IL; ³Neurobio., ²The Univ. of Chicago, Chicago, IL; ⁴Organismal Biol. and Anat., Univ. Of Chicago, Chicago, IL

**Abstract:** Studying the brain during natural ethologically relevant behavior will yield important insights into cortical function. This goal presents substantial technical challenges for the acquisition of neurophysiological data particularly in non-human primates. The common marmoset presents a number of advantages for the neurophysiological study of natural behavior [1]. Here we report the design and implementation of an acrylic free surgical preparation for implanting multielectrode arrays to chronically record sensorimotor cortical population activity from common marmosets across a range of behaviors and brain states. Moreover, our approach is both flexible and robust, and has allowed us to record sensory motor cortical responses for multiple years. Additionally, we present a wireless headstage configuration that minimizes the need for excessive handling of the marmosets while maximizing the ease of replacing batteries to facilitate long recordings. We show that we are able to use these surgical techniques and headstage configuration to record across the marmoset’s behavioral repertoire, such as during locomotion, foraging, grooming and during sleep. Coupled with the in home-cage approach to behavioral training we have developed, this wireless recording preparation provides a viable alternative to neurophysiology with chair restraint for recording neural activity from marmosets during behavioral training paradigms.


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**Poster**

313. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates I

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Topic: E.04. Voluntary Movements

Support: NIH R01 N545853-01

Title: Examination of shared and non-shared neural subspaces in the primary motor cortex between active movement and brain machine interface control

Authors: S. GUPTA¹, S. SHEEN¹, V. PAPADOURAKIS³, K. TAKAHASHI³, N. G. HATSOPOULOS³, *A. J. SUMINSKI²;¹Computer Sci., ²Neurolog. Surgery, Univ. of Wisconsin-Madison, Madison, WI; ³Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: The notion that the primary motor cortex (M1) is simply a driver of voluntary movements has long masked its true identity as the major hub of sensorimotor processing in the cerebral cortex. Indeed, the activity of single neurons in M1 is highly heterogeneous often varying greatly based on the state of the limb, the presence and fidelity of sensory feedback, and the type of end effector being controlled. However, it remains unclear how variations in motor task and sensory context are represented by neural ensembles in the high dimensional space that describes M1 activity. Here we take advantage of dimensionality reduction methods to characterize shared and non-shared neural subspaces between these conditions. Two monkeys (Macaca mulatta) were trained to move a visual cursor to hit a sequence of randomly placed targets while resting their arm in a two-link robotic exoskeleton. They performed the task under four different conditions. During active movement (AM), they used their arm to move the exoskeleton. In the visual observation (OBS) condition, the monkeys observed replay of AM task performance while holding their arm still. In the BMI conditions, the monkeys used a neural decoder to hit targets while holding their arm still (V BMI) and while the exoskeleton moved their arm along with the BMI controlled visual cursor (V+P BMI). Single unit activity was recorded using a 10x10 micro-electrode array chronically implanted in M1. We identify a set of latent variables that define the neural activity in M1 using dimensionality reduction. A novel unsupervised machine learning technique then structures combinations of latent variables as feature vectors and separates the conditions using a similarity metric. It finally quantifies and ranks the separability of the subspaces for all conditions. While much of the variance in population activity is shared across the conditions, we find significant non-shared subspaces between the four conditions. In particular, AM condition often occupies an entirely different subspace than the OBS and BMI conditions possibly due to the activation of muscles to move the arm. In addition, the two BMI conditions also seem to have separable subspaces as we find significant non-shared variance between the V BMI and V+P BMI conditions, suggesting that differences in the availability of sensory feedback modulates the encoding properties of neural ensembles in M1.

Temporal basis functions reveal systematic variation in rotational dynamics in monkey M1

Authors: *D. A. Sabatini*¹, M. T. Kaufman²;  
¹Organismal Biol. and Anat., The Univ. of Chicago, Chicago, IL; ²Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: Neurons in monkey motor cortex (M1) exhibit complex, multiphasic patterns of activity over time during reaching. These patterns are not fully explained by correlations with kinematic variables or muscle activity, and are incompletely fit by dynamical models of pattern generation. Here, we present a simple application of Principal Component Analysis (PCA), which we name Temporal Component Decomposition (TCD). TCD provides a new view of motor cortex activity. Typically, in the motor literature, PCA is performed on a neural data matrix organized as neurons by time and condition (N x TC), focusing on patterns of activity across neurons. TCD instead applies PCA to a matrix that is T x NC. TCD decomposes the data into a small number of temporal basis functions that are combined in a potentially unique way for each neuron in each condition (reach shape). Applying TCD to previously analyzed Utah array recordings from monkeys making curved reaches, we find several novel results. First, nine Temporal Components (TCs) account for ~90% of the variance in firing rates, compared to ~60% with nine components when using PCA over neurons. Six of these TCs comprise three rotational planes. This low-dimensional response cannot be accounted for by smoothing of a Poisson processes, 1/f spectral properties, or as a consequence of instantaneous encoding for muscle activity or kinematic variables. Second, the orientation of the TCs in neural state space (embeddings) varies substantially across reach conditions. The structure of these embeddings is systematic, with similar embeddings for similar reach conditions and low overlap in embedding between dissimilar conditions. This confirms previous findings that M1 has several rotational planes with preserved temporal structure, but shows that these planes are oriented differently for distant conditions. Finally, TC embeddings in state space have a simple, linear relationship with coarse-level parameterizations of the executed reach, revealing a relationship between motor cortex activity and kinematics not observable at the level of individual neurons. These results demonstrate that temporal decomposition can reveal variations in the neural dynamics across conditions that have confounded previous analyses.

Disclosures: D.A. Sabatini: None. M.T. Kaufman: None.
**Title:** The spatio-temporal organization of M1 activity during reaching and grasping in monkeys

**Authors:** *N. CHEHADE, O. A. GHBAWIE;* 
Dept. of Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

**Abstract:** Primary motor cortex (M1) is crucial for controlling arm and hand movements in primates. The spatial organization of M1 outputs is reflected in the somatotopic organization of the forelimb representation. Even though the arm and hand representations can be readily defined with intracortical microstimulation, the spatio-temporal organization of M1 activity that supports coordinated arm-hand actions is less clear. The present study is part of an effort to understand how the neural activity that supports reaching and grasping is encoded in the M1 forelimb representation. To achieve this objective, we trained a macaque monkey to perform a reach-to-grasp task while head-fixed. Target objects were presented in near (200 mm) and far locations (250 mm). Power grip and precision grip were elicited by large (32 mm diameter) and small (13 mm diameter) target objects. A chronic optical window provided access to M1, which we investigated with intrinsic signal optical imaging (ISOI, 630 nm illumination) and linear electrode arrays. ISOI (10 frames/s, 8 s/trials) revealed at least 3 domains (~1 mm²/domain) that were activated in M1 in response to reaching and grasping. M1 domains expanded spatially (up to 8 mm²/domain) even after the end of movement. Systematic shifts in the ‘go’ cue showed that peak reflectance change was temporally-locked to reach onset (~5.5 seconds) thus confirming that ISOI modulation was driven by task-related movements. Intracortical microstimulation in the same optical window showed that the activation domains spatially coincided with parts of the arm and hand representations. Unit activity recorded from linear electrode arrays in dozens of penetrations showed that ISOI domains contained units that were reach-modulated (18% of recorded units), grasp-modulated (38% of recorded units) and task-modulated (32% of recorded units). A preliminary map of the spatial locations of the recorded single units showed that reaching and grasping are encoded throughout the forelimb representation. Our findings thus far show that combining ISOI with neurophysiology has the potential to reveal the spatial-temporal organization of movement encoding in cortex.

**Disclosures:** N. Chehade: None. O.A. Gharbawie: None.
Title: Emergence of lateralized population activity in motor cortex across instructed-delay and execution phases of reaching

Authors: *T. C. DIXON*¹, C. M. MERRICK², R. T. KNIGHT², R. B. IVRY², J. M. CARMENA³;
¹Bioengineering, ²Psychology, ³Electrical Engin. and Computer Sci., Univ. of California, Berkeley, CA

Abstract: While motor cortex predominantly drives movements of the contralateral arm, there is growing appreciation for the bilateral representation of arm movements within a single hemisphere. This apparent conflict between shared and independent representation of movements for either side of the body may be partially resolved by considering the phase of processing and level of information coding. Indeed, previous and ongoing studies have begun to reveal the contribution of each of these factors. Yet, it remains unclear how the lateralized structure of population activity in motor cortex evolves during the course of reach preparation and execution. In this study, we record ensemble spiking activity in bilateral primary motor (M1) and dorsal premotor (PMd) cortices in one macaque monkey performing an instructed-delay reaching task with one arm at a time. During the instructed-delay, both the reaching target and arm are cued. We analyze the time course of this population activity as it transitions from early integration of reach-related stimulus parameters to execution of the actual action. In particular, we ask how these signals are unique (or not) to each arm, and how that changes across phases of processing. Additionally, we characterize the structure of lateralization whenever present, aimed at describing the degree to which hemispheric divisions (ipsi vs contra) and sparsity of the network representation contribute to the segregation of arm signals. We find that, while information about both arm and target are represented during the full time course of the task, the organization of population activity underlying these lateralized representations is quite different before and after movement onset.
Towards a neural population-level understanding of the effects of methylphenidate (Ritalin) in motor cortex of reaching monkeys

Authors: *J. R. VERHEIN*¹, S. VYAS², K. V. SHENOY³;

Abstract: A fundamental goal of motor neuroscience has been to understand how patterns of motor cortical activity drive behavior. One successful strategy has been to “perturb” neural activity and/or behavior using, e.g., electrical or optogenetic stimulation, task-related (e.g., mechanical or stimulus) perturbations, or pharmacological agents. Many of these studies to date have focused on the effects of local pharmacological manipulation (e.g. with muscimol) of particular cortical regions. However, research regarding the relationship between population-level cortical activity and behavior has not rigorously addressed the effects of common cognition-altering drugs. Here we take a first step towards this goal by studying the effects of methylphenidate (MPH), a common stimulant which inhibits catecholamine reuptake, on motor control in rhesus macaques. This approach provides both a precise behavioral assay and the opportunity to study the correlates of the behavior in populations of single neurons.

We administered either oral MPH or vehicle alone (cookie filling) as a control to two adult male macaques (U and P) 15 min prior to the start of a center-out delayed reaching task, and measured two classic motor variables: peak reach speed and reaction time (RT), as well as a measure of early reach variability (the distribution of hand position at peak speed on each trial). We tested the effects of two doses in each animal. Preliminary results show significantly faster speeds during MPH treatment at individually optimized doses (U: 6 mg/kg, P: 1.3 mg/kg) compared to control sessions in both animals (U: p < 0.001, P: p < 0.02). RTs and reach variability were significantly reduced in U only (RT: p < 0.01, variability: p < 0.03). Our results show some dose
dependence, with increased reach variability (p < 0.001) and a trend toward decreased speed in P at a higher MPH dose (3 mg/kg); as well as attenuated effects on RT, speed, and reach variability in U at a lower dose (4.5 mg/kg).

Concomitant to the behavioral measurements, we recorded motor cortical neural activity (in U through 3 96-channel Utah arrays in premotor and primary motor cortex, and in P through V-probes in premotor cortex). Given our observed significant behavioral effects of MPH, this presents an opportunity to study population-level effects of the drug in motor cortex, including its effects on previously described neural population correlates of RT and speed. Preliminary results in U suggest that MPH reduces motor cortical variability during reach preparation and execution. To our knowledge, this study constitutes one of the first investigations of the effects of MPH on reaching behavior and neural population activity.

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Poster

313. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 313.22/DP08/O22

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster

Topic: E.04. Voluntary Movements

Support: HHMI
DARPA
Simons Foundation
NIH

Title: Spatially heterogenous tuning in rhesus motor cortex revealed using neuropixels probes

Authors: *E. TRAUTMANN¹, D. J. O'SHEA², X. SUN², S. VYAS³, S. RYU⁴, K. V. SHENOY⁵;

Abstract: Recent advances in multichannel extracellular recording technologies have enabled dense sampling from large populations of neurons in-vivo. Here, we used high-density silicon Neuropixels probes to record in premotor and primary motor cortices (PMd/M1) of awake rhesus macaque monkeys performing delayed reaches to radially spaced targets. Clustering of neural tuning properties has been commonly observed in visual areas of higher-order mammals such as
cats and rhesus macaque monkeys [1,2]. The motor cortex displays large-scale somatotopic organization (e.g.: [3,4]), though it is not well understood how macroscopic functional organization relates to fine-scale organization of neuronal tuning properties.

Results: Using high-density silicon Neuropixels probes, we quantified the pairwise correlation between tuning curves and PSTHs for reaches to radially-spaced targets. We found no relationship in the pairwise correlation between PSTHs or tuning for reach targets and the distance between neurons. This suggests that A) functional properties of neurons are not spatially clustered at the level of individual cortical columns in PMd and M1, and B) random sampling (i.e.: non-spatially targeted sampling) of neurons in PMd/M1 may be sufficient to fully capture relevant dynamic patterns in motor cortex [5].

Methods: A Neuropixels probe was inserted normal to the brain surface to sample neurons across cortical lamina. Neuropixels probes feature a thin, silicon shank specifically designed for use with mice and rats, creating several challenges for use with larger animals. In particular, the shank is not sufficiently stiff to penetrate the dura, and too short for insertion through a guide tube. We addressed this challenge by sharpening the tip of the Neuropixels probe as well as by creating a small perforation in the dura. In addition, we introduced gentle mechanical stabilization to the surface of the dura, 2-3mm from the recording site in order to reduce tissue motion due to pulse, respiration, and unconstrained motion. Together, these techniques enable acute recording of 200-400 neurons per probe from 384 recording channels, distributed 3.84mm along the length of the probe shank. Using this approach, repeated insertions in one recording site for up to two weeks were possible.


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Poster

313. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program#/Poster #: 313.23/O23

Topic: E.04. Voluntary Movements

Support: DP2-NS111817
        NINDS-Javits (NS25074)

Title: Inter-areal functional neuronal network dynamics in primate primary and premotor cortex during reaching and grasping movements
Authors: *J. B. HYNES*¹, C. E. VARGAS-IRWIN², J. P. DONOGHUE²;
¹Neurosci., Brown, Providence, RI; ²Neurosci., Brown Univ., Providence, RI

Abstract: Classical models of movement processing propose that the reaching and grasping components of object-driven movements are independently processed along distinct dorsal-reach and ventral-grasp circuits before converging in the primary motor cortex (M1). More recent studies have provided evidence to suggest that dorsal and ventral premotor (PM) neurons directly influence one another during object-driven movement generation; however, little is known about the organizing principles and computational properties of these inter-areal functional networks. Mapping the functional structure of dorso-ventral networks is important for understanding how the cortex implements the complex computations underlying the object-driven movements. Previous work has shown that M1 and PM neurons exhibit moment-to-moment fluctuations in their functional properties across different computational stages of movement processing. Our goal with the current work was to determine if M1, PMd, and PMv neurons also exhibit moment-to-moment fluctuations in their functional relationships in a computation-dependent manner or exhibit fixed relationships. We simultaneously recorded primate M1, PMd, and PMv single unit activity during skilled reach and grasp behaviors using multi-electrode arrays. We then used a novel unsupervised mathematical approach to generate low-dimensional neuron functional similarity maps across different phases of the movement planning and reach-to-grasp actions. Our approach allowed us to visualize the functional relationships of the recorded neurons across different stages of movement processing without having to impose tuning functions a priori. Our analyses revealed that groups of neurons with common computational motifs (i.e. functional sub-networks) were not confined to single anatomical areas; instead, the detected functional sub-networks were typically comprised of neurons from all three areas. We also observed that the relationships between neurons changed dynamically across different stages of movement generation. Collectively, our results suggest that reach-and-grasp movements engage a distributed network of neurons that can dynamically alter their associations to meet the computational demands of the movement context. These findings support the emerging view that object-driven movements engage a densely interconnected network of neurons that span both dorsal and ventral motor cortical areas. Our results suggest that characterizing functional sub-networks spanning multiple areas may be a more accurate way to describe computation in cortical networks than comparisons performed based on gross anatomical location.

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Poster

314. Motor Cortex and Motor Learning

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 314.01/O24

Topic: E.05. Brain-Machine Interface
**Title:** Intracortical paired pulse conditioning for *in-vivo* human motor cortex plasticity induction

**Support:** NSF ERC 100001176

**Authors:** D. J. CALDWELL¹, J. A. CRONIN², C. J. PASCHALL³, A. B. BROWN⁹, V. MARTINEZ⁴, K. E. WEAVER⁵, R. P. RAO⁶, S. I. PERLMUTTER⁷, A. L. KO⁹, *J. G. OJEMANN⁸;


**Abstract:** Timed cortical stimulation protocols can induce changes in cortex. Non-human primate studies have shown cortical evoked potentials (CEPs) can be enhanced with short-delay paired pulse stimulation of two cortical regions. We implemented a paired pulse conditioning paradigm intraoperatively in patients receiving DBS implants through acutely implanted macroscale intracranial EEG electrodes to induce changes in cortical plasticity. Electrodes with identifiable CEPs were used, with a primary stimulated electrode (A) and the site of strongest CEP (electrode B) utilized. We compared paired site cortical stimulation (AB) relative to single site stimulation (AA), and time lags including 25 ms and 200 ms between conditioning trains. In fourteen (14) subjects, conditioning paradigms with 200 ms delays resulting in greater degrees of change than 25 ms conditions, and with paired stimulation between sites being more effective than single site stimulation (p<.001). Additionally, longer conditioning sessions result in greater potentiation of CEP magnitude relative to shorter sessions. We found no significant differences between disease conditions, supporting the generalizability of these results beyond a particular disease process. Our findings of enhanced CEPs using existing medical devices may offer a path for enhanced use of cortical stimulation for recovery, as from stroke. The timing of optimal paired stimulation (200msec) was a longer delay than found in non-human primates. This may reflect the larger scale of stimulation with clinical macroscale electrodes (2.4mm diameter) with a larger extent of depolarized cortex, yielding compound and multiplexed effects.
Evoked potential amplitude was enhanced with a paired pulse stimulation conditioning paradigm using a 200msec delay between regions. A shorter delay and same-electrode stimulation had weaker effects.

**Disclosures:**  

**Poster**

314. Motor Cortex and Motor Learning

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 314.02/O25

**Topic:** E.05. Brain-Machine Interface

**Support:**  
Dutch Technology Foundation STW (grant UGT7685)  
ERC-Advanced ‘iConnect’ project, grant ADV 320708  
NIDCD of the National Institutes of Health (award number U01DC016686)

**Title:** Auditory spelling by an ALS patient with a fully implanted brain-computer interface
Authors: *E. J. AARNOUTSE, S. LEINDERS, Z. V. FREUDENBURG, B. VAN DER VIJGH, E. G. M. PELS, M. P. BRANCO, M. J. VANSTEENSEL, N. F. RAMSEY; UMC Brain Center, University Med. Ctr. Utrecht, Utrecht, Netherlands

Abstract: Late stage ALS patients have, with progression of the disease, increasing difficulty to communicate. Assistive and augmentative communication methods help them to keep autonomy and independence. One method is a Brain-Computer Interface, which translates brain signals to a means to spell words. However, most BCI methods rely on eye sight, something many ALS patients develop problems with. Here we report on an ALS patient with an implanted BCI (Utrecht Neuroprosthesis; UNP) who has been using the system at home for almost 3 years. The UNP is fully implantable and wirelessly interfaces with a tablet with speech synthesis for communication (Vansteensel, Pels, Aarnoutse et al. 2016). The patient has until now used the UNP with visual feedback, but given that her eye and eye lid movement control deteriorates we investigated feasibility of auditory system interaction. The patient is a woman with late stage ALS, who was diagnosed with the disease in 2008. In October 2015 the participant (then 58 y) was implanted with the UNP. The UNP records ECoG signal from 2 subdural electrodes strips (Resume II®, Medtronic, 4 electrodes each, 4 mm diameter, 1 cm distance, off label use) which were implanted, after fMRI prelocalization, over the hand area of the left motor cortex and the left dorsolateral prefrontal cortex. The strips were connected subcutaneously to an amplifier (Activa® PC+S, Medtronic, off label use), implanted under the clavicle. ECoG data is streamed to a tablet computer running BCI software. The participant generates a distinguishable signal by attempting to move her hand. Bandpass filtered data with center frequencies of 20 Hz and 80 Hz are streamed 5 times per second to the tablet where the BCI software filters the data and translates the data into a click and subsequently to a keypress signal in Communicator-5 AAC software (Tobii Dynavox). The AAC software uses a visual or auditory scanning paradigm to select letters. Auditory feedback required a slower scan rate to allow for pronouncing the selected rows and letters. 12 runs of purely auditory spelling was performed. In 9 runs the word was spelled correctly, 2 runs were aborted to decrease the scan rate, 1 run was aborted for technical reasons. In all runs, corrections (erasing the last spelled letter) were allowed. The total accuracy was 90.2%. On average 1.3 correct characters were spelled per minute. This study shows that auditory BCI with an implant is feasible. Although the speed was reduced in comparison with visual feedback, the speed is encouraging and higher than other auditory BCIs. For patients who are at risk of losing eye sight an (implanted) auditory BCI based on scanning might be a solution for sustaining communication.

Disclosures: E.J. Aarnoutse: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Funded by the Dutch Technology foundation STW with co-funding from Medtronic Europe. S. Leinders: None. Z.V. Freudenburg: None. B. Van der Vijgh: None. E.G.M. Pels: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Funded by the Dutch Technology foundation STW with co-funding from Medtronic Europe. M.P. Branco: None. M.J. Vansteensel: None. N.F. Ramsey: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Funded by the Dutch Technology foundation STW with co-funding from Medtronic Europe.
314. Motor Cortex and Motor Learning

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Diversity supplement to parent grant

**Title:** Oligodendrocyte and myelin loss impairs recording performance of neural interfaces in cuprizone-induced model of demyelination

**Authors:** *S. M. WELLMAN*¹⁻⁵, K. GUZMAN⁶, L. E. BRINK⁶, S. SRIDHAR⁶⁻², J. CHARLES⁶, L. LI¹, F. CAMBI²⁻⁷, T. D. Y. KOZAI¹⁻³⁻⁸⁻⁵;  
¹Dept. of Bioengineering, ²Dept. of Neurol., ³Ctr. for Neurosci., ⁴McGowan Inst. of Regenerative Med., Univ. of Pittsburgh, Pittsburgh, PA; ⁵Ctr. for Neural Basis of Cognition, Pittsburgh, PA; ⁶Veterans Admin. Pittsburgh, Pittsburgh, PA; ⁷Dept. of Neurol., Univ. of Kentucky, Lexington, KY; ⁸NeuroTech Ctr., Univ. of Pittsburgh Brain Inst., Pittsburgh, PA

**Abstract:** Neural electrode technology used to interface with the central nervous system (CNS) holds the potential to answer basic, fundamental neuroscience questions and offer critical therapeutic rehabilitation for patients afflicted with neurological disorders. However, intracortical electrode implantation induces overwhelming biological inflammation which can reduce the quality of recordable neural signals over time. Oligodendrocytes, the myelin producing cells of the CNS, regulate neuronal health and signaling by providing neurotrophic and metabolic support as well as axonal myelination. Due to their high energy demands, oligodendrocytes can be susceptible to the oxidative and metabolic stresses that occur during electrode implantation, which brings into question their contribution to the biological failure modes of neural interfaces. Cuprizone-induced demyelination was used to probe the effects of oligodendrocyte and myelin loss on the recording performance of intracortical microelectrode devices. A Michigan-style electrode array was implanted into the visual cortex of C57BL/6J wild-type mice 5 weeks after cuprizone administration in order to completely demyelinate the cortex prior to insertion. Cuprizone diet was continued alongside electrophysiological recording for 7 weeks post-insertion. Control mice were implanted similarly while being maintained on normal rodent diets. Prior to electrode insertion, immunohistochemistry was used to confirm reduced oligodendrocyte densities and myelin loss in the cortex of cuprizone-treated animals compared to control mice. At the onset of implantation, cuprizone-treated mice exhibited significantly reduced recording performance yields, amplitudes, and signal-to-noise ratios compared to control mice, remaining relatively low but stable over time. Interestingly, control
mice demonstrated elevated recording metrics within the acute period during implantation before declining to the chronic performance levels of cuprizone-treated mice. This suggests that oligodendrocytes residing within the recording radius of the electrode microenvironment in control mice undergo a functional and/or anatomical decline similar to that of the cuprizone-treated mice, implicating the preservation of oligodendrocytes and myelin as necessary tissue components for neural recording quality. These results reveal an oligodendrocyte-dependent regulation of neuronal electrophysiological properties, contribution to the quality of intracortical recording devices, and sensitivity to biological inflammation that can impact wound healing and repair following neurodegenerative injury.


Poster

314. Motor Cortex and Motor Learning

Location: Hall A

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DARPA HR0011-15-2-0006
McNight Foundation Technological Innovations in Neuroscience Award [JMC, MMM]
Chan Zuckerberg Biohub [MMM]

Title: Transcranial wireless power and MIMO backscatter communication with multiple neural dust recording sites

Authors: *D. K. PIECH¹, J. M. CARMENA², M. M. MAHARBIZ³; ¹UC Berkeley - UC San Francisco Grad. Program in Bioengineering, Univ. of California Berkeley, Berkeley, CA; ³EECS, ²UC Berkeley, Berkeley, CA

Abstract: Freely-floating wireless implantable neural recording and stimulation devices are an emerging type of neural interface with promise for scalable distributed recording, reduced micromotion-induced gliosis, and very low risk implantation modalities. The neural dust recording interface is an ultrasonically-powered untethered recording system consisting of sub-mm sensors implanted in the brain which wirelessly relay extracellular voltage signals to an external transceiver through ultrasonic backscatter communication. In order to scalably record from these sensors (‘motes’), methods for achieving a high SNR wireless link, many-device simultaneous communication, and low-volume implant size must be realized. Furthermore, the
skull provides a formidable challenge for ultrasonic communication, as it is attenuating and scattering.

Here we show that by utilizing extremely short pulses of ultrasound to interrogate the dust motes, the reflection from a mote can be resolved by time-of-flight from other motes and from the skull. Separation of the skull reflection and mote reflection in the time domain enable high gain digitization of the mote reflection without saturating the transceiver front-end with the very high skull reflection amplitude, enabling high SNR recovery of neural dust signals through the skull. We develop a backscatter communication algorithm which isolates backscatter signals independently on many external transceiver elements to achieve multiple-input multiple-output communication, improve SNR, and help to overcome scattering through the skull. We demonstrate ultrasonic wireless communication through an ex-vivo primate skull, and communication simultaneously between multiple sensor sites and many external transceivers. This technique is applicable to neural sensor motes with either sophisticated modulators and encoding schemes, or simple modulators consisting of only an impedance change. These results demonstrate that improved signal processing on the external side of the wireless link can enable extremely small and simple wireless neural sensor motes.


Poster

314. Motor Cortex and Motor Learning

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 314.05/O28

Topic: E.05. Brain-Machine Interface

Support: NIH/NINDS 7R01NS036692-16
NIH/NINDS 7R01NS082851-04
NSF 1847436

Title: Deep brain spatially expanded multifunctional fiber-based neural probe arrays for chronic neural interfacing

Authors: *S. JIANG¹, D. C. PATEL⁴, J. KIM¹, S. YANG¹, W. A. MILLS, III², Y. ZHANG¹, Z. FENG¹, A. WANG¹, Y. GUO⁵, I. KIMBROUGH³, H. SONTHEIMER⁶, X. JIA¹;
¹The Bradley Dept. of Electrical and Computer Engin., ²Translational Biology, Medicine, and Hlth., ³Sch. of Neurosci., Virginia Tech., Blacksburg, VA; ⁴Fralin Biomed. Res. Inst., Roanoke,
Abstract: Functional interactions between neurons in different brain regions has been studied using a variety of technologies. For example, metal-based microwires, silicon-based multichannel arrays and electrode arrays with flexible substrates have been shown to provide high-resolution electrophysiological recording with high signal-to-noise ratio. However, with single implantation, the physical properties of these devices limit their access to a single, small brain region. To overcome this limitation, we developed a platform that provides three-dimensional coverage of brain tissue through multifunctional polymer fiber-based neural probes that can interface simultaneously with neurons in multiple sites. To achieve electrode expansion after implantation, a scaffold with spiral hollow channels is utilized here to direct multifunctional fiber probes into brain tissue at specified angles. For multisite interfacing within a single branch, we can expose electrode recording sites, microfluidic channel opening, and waveguide windows along the fiber length. Our femto-second laser micromachining technique allows us to fabricate these spatial interfacing sites based on the specific biological application. We obtained recordings from transgenic mice to show application of optical stimulation causing distinctly different brain activities from spaced electrodes. Similarly, we were able to detect varying electrophaphic activities from different brain regions during ictal and interictal periods in a mouse model of chronic epilepsy. Finally, chronic recordings validate the ability of these fibers to provide long term neuronal readout with little or no rejection. Our data suggest that this three-dimensional multiplexing brain interface has the potential to allow for multimodal analysis of brain circuitry activity between brain regions under physiological and pathological state.


Poster

314. Motor Cortex and Motor Learning

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 314.06/O29

Topic: E.05. Brain-Machine Interface

Support: NIH Grant R01NS089688,
NIH Grant R01NS094396
NIH Grant R01NS062019
Different coating approaches to address the inflammation around the implanted neural electrode

Authors: *A. GOLABCHI, Z. J. DU, T. CUI;
Dept. of Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Foreign body response (FBR) around the neural implant is a key factor that affects the long term performance of these devices. These limitations can be addressed through an advanced surface coating which provides a combination of recording and stimulation advantages, including lowered impedance and increased charge transfer as well as the ability to modulate the inflammatory tissue response. Here we present two coating approaches targeted to the conducting and insulation regions of the electrode, respectively, and explore the impact of these coating technologies on chronic recording performance of neural implants. Firstly, we used multi-walled carbon nanotubes (CNTs) and anti-inflammatory dexamethasone doped poly(3,4-ethylenedioxythiophene) (PEDOT) as electrode coatings to improve the chronic neural interface. Previously, we have reported PEDOT/CNT/Dex coated electrodes lowered impedance and reduced inflammation after 14 days of implantation in rat dorsal root ganglion compared to uncoated electrodes. Here, we report the electrochemical behavior of the coated electrode/tissue interface during the prolonged implantation period of 12 months. Secondly, the insulation of the neural probes was coated with neuronal adhesion molecule L1 using silane chemistry. We have previously shown L1 when covalently bound to the surface of the electrode, reduces the initial microglial attachment, gliosis, and promote electrode-neuron integration at 1, 4 and 8 weeks. The chronic recording performance of L1 coated arrays was compared to uncoated arrays over 16 weeks. The PEDOT/CNT/DEX coated electrodes demonstrated stable impedances and recorded high quality visually evoked a neural response with minimum degradation even at time points past 1 year. Meanwhile, L1-coated probes showed significantly higher SU yield compared to the control group over 16 weeks of the implantation. Additionally, by aligning the implant depth across animals between 2-16 weeks to layer IV depth and identifying signals from two depths (cortex and hippocampus), we observed significantly higher SU yield in L1-coated compared to the control in both depths, with the more dramatic difference found in the hippocampus. Finally, quantitative image analysis demonstrated significantly reduced expression of microglia, astrocytes and BBB leakage within 40 μm zone around the L1 probes compared to the control at 16 weeks. Taken together, these results demonstrated the feasibility of reducing inflammatory tissue response and improving chronic neural recording via surface coatings. The combination of the coating approaches should further improve the quality and longevity of neural implants.

Disclosures: A. Golabchi: None. Z.J. Du: None. T. Cui: None.
Poster

314. Motor Cortex and Motor Learning

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 314.07/O30

Topic: E.05. Brain-Machine Interface

Support: NIH Director’s New Innovator Award Program, grant number [1 DP2 HD087955]

Title: Examining deep neural network decoders for BCI motor control in tetraplegia

Authors: *N. F. HARDY¹, D. B. SILVERSMITH³, S. REDDY⁴, R. ABIRI⁵, E. F. CHANG², S. LEVINE⁴, A. DRAGAN⁴, K. GANGULY¹;
¹Neurosurg., ¹UCSF, San Francisco, CA; ³Bioengineering, Univ. of California San Francisco, San Francisco, CA; ⁴UC Berkeley, Berkeley, CA; ⁵Dept. of Neurol., Univ. of California, San Francisco, San Francisco, CA

Abstract: Recent advances in Brain Computer Interface (BCI) research have enabled severely impaired patients to perform complex tasks, including composing emails, navigating the internet, and controlling robotic limbs. While significant progress has been made, developing BCI algorithms that are robust and feel intuitive to patients remains challenging. Current state of the art BCI decoding algorithms rely on linear decoders, e.g. Kalman Filters, to map spiking activity to output commands. Linear decoders are well suited to tasks with relatively small datasets and simple outputs, but can struggle when applied to complex tasks. On the other hand, nonlinear decoding algorithms such as deep neural networks can find complex relationships between input features and outputs, but require very large datasets to train.

In this work, we tested the performance of deep neural networks (DNN) as decoders in electrocorticography (ECoG) based BCI. ECoG has several advantages over spikes-based BCI: ECoG does not penetrate cortical tissue, can sample from many brain regions at once, and can potentially maintain stable recordings over a long period of time. Recent work has shown that ECoG BCI with linear decoders allows partially paralyzed patients to perform a 3D cursor control task (Degenhart et al., 2018). In addition, Anumanchipalli and colleagues (2019) demonstrated that DNNs can decode speech from ECoG signals recorded from epileptic patients.

Here, we examined DNNs as BCI decoders of ECoG data recorded chronically from a human subject with tetraplegia caused by a bilateral pontine stroke. The subject was chronically implanted with a 128-channel ECoG grid over the left motor and somatosensory cortices. We then trained multiple DNN architectures on data recorded over multiple months during real time 2D cursor control with a velocity Kalman Filter. Results indicated that DNN decoders might outperformed Kalman Filters in the 2D cursor control task. Further, we applied our DNN decoding framework to a typing task with an assistive agent. These findings provide support for
the use of chronic ECoG recordings for neuroprosthetic control, and for using DNN decoders to improve BCI control in complex environments.


Poster

314. Motor Cortex and Motor Learning

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 314.08/O31

Topic: E.05. Brain-Machine Interface

Support: DARPA-PA-18-02-04-INI-FP-006
OSUWMC Neurological Institute

Title: Neural signal quality and decoding performance following intracortical brain-computer interface hardware failure

Authors: C. F. DUNLAP¹, S. C. COLACHIS, IV³, N. V. ANNETTA³, D. A. FRIEDENBERG⁴, P. D. GANZER³, G. SHARMA³, *M. A. BOCKBRADER²;
¹Biomed. Engin., ²PM&R/Biomedical Engin., The Ohio State Univ., Columbus, OH; ³Med. Devices & Neuromodulation, ⁴Advanced Analytics, Battelle Mem. Inst., Columbus, OH

Abstract: Introduction: Signal quality of chronic recordings from intracortical microelectrode arrays (MEA) may progressively degrade due to tissue-implant interactions, including glial scarring and neuronal death at the neural interface. MEAs are also susceptible to hardware failures such as electrode corrosion and wire bundle or connector damage. Each of these bioelectronic failure modes may decrease neural decoding performance, but once identified, may also be amenable to different strategies for compensation. Our goals were to identify signal metrics characteristic of failure modes in an aging array and also evaluate solutions to maintain decoding performance. Methods: Five years of neural recordings were evaluated from a participant in the Reanimation in Tetraplegia clinical trial (NCT01997125). The participant is a 28-year-old man with C5 ASIA A spinal cord injury who used an investigational, intracortical BCI with a left motor cortex Utah MEA (Blackrock Microsystems, Utah). Neural activity (mean wavelet power across 234-3750Hz) was decoded with machine-learning algorithms. Decoding accuracy and signal metrics were characterized for a 4-movement motor imagery task that the participant regularly performed. Results: Overall, neural decoding performance remained high across 5 years, with decoder accuracy exceeding 85%, even though impedance and signal-to-noise declined over time. Four potential failing channels were identified in the 96-channel array. They had weak signal correlations (<0.5) with other channels, variances greater than 2 standard deviations from the array-wide mean, and highly variable session-to-session impedances.
Malfunctioning recording channels were associated with a damaged amplifier connector and a pedestal connector failure. Excluding the flagged channels improved offline decoding accuracy by up to 9%. **Conclusion:** Chronic impedance and signal-to-noise decline in an aging array impacted decoder performance less than isolated malfunctioning channels. When problem channels with high signal variance, low signal correlation, and high session-to-session impedance were identified and excluded during decoding, BCI performance recovered. Automating detection of and compensation for these types of bioelectronic failures is important for translating BCIs out of the laboratory where a technician may not be available to check signal quality.

**Disclosures:**  
**C.F. Dunlap:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute.  
**S.C. Colachis:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute.  
**N.V. Annetta:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute.  
**D.A. Friedenberg:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute.  
**P.D. Ganzer:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute.  
**G. Sharma:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute.  
**M.A. Bockbrader:** A. Employment/Salary (full or part-time):; The Ohio State University.  
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**Poster**  
**314. Motor Cortex and Motor Learning**  
**Location:** Hall A  
**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM  
**Program #/Poster #: 314.09/O32**  
**Topic:** E.05. Brain-Machine Interface  
**Title:** Simultaneous exploration and exploitation of neural strategies during neuroprosthetic learning  
**Authors:** *A. YOU¹, B. LIU², A. SINGHAL², S. GOWDA², H. G. MOORMAN³, A. L. ORSBORN³, J. M. CARMENA⁴;  
¹The UC Berkeley - UCSF Grad. Program In Bioengi, Berkeley, CA; ²Univ. of California,
Abstract: Brain-machine interfaces (BMI) allow users to interact with their external environments by decoding neural signals to control external effectors ranging from computer cursors to robotic arms. In situations where decoders are held constant, it has been shown that neural population activity consolidates and generates more coordinated patterns over learning. Furthermore, previous work has shown that BMIs can be “two-learner” systems, in which both neurons and decoders co-adapt over the course of learning. This is particularly useful in situations where decoders need to be refit over the course of training due to neural drift or changes in neural ensembles available for decoding. However, it is unclear how population firing patterns may change as a result of these perturbations. In this study, we analyzed previous BMI experiments with nonhuman primates and found neural activity to continuously consolidate and generate more coordinated patterns over learning. Changes in decoder weights or neurons did not hinder the formation of these patterns. Instead, neurons exhibited exploratory behaviors that were separate from previously consolidated strategies. Our results indicate that neurons may be simultaneously exploring and exploiting neural strategies by modifying learned patterns rather than generating completely new ones even from the onset of learning a new task.

communication between cortical neurons disconnected by the lesion. We hypothesized that an
integrate-and-fire (IAF) neural network could be used to artificially communicate information
from one population of cortical neurons to another. To investigate the effects of such a closed-
loop brain-computer interface on neural dynamics, we recorded the spontaneous activity of
multiple single neurons with a Utah array implanted in primary motor cortex of a pigtail
macaque. Each experimental session was divided into ‘pre stimulation’ and ‘closed-loop
stimulation’ epochs. Several recorded neurons served as inputs to a simple two-layer IAF
network. When these “input” neurons spiked, a virtual “post-synaptic potential” was generated
in each of its connected IAF units in the neural network. An IAF unit spiked whenever its
“membrane potential” reached a predefined threshold, which subsequently generated a trigger to
deliver an electrical stimulus (cathodal, biphasic stimulation; 15 µA amplitude; 200 µs pulse
width) at a cortical site adjacent to a subset of the input neurons. The stimulation evoked
distinctive excitatory and/or inhibitory responses at each of the nearby input neurons. We
compared the spike dynamics of the input neurons between the pre and closed-loop stimulation
epochs using auto- and cross-correlograms. This allowed us to measure the effects of each of the
artificial connections on the spike dynamics of each of the input neurons. During the closed-loop
stimulation epoch, we found that if an input neuron excited/depressed another input neuron
through an artificial connection, there were corresponding peaks/troughs in their cross-
correlograms following the stimulation trigger. If an input neuron artificially activated itself, the
resultant positive feedback loop sustained activity. Over sessions lasting a few hours there was
no evidence that the monkey altered the firing properties of the input neurons. Our results show
that an IAF neural network can be used in a closed-loop brain-computer interface to artificially
connect a set of cortical neurons. The changes in spike dynamics resulting from the artificial
connections were dependent on the connectivity of the neural network and effects of outputs on
the input neurons. Taken together, this suggests that IAF neural networks provide a flexible
means of connecting neural populations to modulate their neural activity.

Fetz: None.

Poster

314. Motor Cortex and Motor Learning

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 314.11/O34

Topic: E.05. Brain-Machine Interface

Title: Chronic large-area neural recording using microwire arrays

Authors: *A. M. O. OBAID¹, Y.-W. WU², M.-E. HANNA¹, J. B. DING⁴, N. MELOSH³;
¹Stanford Univ., Stanford, CA; ²Dept. of Neurosurg., Stanford Univ., Palo Alto, CA; ³Materials
Abstract: Mammalian brains typically consist of billions of neurons operating at millisecond time scales, of which current recording techniques only capture a tiny fraction. Electrical recording techniques capable of sampling neural activity at such timescales have been difficult to scale due to the need for application specific designs as well as the mismatch between the three-dimensional architecture of the brain and largely two-dimensional microfabrication techniques, limiting both neurophysiological research and neural prosthetics. Recent advances in CMOS device design have led to high-recording quality planar probes, with diminishing sizes to ameliorate the extent of tissue damage. Matching these powerful silicon electronics to the inherently three dimensional architecture of the brain has remained challenging however, as devices are constrained to the planar two dimensional surfaces required for silicon processing. Here we describe a new methodology whereby we perform a heterogeneous integration of a bundle of microwires to CMOS chips, such as pixel arrays found in modern camera chips or displays, and precise microwire spacing, critical for mitigating tissue damage. Microwires have long been known to have low insertion damage and good electrical recording performance yet required individual mounting and connectorization. Arranging them into bundles controls of the spatial arrangement and three-dimensional structure of the distal (neuronal) end, while providing a robust parallel contact plane on the proximal side which is interfaced to a planar pixel array. The modular nature of the design enables a variety of microwire types and sizes to be integrated with different types of silicon-based arrays, allowing channel counts to be scaled from a few dozen to thousands of electrodes using the same fundamental platform. This system has excellent recording performance, demonstrated via single unit and local-field potential recordings in isolated retina, and in the motor cortex and striatum of awake moving mice. This concept links the rapid progress and power of commercial multiplexing, digitisation and data acquisition hardware together with a chronic three-dimensional neural interface.

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Poster
314. Motor Cortex and Motor Learning
Location: Hall A
Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM
Program #/Poster #: 314.12/O35
Topic: E.05. Brain-Machine Interface
Title: A novel bi-directional implantable brain machine interface with versatile stimulation capabilities
Authors: *J. RICKERT, S. MARTIN, C. STOLLE, F. WENZEL, O. BRUNNER, J. HOLUB, N. GRIGAT, M. OBERT, M. LEISTNER, D. GRIESHABER, J. HUMMEL, S. RIEGER; CorTec GmbH, Freiburg, Germany

Abstract: Bi-Directional implantable brain machine interfaces not only read from the brain but also provide options to write to the brain. Electrical stimulation is a well-established method of interacting with the nervous system, applied to the brain, it leads to modulation of neural network function (as it is done e.g. in deep brain stimulation for treatment of M. Parkinson). This effect is strongly dependent on the stimulation intensity as well as stimulation frequency. It also depends on the location at which the stimulus is delivered, and the electrical field spatial distribution associated with stimulus current.

The Brain Intercharge system developed by the authors generates current-controlled, charge balanced pulses with amplitude of up to 6 mA and pulse widths of up to 2.5 ms. The compliance voltages available for driving the current are asymmetric -12 V as negative and +6 V as positive rail. The current can be directed to any of its 32 electrode contacts the system also uses for brain recording. The current return electrode can be selected to also be any of the 32 electrode contacts or subsets of them, or an additional counter electrode. These options make the system very versatile to use. Electrical patient safety is warranted by the use of blocking capacitors switched in between electronics and electrodes.

Disclosures: J. Rickert: A. Employment/Salary (full or part-time); CEO of CorTec. S. Martin: A. Employment/Salary (full or part-time); CEO and CTO of CorTec. C. Stolle: A. Employment/Salary (full or part-time); Employee of CorTec. F. Wenzel: A. Employment/Salary (full or part-time); Employee of CorTec. O. Brunner: A. Employment/Salary (full or part-time); Employee of CorTec. J. Holub: A. Employment/Salary (full or part-time); Employee of CorTec. N. Grigat: A. Employment/Salary (full or part-time); Employee of CorTec. M. Obert: A. Employment/Salary (full or part-time); Employee of CorTec. M. Leistner: A. Employment/Salary (full or part-time); Employee of CorTec. D. Grieshaber: A. Employment/Salary (full or part-time); Employee of CorTec. J. Hummel: A. Employment/Salary (full or part-time); Employee of CorTec. S. Rieger: A. Employment/Salary (full or part-time); Employee of CorTec.

Poster

314. Motor Cortex and Motor Learning

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 314.13/O36

Topic: E.05. Brain-Machine Interface

Title: Clinical use of laser-micromachined cortical grid electrodes
**Authors:** *M. SCHUETTLER*¹, S. BENSCH¹, N. F. INCE², S. PRABHU⁴, J. RICKERT¹, C. HANSER¹, C. HENLE¹, A. MARX¹, P. ASMAN³;
¹Cortec GmbH, Freiburg, Germany; ³Dept. of Biomed. Engin., ²Univ. of Houston, Houston, TX; ⁴Dept. of Neurosurg., MD Anderson Cancer Ctr., Houston, TX

**Abstract:** Cortical grid electrodes are widely used for mapping of the human brain, especially as a diagnostic tool to permit geometrically exact resection of brain tissue in case of surgical therapy of brain tumors and epilepsy.

Although cortical grids are commercially available and established since many years, the market provides devices that are hand-made and rather stiff, compared to the brain tissue, leading to difficulties when larger areas of the brain need to be covered by a planar grid that the surgeon tries to put on the natural curvature of the brain.

To overcome problems with current electrodes, we fabricated cortical electrodes using laser-based micromachining of traditional implant materials (silicone rubber and platinum–iridium alloy) that are highly reproducible and precise in dimensions but also are very soft. In spring 2019, a set of cortical electrodes produced with our novel methods received pre-market notification 510(k) by the FDA. The following briefly describes some initial clinical findings.

To map the sensory-motor cortex, the electro-corticogram (ECoG) was recorded intraoperatively during awake brain surgery from a patient (Male, 45 years old) with high grade glioma. The tumor was located anterior to the motor cortex. Vibrotactile simulation was delivered using coin vibration motors, individually placed on the fingertips and palm. The stimulation was delivered for 1s at 250Hz with an inter trial interval from 2 to 4s between consecutive stimulations. ECoG was recorded from 53 channels using a cortical grid with 4 x 8 contacts of 2.7 mm diam. spaced in check-board pattern with 1 cm, 3 x 7 contacts of 1 mm diam. spaced in between. The grid was placed adjacent to the tumor and extended posteriorly towards the hand knob overlapping with anatomically (or imaging) defined motor and sensory regions. Recordings were obtained intraoperatively in awake state at 2.4kHz sampling frequency with gHIAmp (gTec, Austria) bioamplifier. In total 118 trials were recorded. The vibrotactile stimulation was well identified in the ECoG and its strongest representation was localized. The majority of the neural responses were located posterior to the central sulcus. A broadband energy increase reaching up to 800Hz was observed immediately after the stim onset. 200 ms later this energy increase was limited to a frequency range between 60-200 Hz overlapping with traditional gamma band and lasted for the duration of the vibrotactile stimulation.

**Disclosures:** **M. Schuettler:** A. Employment/Salary (full or part-time); CEO and CTO of CorTec. **S. Bensch:** A. Employment/Salary (full or part-time); Employee of CorTec. **N.F. Ince:** None. **S. Prabhu:** None. **J. Rickert:** A. Employment/Salary (full or part-time); CEO of CorTec. **C. Hanser:** A. Employment/Salary (full or part-time); Employee of CorTec. **C. Henle:** A. Employment/Salary (full or part-time); Employee of CorTec. **A. Marx:** A. Employment/Salary (full or part-time); Employee of CorTec. **P. Asman:** None.
Poster

314. Motor Cortex and Motor Learning

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 314.14/O37

Topic: E.05. Brain-Machine Interface

Support: STW Grant UGT7685
ERC-advanced i-Connect grant ADV 320708
Implanted hardware provided by manufacturer Medtronic

Title: Low frequency spectral responses in dlPFC measured with fully implanted BCI during working memory task differ between two LIS users with different etiologies

Authors: S. LEINDERS¹, K. VAN DEN BERG², E. J. AARNOUTSE¹, M. P. BRANCO¹, E. G. PELS¹, B. VAN DER VIJGH¹, M. J. VANSTEENSEL¹, *N. F. RAMSEY¹, Z. V. FREUDENBURG¹;
¹UMC Utrecht-Rudolf Magnus Inst., Utrecht, Netherlands; ²Univ. Utrecht, Utrecht, Netherlands

Abstract: People with severe paralysis may be fully conscious but unable to communicate without some communication aid, a condition commonly referred to as locked-in syndrome (LIS). Whereas traditional communication aids require some minimal motor control, brain computer interface (BCI) technology uses brain activity for computer control, and is therefore a promising communication method for some people with LIS. As part of the Utrecht NeuroProsthesis project (Vansteensel et al., NEJM, 2016), two people with LIS caused by late-stage ALS or pontine stroke were implanted with a BCI, to test the feasibility of independent home use for communication. For BCI control, our users use activity measured from sensorimotor hand cortex by subdural electrocorticography (ECoG) electrodes. Correctly timed attempted hand movement allows them to produce clicks in scanning-based software to select on-screen options in games or spelling applications. Using sensorimotor activity for control is the current paradigm for self-paced BCI systems. However, sensorimotor-based BCIs may not work well for all end-users. Partly as a backup in case sensorimotor signals would deteriorate due to disease progression, but also to investigate its potential as a BCI control signal by LIS users, our participants also received ECoG electrodes over their left-dorsolateral prefrontal cortex (dlPFC). We have shown previously that mental arithmetic consistently increased high frequency band (65-95Hz) power in the dlPFC which can be used for 1-d cursor control (Vansteensel et al., Ann Neurol 2010). Here, we focus on low frequency band power during mental arithmetic in two LIS users. In summary, taken across all runs, the pontine stroke user showed a decrease in low frequency band power (alpha: 8-12Hz) during mental arithmetic (p = 0.0004), whereas the ALS user showed a clear increase (p < 0.0001). The fact that different LIS etiologies affect brain signals differently and in unexpected ways is not surprising, as research has shown low
frequencies are affected in ALS patients (Shantosh et al., Neurol. India, 2005). Also, electrophysiology work with brainstem stroke patients has established clear but heterogeneous brain signal changes in the majority of cases (Patterson & Grabois, Stroke, 1986). Changes in these basic brain signal characteristics can affect BCI performance. Ideally, signal characteristics relevant for BCI control are quantified before implantation of future systems and should inform BCI system details (e.g. sensor location), leading to BCI systems personalized for optimal performance.


Poster

314. Motor Cortex and Motor Learning

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 314.15/O38

Topic: E.05. Brain-Machine Interface

Support: NRF Grant 2016M3C7A1904988

Title: Networking in primary motor cortex predicts arm-reaching speed variation

Authors: *M.-K. KIM, S. CHAE, T. KIM, S.-P. KIM; UNIST, Ulsan, Korea, Republic of

Abstract: Neuronal ensemble activity of the primary motor cortex (M1) produces intricate patterns associated with the speed of arm movements. Unlike directional tuning properties of single neurons, collective changes of the population of neurons are known to characterize speed. However, such a collective activity may or may not contain neurons directly coding speed information. For example, trial-by-trial variability of the speed profile of arm movements is predominantly predicted by preparatory activity, but without detailed aspects of the maximum speed and its latency. To address these issues, we investigated kinematics-related latent components of neuronal populations and their interactive patterns. Kinematics-related latent components were estimated by canonical correlation analysis (CCA). Then, we analyzed connectivity between latent variables using Pearson correlation coefficients, from which we formed a network of latent components. We further quantified the characteristics of the network by a clustering coefficient based on the graph theory, which reflects the degree of clustered networks and node density. Dataset for this study was acquired from M1 of a rhesus macaque that performed a two-dimensional center-out reaching task, which is available at a public database on the collaborative research in computational neuroscience (CRCNS). To analyze the variability of the speed profile, we separated individual profiles into three disjoint groups having
early- (≤ 25 %), mid- (> 25 % or < 75 %) and late- (≥ 75 %) latency of the maximum speed regardless movement direction. Plus, the trials of the speed magnitude were also separated in the same manner into three groups. We found that the clustering coefficient increased as it took longer to reach the maximum speed in arm movements. We also found that the clustering coefficient increased as the maximum speed increased. Our results suggest that the property of networking among latent components of peri-movement M1 population activities may elucidate the variability of the speed profile of point-to-point arm movements with an identical distance.

Disclosures: M. Kim: None. S. Chae: None. T. Kim: None. S. Kim: None.

Poster

314. Motor Cortex and Motor Learning

Location: Hall A

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Program #/Poster #: 314.16/O39

Topic: E.05. Brain-Machine Interface

Support: NIH

Title: In vivo characterization of the stability of cortical response to electrical microstimulation

Authors: *X. S. ZHENG¹, Q. YANG¹, A. VAZQUEZ¹, T. CUI²;
²Dept. of Bioengineering, ¹Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Intracortical microstimulation is not only a useful tool in neuroscience research for dissecting circuitry and modulating behaviors, it has also shown promise in restoring sensory functions in humans with neural deficits. While efficacious, it is unclear how the cortex dynamically responds to electrical stimulation over time, and how different electrode materials differ in effectiveness and safety. Traditionally, In vivo characterizations of stimulation performance have been largely based on electrophysiological, behavioral, or end point histological outputs. Using meso-scale fluorescent microscopy and two photon microscopy (TPM), we can observe and quantify the result of microstimulation in transgenic animals in real time, chronically.

NeuroNexus electrodes with iridium sites (703 µm²) were dual-modified with iridium oxide (n = 8) and PEDOT/fCNT (n = 8 ), and were implanted in Thy1-GCaMP6s mice. Electrical stimulations (biphasic charge balanced waveforms with 100 µS cathodic pulse, 100 µS interphase delay and 200 µS anodic pulse half the cathodic amplitude) between 0.5nC/phase to 6nC/phase at 50Hz were applied sequentially to each electrode site at 1s on 3s off (25% duty cycle), repeated 6 times. This stimulation was applied on day 1 and weekly thereafter. The GCaMP responses were imaged.

Preliminary results showed a dynamic change in cortical response to stimulations over time. Specifically, on day 1, higher GCaMP response was evoked at a lower stimulating current
amplitude. This suggests that the acute damage by the implant severed axonal (likely inhibitory) connections, resulting in elevated cortical response at lower stimulation intensities. Additionally, on day 1, we observed a drop off in GCaMP response in 62% channels at stimulating amplitudes higher than 4nC/phase, indicating that the acute neuronal damage hindered the brains’ ability to endure metabolic demand at higher stimulation amplitudes. Current intensity to evoke a maximum cortical response significantly increased from day 1 to 5.5nC/phase on day 21 (p = 0.0043, n = 16), suggestive of reconstitution of local neuronal network. Furthermore, we observed qualitative chronic inconsistencies in cortical response recruitments in 37.5% channels. This is likely due to the micromotion between the electrode and the neural tissue which lead to changes in the immediate tissues activated. Notably, we observed no neuronal recruitment differences in the meso-scale between material types despite PC being significantly lower in impedances and higher in charge injection limits. Results on changes to neuronal soma distributions nearby stimulating sites will be reported from TPM studies.


Poster

314. Motor Cortex and Motor Learning

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 314.17/O40

Topic: E.05. Brain-Machine Interface

Support: Neural Engineering SEED Grant
NIH 1DP2EB022357

Title: Glial cell mediated neurodegeneration surrounding the Utah electrode-tissue interface

Authors: *C. BENNETT, A. ALVAREZ-CIARA, A. PRASAD;
Univ. of Miami, Miami, FL

Abstract: Relaying valuable neural signals from the central nervous system is made possible with the use of intracortical microelectrode arrays. However, the presence of an intracortical electrode within the tissue creates a reactive local microenvironment, which leads to a cascade of proinflammatory and prooxidant factors, affecting the transcriptional rates of glial cells. Quantitative real time polymerase chain reaction (qRT-PCR) and immunohistochemistry (IHC) was used to quantify gene and protein expression, respectively, at acute to chronic time-points of 48-hr, 1-wk, 2-wk, and 4-wk. Genes that mitigate the reactivity of glial cells (astrocytes and microglia), which act as the main supporting cells of neurons, were monitored in rats implanted with a non-functional 4x4 Utah microelectrode array in the somatosensory cortex. Electrode implantation alters transcriptional rates of astrocytes and microglia as compared to unoperated controls, leading to the activation and prolongation of the complement cascade, which
chronically tags and phagocytoses cellular debris and neuronal axons surrounding the electrode-tissue interface. Moreover, chronically stimulated pathways such as the complement pathway could prolong the initial implant-induced trauma and become deteriorative to the brain parenchyma, potentially affecting chronic neuronal recordings.

**Disclosures:** C. Bennett: None. A. Alvarez-Ciara: None. A. Prasad: None.

**Poster**

**314. Motor Cortex and Motor Learning**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 314.18/O41

**Topic:** E.05. Brain-Machine Interface

**Title:** Decoding reach speed using a hybrid artificial neural network decoder for brain-computer interfaces

**Authors:** *H. MAO*¹, Y. INOUE⁴, S. B. SUWAY², J. ORELLANA⁵, A. B. SCHWARTZ³;
¹Systems Neurosci. Inst., ³Dept. of Neurobio., ²Univ. of Pittsburgh, Pittsburgh, PA; ⁴Dept. of Neurosurg., Osaka Univ. Grad. Sch. of Med., Osaka, Japan; ⁵Carnegie Mellon Univ., Pittsburgh, PA

**Abstract:** Neuronal firing rates in the primary motor cortex encode the direction and speed of arm reaching movement. Neural prostheses that extract this encoded information are used by paralysed subjects to regain lost arm movement. Although the performance of moving the prosthetic arm approximates that of normal subjects moving their native arms, stopping at the end of a reach as a target is acquired is still challenging. This difficulty is due to the way speed modulates firing rate of individual neurons: Speed affects the amplitude and offset of the tuning function. The speed-dependent offset acts in a nonlinear manner on linear decoders. Therefore, typical decoders, for example the Population Vector Algorithm (PVA) and some variations of the Optimal Linear Estimator (OLE), could be suboptimal as they work by inverting linear tuning functions without taking the speed-offset effect into account. We demonstrate the influence of the speed-offset effect on decoding with both simulated activity and empirically recorded activity as monkeys performed center-out arm reaching movement. One solution to this nonlinear tuning function problem is an artificial neural network (ANN) decoder that finds a direct nonlinear mapping from neuronal firing rates to velocity. On both the simulation and monkey arm reaching data, the ANN decoder improved decoding accuracy compared with the PVA and OLE decoder. For brain-computer interface (BCI) applications, we propose a hybrid artificial neural network (hANN) algorithm that combines neuronal tuning function modelling and the decoding power of the ANN. First, recorded data are used to fit empirical tuning functions for individual neurons that account for both the gain and the offset effect of speed. Then, an ANN decoder is trained with velocity and simulated neural data according to these empirical tuning functions. When a
monkey performed a center-out cursor movement task via a BCI, the hANN decoder led to skillful control of both direction and speed as demonstrated by stereotypic bell-shaped speed profiles, straight trajectories, and steady cursor positions before and after the movement. This work suggests that hybrid decoders that combine detailed encoding models with nonlinear computational approaches are likely to provide substantial gains in BCI performance.


Poster

314. Motor Cortex and Motor Learning

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 314.19/O42

Topic: E.05. Brain-Machine Interface

Support: NIH Grant 1UH3NS107714

Title: Neural dynamics in primary motor cortex during BCI calibration in a person with tetraplegia

Authors: *J. E. DOWNEY1, J. M. GOODMAN, JR1, A. K. SURESH1, J. L. COLLINGER3, S. J. BENSMAIA2;
2Dept. of Organismal Biol. and Anat., 1Univ. of Chicago, Chicago, IL; 3Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Rotational dynamics have been observed in primary motor cortex (M1) during reaching in mice, monkeys, and humans. In the present study, we investigated whether such dynamics were also observed during calibration of a Brain-Computer Interface (BCI) a subject used to move a cursor based on intracortical recordings in M1. Indeed, one might hope to exploit orderly neural dynamics when decoding intended movements from M1 signals. Before we can exploit them, however, we must verify that they are present when the subject cannot make overt movements.

The subject in this study has a C5/C6 spinal cord injury resulting in the paralysis of his hands. For the clinical BCI study, we placed 2 Utah arrays with 88 recording channels each in the hand and arm area of M1. Unsorted threshold crossings on each channel were counted in 20 ms bins and smoothed with a 25-ms Gaussian kernel. Data were collected during the first phase of a two-phase decoder training process. During this first phase, the subject attempts to move the cursor to one of 8 targets in a standard center-out task while the computer actually moves the cursor to the targets. The resulting neural data and observed cursor velocities are used to develop the decoder. We assess whether M1 exhibits orderly dynamics during this phase, and compare these to the dynamics observed in the presence of overt movements in able bodied organisms.

Poster

314. Motor Cortex and Motor Learning

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 314.20/O43

Topic: E.05. Brain-Machine Interface

Support: UF Pre-eminence Start-up Funds
W.M.Keck Foundation
NSF Grant 1636007

Title: Electrophysiological and histological characterization of the foreign body response to implantable neural interfaces in the African spiny mouse brain


Abstract: Mammalian tissue injury typically results in the formation of a collagenous scar at the site of injury. In the field of neural interfaces, chronically implanted neural interfaces in the central nervous system (CNS) lead to an encapsulating glial scar. Glial encapsulation serves as an ionic barrier, thus reducing the signal-to-noise ratio of electrophysiology and overall functionality of the device. While other non-mammalian species have shown remarkable ability to fully regenerate tissue, such as the axolotl, the African Spiny Mouse (ASM) is the only known mammal able to fully regenerate injured tissues with minimal scarring. The unique regenerative abilities of this species make it a prime candidate for investigating the foreign body response (FBR) to implanted devices, which has traditionally been a highly variable and complex system to understand. Without a clear understanding of the FBR’s biological mechanism, research focused on FBR minimization has typically focused on altering the properties of the device. While this can be effective in FBR mitigation, it can negatively impact the functionality of the device. If the biological mechanism of the FBR could be identified and targeted, the FBR could be reduced or eliminated completely, allowing next generation device design to be optimized while achieving a favorable tissue response. Here we take a novel approach to CNS FBR mitigation by comparing the FBR of the ASM to that of the C57BL/6 mouse. Marked discrepancies in cytokine expression and macrophage activation have been observed between the ASM and C57BL/6 mouse in endothelial tissue, indicating that there may be a
similar difference in the FBR of the brain. This study aims to draw a similar comparison in the brain within the context of implantable electrodes. Our preliminary data indicates reduced astrocytic response to neural implants in the ASM, motivating further investigation. Here, we present a histological and electrophysiological examination of the FBR in the ASM brain. Electrophysiology was recorded from functional, single-shank electrodes at longitudinal time points and immunohistochemistry was performed on cryosectioned tissue from tissues harvested at several relevant FBR endpoints.


Poster

314. Motor Cortex and Motor Learning

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 314.21/O44

Topic: E.05. Brain-Machine Interface

Title: High-bandwidth wireless implantable multichannel neural recording system

Authors: *B. CROFTS, A. WILDER, D. MCDONNALL, S. HIATT; Ripple Neuro, Salt Lake City, UT

Abstract: High channel count microelectrode arrays provide access to spatially and temporally selective neural data, given a method for multichannel data recording and high bandwidth transmission. Percutaneous implementations are limited by large connectors that commonly fail, require heavy pedestals, and are prone to infection. An implantable neural recording system is proposed as a chronically viable system for digitizing and wirelessly transmitting electrode array data at sample rates appropriate for cortical neuron extracellular spike events. This work extends a developed and extensively tested implantable system for recording electromyographic data. A proof-of-concept study illustrates successful data transmission at high sample rates. The implant is constructed with an electronic circuit board with multichannel biopotential amplifier, hermetically sealed ceramic enclosure, brazed titanium ground band, electrode connection feedthrough structure, lead attachments, and silicone-filled epoxy header. Implant electronics are wirelessly powered via a pair of loosely coupled printed circuit board-based coils in highly resonant mutually inductive circuits. Digitized data are transmitted over a high-speed infrared (IR) link that exploits a near IR epidermal/adipal transparency window. An external transceiver device drives the implant power coil and receives and decodes IR data. The implantable system was configured for thirty-two electrode channels digitized at 30,000 samples per second. These specifications represent an order of magnitude rate increase from an existing implantable electromyographic recording system. The implant’s power consumption and IR transmission configuration, external transceiver’s data reception circuitry, and IR decoding algorithm are key
aspects to this design. Benchtop data transmission performance was verified over a volume defined by a viable range of implant to external transceiver displacements and depth using a robot positioner and tissue representative light obstruction and in-air media. Biopotential amplifier and digitization performance have been previously verified in other studies. A chronically implantable system for wireless transmission of multichannel neural data extends the possibilities for a wide range of research including brain-machine interfaces, cortical mapping, and electrode efficacy.

Disclosures: B. Croft: A. Employment/Salary (full or part-time); Ripple Neuro. A. Wilder: A. Employment/Salary (full or part-time); Ripple Neuro. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ripple Neuro. D. McDonnell: A. Employment/Salary (full or part-time); Ripple Neuro. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ripple Neuro. S. Hiatt: A. Employment/Salary (full or part-time); Ripple Neuro. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ripple Neuro.

Poster

314. Motor Cortex and Motor Learning

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 314.22/P1

Topic: E.05. Brain-Machine Interface

Title: In-vitro mechanical and electrical validation of a flexible microelectrode


Abstract: Neuromodulation aims to treat various disorders and diseases related to autonomic function by stimulating, blocking, and recording activity from peripheral nerves. The efficacy and success of neuromodulation depend heavily on the quality of the interface (electrode) used. Unfortunately, commonly used interfaces are either stiff or rigid, which leads to both biotic and abiotic breakdown of the bioelectronics. To address this point, we are working on refining bench test procedure with a goal of clinical translation. Currently, no effective interface exists to monitor and/or stimulate autonomic nerves chronically in the mouse model. The implicit design challenges for chronically interfacing with the mouse cervical vagus nerve include developing electrodes that can conform to tight curvature and still maintain mechanical and electrical integrity. To validate the in-vivo longevity of the polyimide-based microfabricated flexible electrodes we have carried out in-vitro studies.
Multiple layouts of flexible electrodes were fabricated using polyimide (PI-2611) as substrate and insulator, and state-of-the-art iridium oxide (SIROF) for the electrode interface. To evaluate the electrical integrity of the polyimide, an accelerated soak test was carried out by soaking the insulated test structure in 67°C Phosphate-buffered solution (PBS) for 30 days. Over the course of (specific time frame), we will periodically carry out electrical impedance spectroscopy (EIS) over the course of time to measure the longevity of the devices.

Sputtered Iridium oxide (SIROF) stability testing was also carried out on multiple layouts with the help of stimulation stability tests. This was done by placing SIROF electrodes in PBS. The electrodes were tested for up to 100 million biphasic pulses (166µs phase with 66µs interphase delay) with current amplitudes ranging from 100 µA to 1000 µA. EIS and cyclic voltammetry (CV) was measured before and after the pulse trains were applied. Initial results suggest that iridium oxide is a stable metal for long-term stimulation applications.

To increase the longevity of the fully assembled device, we have developed stretchable helical coiled interconnects; these interconnects will help absorb micro motion, and eventually increase the longevity of the devices. To date, formal tests have not yet been carried out to provide structural support for our hypothesis.

These bench testing strategies will help us to achieve successful in-vivo chronic interface with the mouse cervical vagus nerve. The data we are going to present here are from rats chronic in-vivo impedance spectroscopy and SNR.

**Disclosures:**  

**Poster**

**314. Motor Cortex and Motor Learning**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:/Poster #:** 314.23/P2

**Topic:** E.05. Brain-Machine Interface

**Support:**  
DARPA Biological Technologies Office (BTO) Program: Targeted Neuroplasticity Training (TNT) HR0011-17-2-0051  
NIH 1R21EY029458-01 and the Boettcher Foundation Webb-Waring Biomedical Research Awards

**Title:** Temporally-precise vagus nerve stimulation increases motor learning of a dexterous reach task through cholinergic neuromodulation

**Authors:**  
*S. G. BOWLES, J. L. HICKMAN, X. PENG, W. R. WILLIAMSON, C. G. WELLE;*  
Univ. of Colorado Sch. of Med., Aurora, CO
Abstract: Vagus nerve stimulation (VNS) has been shown to increase the effectiveness of motor rehabilitation after a stroke in animal models. Additionally, lesions of cholinergic basal forebrain (BF) neurons prevented VNS driven cortical plasticity. However, it remains unknown if VNS can improve motor performance in healthy animals, and by what mechanisms VNS acts to improve motor outcomes. To address these questions, we implanted chronic stimulating electrodes on the left cervical vagus nerve of the mouse (Cortec; Microleads). Animals were trained to perform a dexterous forelimb reach for a food pellet using an automated behavioral system, CLARA. Reach kinematics were tracked using a deep learning network (DeeplabCut) to allow for 3D markerless tracking of multiple digits. Through a combination of the CLARA and DeepLabCut systems, we performed closed-loop stimulation based on reach outcome. VNS paired with successful completion of a dexterous reach task in healthy animals enhances acquisition of the task (30hz, 100us pulse width, 0.6mA, 0.5s train). To explore the role of cholinergic modulation in mediating the VNS behavioral effect, cholinergic cells in the basal forebrain were either excited through activation of virally-mediated channelrhodopsin (20hz, 10ms pulse width, 0.5mW, 0.5s duration) or inhibited by activation of virally-mediated archaerhodopsin (continuous, 0.5mW, 0.5s duration). We found that excitation of cholinergic projection neurons improved motor learning in a manner similar to VNS models. Moreover, cholinergic inhibition was sufficient to prevent VNS enhancement of motor learning. Together these results show that VNS, when paired with the successful outcome of a dexterous reach, can increase motor learning in healthy animals. These data implicate activation of the BF cholinergic system as one mechanism by which VNS enhances motor learning.


Poster

314. Motor Cortex and Motor Learning

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 314.24/P3

Topic: E.05. Brain-Machine Interface

Support: 1F32MH118714-01
5U19 NS104649

Title: Neural dynamics underlying generalization in motor cortex

**Abstract:** The repertoire of motor commands that are sent to arm muscles to generate one set of behaviors, such as straight trajectories, may also be used in different temporal sequences to drive the arm in new trajectories. The motor commands sent to the muscles are generated by neural activity patterns. Are the neural activity patterns used to generate a specific motor command reused when the command appears in a new behavioral sequence? To address this question, rhesus macaques are trained to operate a brain-machine interface (BMI) that causally maps high dimensional neural activity patterns to two-dimensional motor commands that control the x and y velocity of the BMI cursor. Monkeys proficiently perform two different tasks with the BMI; a center-out task composed of straight movements and an obstacle avoidance task composed of curved movements. We find that the neural activity patterns used to generate motor commands in the obstacle task deviate from the activity patterns used in the center-out task. Online results show that calibrating decoders with task-specific neural activity patterns improves the animals’ ability to perform that task. What then, is the neural policy used to select a neural activity pattern for a given motor command? By using statistical modeling, we find that differences in neural activity patterns are not accounted for by modeling the kinematic state of the cursor, but rather can be explained by linear neural dynamics that are general to both classes of behavioral trajectories. Across different movement trajectories, a given motor command is followed by different future motor commands. Being in different neural states to generate the same motor command may be advantageous by allowing the neural dynamics to assist in reaching an appropriate neural activity pattern for the generation of the next motor command. We demonstrate that the neural activity patterns used for a motor command leverage the neural dynamics for generation of the next motor command. Thus we find that in motor cortex, a neural activity pattern generating a motor command is not a static map that is re-used in the middle of diverse neural and behavioral trajectories, but rather that motor cortex flexibly generates neural activity patterns for the same motor commands in a way that leverages its high dimensional dynamics. These results suggest the practical application of using the neural dynamical state as a BMI control signal to enable better generalization to complex movements.


**Poster**

314. Motor Cortex and Motor Learning

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 314.25/P4

**Topic:** E.05. Brain-Machine Interface

**Support:** eu h2020 fetproact-16 732266-plan4act, dfg-for1847
Title: Home-cage based behavioral and wireless neural recording setup for unrestrained rhesus macaques

Authors: *N. S. AGHA*¹, A. TRUNK², M. BERGER³, A. M. GAIL⁴;
²Vat: 15329080-2-41, ³Cognitive Neurosci. Laboratory, Sensorimotor Group, ¹German Primate Ctr., Göttingen, Germany; ⁴German Primate Ctr., Goettingen, Germany

Abstract: System-level neurophysiology studies in awake behaving monkeys usually require partial movement restraint and separation from social groups during experiments. This allows detailed control over or surveillance of as many behavioral parameters as possible, together with tethered neural data acquisition at high bandwidth. Recent developments, like our Reach Cage (Berger et al. 2018, bioRxiv), use wireless neural recordings and allow studying neural correlates of motor behavior in less restricted experimental settings, thereby alleviating the movement restraint requirement. However, animal transport and social separation is still required during the experiment. Other recent developments allow animals to perform increasingly advanced cognitive tasks in a self-paced manner, while remaining within their home environment. Multiple monkeys in our facility have previously used our XBI (eXperimental Behavioral Instrument) as an interactive home-cage based device for automated behavioral training and testing (Calapai et al., 2017; Berger et al. 2018). Here, we combine both our previous experimental approaches towards system-level neurophysiological recordings within the housing environment. For this, we incorporated a wireless neural recording system into our XBI for synchronized behavioral and neural recordings. We recorded up to 192 broad-band neural signals (upgradeable to 256) at single unit resolution from three cortical sensorimotor areas from two animals while they conducted a visually instructed, goal-directed, memory-guided reach task. We were able to use our portable wireless system to extract reach-goal selective movement planning activity from simultaneously recorded neural and behavioral activity. In conclusion, our new system allows for wireless recording of neural data correlated with spatially and temporally precise instructed behavior in rhesus monkeys without physical restraint of the animal, but otherwise highly similar to conventional chair-seated touch screen experiments. With this, we achieved system-level neurophysiological recordings during trained cognitive task performance in the animals housing environment.

Disclosures: N.S. Agha: None. A. Trunk: None. M. Berger: None. A.M. Gail: None.

Poster

314. Motor Cortex and Motor Learning

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 314.26/P5

Topic: E.05. Brain-Machine Interface

Support: DARPA Contract with DOE DE-AC52-07NA27344
Title: Next-generation high-channel count neural implant arrays and modular head-mounted package for chronic human-use neuromodulation applications

Authors: *R. HAQUE, S. PATRA, J. ZHOU, M. TRIPLET, J. PEBBLES; Lawrence Livermore Natl. Lab., Livermore, CA

Abstract: The development of human-grade implants for treating a variety of neural disorders has garnered much interest recently. These chronic solutions provide recording and stimulation capability while also providing a higher channel count than currently commercially available. Here we present two types of microfabricated flexible polymer arrays and head-mounted titanium packages for electronics encapsulation as a modular platform for human neuromodulation applications. Each of the polymer arrays are attached to a small, pill-size titanium can (29mm x 6.3mm x 6mm) with a ceramic base providing 64 high-density platinum feedthroughs in a hermetically-sealed package. A 6-pin platinum-iridium cable attached to the pill-size can terminates in a 0.9mm BalSeal SYGNUS connector. This module (array, pill-sized can, and cable) is called a SmartLead. The 64 channel cortical and subcortical arrays are permanently-attached to the SmartLead and are constructed of thin-film metals encapsulated in polyimide, coiled, and molded in silicone. The electrode contacts are 800µm in diameter and made of platinum-iridium and are capable of both recording and stimulation. Measured impedances are typically less than 500Ω. An embedded inner-tube for a removable stylet aids implantation of the arrays through a burr hole. The cortical array is sized 32mm x 14mm and the subcortical array is 1.27mm diameter to match commercial deep brain stimulator probes. The SmartLead can be connected to a head-mounted titanium package, also designed for chronic, long-term use. This module, sized 31.5mm x 40mm x 7mm is designed to be embedded in the skull and provides 5 BalSeal SYGNUS ports and accepts up to 4 SmartLeads of either cortical or subcortical variety simultaneously, providing access to 256 channels for both recording and stimulation enabling the prospect of closed-loop neuromodulation capabilities with the proper electronics. The 5th port can be used for an additional SmartLead, an antenna to provide external communications, or a cable to another implanted module in the chest depending on what the embedded electronics are designed to do. An earlier version of the arrays have separately been tested in an intraoperative setting by collaborators at UCSF under an FDA Non-Significant Risk Designation and with IRB approvals and have demonstrated good functionality matching or exceeding commercial arrays. Combined, we present a modular, head-mounted solution aimed at chronic human use for neuromodulation, enabling new techniques such as distributed closed-loop neuromodulation.

Poster

314. Motor Cortex and Motor Learning

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 314.27/P6

Topic: E.05. Brain-Machine Interface

Support: NIH Grant 1R21EY026365-01
Stanford NTI

Title: Neuroroots: Ultra-low profile implant for brain-machine interface

Authors: *M. D. FERRO*¹, A. GONZALEZ², S. JAYABAL³, M. GAGNON³, E. ZHAO¹, J. L. RAYMOND³, L. M. GIOCOMO², N. MELOSH¹;
¹Materials Sci., ²Neurobio., Stanford Univ., Stanford, CA; ³Stanford Univ. Sch. of Med., Stanford, CA

Abstract: Communication between living brain tissue and engineered devices is the key link to understand the brain fundamental function and to clinically restore neurological deficits. The tools that are currently broadly available for this interface exhibit some limitations such as invasiveness, low channel count, complicated implantation strategies and bulky connectorization, which hinder these devices from optimal performance and widely accepted clinical solutions. Enabled by new materials and device designs, a new generation of brain interface technologies is replacing bulkier, non-compliant systems with the aim of seamless electronic-biological interfaces with lower tissue damage, reduced immunogenicity, high-density, tunable spatial distribution, and long-term stability. Recent successful examples leveraging mechanically compliant materials have demonstrated major breakthrough in brain research using ultra-flexible systems for ECoGs recordings, and for depth electrodes. Yet, surgical implantation damage, scalable channel count and the number of devices implanted simultaneously are still significant challenges.

Neuroroots is a new platform enabling facile implantation of ultra-low damage and scalable channel-count penetrating electrodes for chronic brain recording and stimulation. The platform consists of dangling ‘root’ electrodes only 5 µm wide by 1µm thick, matching both cell-size dimensions and tissue mechanical properties, yet without interconnectivity between electrodes. For insertion, we developed a strategy based on an ultra slim, 35µm diameter microwire as a temporarily shuttle onto which numerous individual electrodes self-align. Once inserted, these wires delaminate, and the shuttle is removed, leaving only the electrodes into the neural tissue. First, we demonstrated chronic implantation of an array of 32 electrodes into the hippocampus of freely behaving rats. Recordings exhibit single-unit with a remarkable stability over a period of 7 weeks. The open structure of the mesh is believed to minimally perturb the ecosystem and nutriment diffusion, thus minimally impacting the sampled neural region. Furthermore, we
implanted NeuroRoots into the cerebellum both in-vitro and in-vivo and recorded clear single-units in both, which demonstrate the unique ability of this platform to be implanted into region which are difficult to access for traditional probes.


Poster

314. Motor Cortex and Motor Learning

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 314.28/P7

Topic: E.05. Brain-Machine Interface

Support: NIH-U01NS099700

Title: Long-term stability of recordings and microstimulation of intracortical microelectrodes is depth-dependent

Authors: *M. E. URDANETA¹, N. G. KUNIGK², S. W. CURRLIN¹, J. D. PEÑALOZA-APONTE², F. DELGADO², K. J. OTTO³;
¹Neurosci., ²Biomed. Engin., ³Univ. of Florida, Gainesville, FL

Abstract: Advances in brain-machine-interfaces (BMI) and implantable technologies have opened the door for bidirectional neuroprostheses capable of restoring function in patients with neurological deficits. Intracortical recordings in motor cortex have allowed tetraplegic patients to control robotic arms with several degrees of freedom. Analogously, touch feedback from these prosthetic limbs has been established via intracortical microstimulation (ICMS) of the somatosensory cortex (S1). However, these implanted devices consist of multi-electrode arrays with a single electrode-site at the tip of each shank, confining the recording and/or stimulating interface to a single layer of the cortex. Moreover, intracortical microelectrodes are prone to biotic factors such as the foreign body response (FBR) that severely reduce the functional life of these devices. In order to assess the optimal cortical depth for longitudinal recordings and microstimulation, a silicon microelectrode with 16 equally spaced electrode-sites spanning all the layers of the cortex was chronically implanted the S1 of rats. Longitudinal neural recordings and microstimulation detection thresholds were obtained for more than 25 weeks. Our results showed that compared to superficial channels, more sites located in layers IV and V remained functional over the course of the experiment. These sites also exhibited higher signal-to-noise ratio (SNR) in electrophysiological recordings. Similarly, microstimulation thresholds obtained via a conditioned avoidance behavioral paradigm showed that ICMS sensitivity was also depth-dependent. In particular, electrode-sites in layers IV and V required significantly less charge to evoke behavioral percepts. Furthermore, these cortical layers displayed the highest stability for
long-term microstimulation. Collectively, our results suggest that cortical depth plays an important role in the functionality and long-term performance of implantable neuroprostheses and bidirectional BMIs.

**Disclosures:** M.E. Urdaneta: None. N.G. Kunigk: None. S.W. Currlin: None. J.D. Peñaloza-Aponte: None. F. Delgado: None. K.J. Otto: None.

**Poster**

**315. Brain-Computer Interface: Intracranial**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 315.01/P8

**Topic:** E.05. Brain-Machine Interface

**Support:** Wu Tsai Neurosciences Institute
Larry and Pamela Garlick
Samuel and Betsy Reeves
NIH NIDCD R01DC009899
NIH NIDCD R01DC014034
NIH NICHD-NCMRR R01HD077220
NIH NINDS 5U01NS098968-02

**Title:** Decoding multiple click signals in a person with tetraplegia to increase brain-computer interface bit rate

**Authors:** *S. N. FLESHER*1,2,3, D. T. AVANSINO1,2,3, L. R. HOCHBERG8,9,11,12,10, J. M. HENDERSON1,3,4, K. V. SHENOY2,5,6,7,3;

**Abstract:** Brain-computer interfaces (BCIs) aim to restore a means of interacting with their environment to those who have lost motor function due to spinal cord injury or disease. They do so by transforming neural signals into movement of external end effectors, such as a cursor used to control a computer or tablet-computer interface by decoding a 2D continuous space and a discrete “click” signal. The utility of such an interface can be estimated with selection rate (bits/s). To date, efforts to increase information throughput have involved adding more targets to the interface or decreasing time required per selection. But adding too many targets can decrease accuracy and thus a maximum information throughput exists. Here we investigate another means
of increasing information throughput: varying the number of click options available to the user. For example, using a mouse with two click options instead of one to act on one of eight targets doubles the number of potential targets, resulting in a maximum 1 bit increase, compared to a maximum improvement of 0.2 bits that would be achieved by adding a ninth target with only one click option. We tested the potential for decoding four click signals in addition to 2D continuous cursor velocity, which has the potential to nearly double the bit rate (relative to a single click). A participant with tetraplegia enrolled in the BrainGate2 clinical trial had two electrode arrays implanted in hand-knob area of motor cortex. He moved a cursor to one of eight peripheral targets in a center-out task using a ReFit Kalman decoder. Once over the target, he was instructed to perform one of four movements to generate one of the 4 clicks. Three different sets of four instructed body movements were tested: (1) hip abduction, adduction, and flexion and ipsilateral elbow flexion, (2) flexion the individual fingers on the contralateral hand and (3) flexion of the thumb, index, middle or little fingers. Offline analysis of the click signals was performed using a naive Bayes classifier. Offline classification of the intended click, using 20 ms bins of thresholded neural spiking activity, predicted the intended click target on held-out data with maximum accuracy of 98.6% for movement set 1, 87.5% for movement set 2 and 90% for movement set 3. Importantly, when the participant was controlling the translation of the cursor, his ability to hold the cursor over the targets was unimpeded by his attempts to generate the click signals. Future work is needed to test this online to identify click state in real time. If successful, this could improve BCI information throughput and meaningfully improve the user’s ability to navigate real-world computer interfaces.


Poster

315. Brain-Computer Interface: Intracranial

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:/Poster #: 315.02/P9

Topic: E.05. Brain-Machine Interface

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Larry and Pamela Garlick Foundation
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Title: Neural representation of attempted movement of a paralyzed limb in a person, and implications for intracortical brain-computer interfaces

Authors: *D. R. DEO1,2,3, F. R. WILLET4,5,2, D. T. AVANSINO4,5,6, S. VYAS7, N. EVEN-CHEN5, L. R. HOCHBERG10,11,13,14,12, J. M. HENDERSON4,2,3, K. V. SHENOY5,7,8,9,2;

Abstract: Intracortical brain-computer interfaces (iBCIs) for people with paralysis have largely built upon work investigating the neural representation of overt reaching movements in monkeys. However, in people with paralysis, iBCIs leverage neural features related to attempted movement of paralyzed limbs. These attempted movements’ neural representation may differ substantially from overt movement. Although prior work in humans has shown proficient iBCI-enabled control of cursors and robotic arms, relatively little is known about how paralysis affects movement representation at a single unit level. Furthermore, it is not known whether neural activity during attempted movement of paralyzed limbs is similar to closed-loop iBCI control in which the neural activity is guiding the cursor. We addressed these questions with neural recordings from two 96-channel microelectrode arrays implanted in “hand knob” area of motor cortex in a participant with a C4 spinal cord injury enrolled in the BrainGate2 clinical trial. We first compared our participant’s neural activity during attempted movement of the paralyzed arm and overt movement of the head to neural activity during monkey arm movement. We found that neural activity during overt head movement had strong modulation to distance and force magnitude, similar to monkey arm movement. In contrast, attempted movement of the paralyzed arm had weak modulation to distance and force. Interestingly, we found that neural activity during closed-loop iBCI control was more similar to overt movement than to attempted movement of the paralyzed limb (i.e. it also had a strong representation of distance). To further clarify the relationship between attempted movement of a paralyzed limb and closed-loop iBCI control, the participant controlled the cursor using a series of different joint movement strategies. We found that neural activity changed during closed-loop iBCI control, becoming more similar across different movement strategies. Surprisingly, we also found that the cursor could be controlled equally well when instructed to control it without attempting (or imagining) any particular movement at all. These results suggest that neural activity may change during iBCI control to reflect a generic “cursor control” signal that somewhat dissociates it from any particular attempted movement representation. Future work will be needed to clarify how and why these neural changes occur, and to elucidate the mechanism behind body-dissociated iBCI control.

Title: Human middle frontal gyrus exhibits activity related to both eye movements and intended hand movements

Authors: *K. G. WILCOXEN¹,²,⁴, T. HOSMAN¹,²,⁴, J. B. HYNES¹, J. SAAB³,⁴,², B. R. BUCHBINDER⁵, N. SHMANSKY⁵, S. S. CASH⁶,⁹, E. N. ESKANDAR⁷, J. D. SIMERAL¹⁰,³,⁶,², B. FRANCO⁶, J. KELEMAN⁶, C. E. VARGAS-IRWIN¹,²,⁴, L. R. HOCHBERG¹⁰,³,⁸,⁹,², L. R. HOCHBERG⁴,³,⁸,⁹,²;

Abstract: Intracortical brain-computer interfaces (iBCIs) record signals directly from the brain and harness those signals to control external devices, such as computer cursors. Typically, iBCIs rely on activity associated with intended movements recorded from the precentral gyrus (PCG). Another promising source of iBCI control signals is the middle frontal gyrus (MFG). However, other signals, such as activity associated with eye movements, may be present along with information about intended hand movements. Here, we compared single unit activity in PCG and MFG in response to eye movements and intended hand movements in a person with tetraplegia. We collected data from participant T10, a 35-year-old, right-handed man with a spinal cord injury (C4 AIS-A), in the BrainGate2 pilot clinical trial. T10 had two 96-channel microelectrode arrays (Blackrock Microsystems, Inc) implanted—one in the left MFG and one in the left PCG. T10 performed an instructed-delay, center-out task with 4 radially-distributed targets. T10 acquired targets using two different control modes in alternating blocks. In eye control blocks, he
acquired targets using eye-gaze (Tobii eye-tracker), while holding a neurally-controlled cursor stationary within a centrally-located target. In hand control blocks, he acquired targets by moving the neurally-controlled cursor and fixating on the central target. Single units with significantly different firing rates for movements to different targets were deemed directionally-tuned (Kruskal-Wallis, p<0.01).

As expected, more units in PCG were directionally-tuned during hand control blocks than eye control blocks. Notably, approximately equal numbers of MFG units were directionally-tuned during hand control and eye control blocks. However, MFG ensemble activity patterns could distinguish between the two control modes. This suggests that MFG, to a greater degree than PCG, represents both eye and hand movements, but may still provide information about intended hand movements for iBCI control.


Poster

315. Brain-Computer Interface: Intracranial

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

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Topic: E.05. Brain-Machine Interface

Support: ALS Association Milton Safenowitz Postdoctoral Fellowship
A. P. Giannini Foundation Postdoctoral Research Fellowship
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Wu Tsai Neurosciences Institute
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Samuel and Betsy Reeves
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Title: Motor cortical representation and decoding of attempted handwriting in a person with tetraplegia

Authors: *F. R. WILLETT*1,2,3, D. T. AVANSINO1,2,4, D. R. DEO5,3, L. R. HOCHBERG10,11,12,13, J. M. HENDERSON1,3,6, K. V. SHENOY2,7,8,9,3,1

Abstract: Handwriting is a fine motor skill in which straight and curved pen strokes are strung together in rapid succession. Because handwriting demands fast, richly varying trajectories, it could be a useful tool for studying how the motor cortex generates complex movement patterns. Additionally, attempted handwriting movements could be decoded by a brain-computer interface (BCI) and then translated to text in real time, restoring the ability to communicate to people with severe paralysis. In healthy adults, the average handwriting speed is 120 characters per minute (Jebsen 1969), which is three times the record rate for point-and-click intracortical BCI typing (Pandarinath 2017).

Here, we investigated the representation and decodability of attempted handwriting movements in a person with C4, ASIA-A spinal cord injury who was paralyzed from the neck down. We recorded from two microelectrode arrays in hand knob of precentral gyrus while the participant attempted to write letters using an imaginary pencil. The participant wrote letters one at a time in an instructed delay task with a 2-3 second delay period followed by a 1 second movement period. Encouragingly, a cross-validated naïve Bayes classifier achieved 87% accuracy across all 26 letters (and 2 punctuation symbols) using only three, 300 ms time bins of neural activity after the go cue. However, this simple classifier doesn’t account for temporal variability across trials. We designed a recurrent neural network classifier to account for how letters can be written at slightly different speeds each time. It achieved 92% accuracy, with the majority of misclassifications confined to a small set of similarly-written letters (e.g. q and g). These preliminary results suggest that a handwriting BCI could be accurate enough to achieve high communication rates.

Finally, to gain insight into how motor cortex contributes to handwriting, we examined the neural activity during the instructed delay period. Recent theories of neural population dynamics during arm reaching suggest that neural dynamics in motor cortex might “unroll” the preparatory state into patterns of activity that generate the movement. Interestingly, we found that preparatory activity represented upcoming letter features only within a short time-horizon. That is, letters that differed only near the end (e.g. m and n) had seemingly identical preparatory representations, suggesting that motor cortex requires additional inputs during the movement to complete the letter. However, letters that begin identically but quickly diverge (e.g. l, t, and k, which all begin with a downstroke but then diverge) had separable (but similar) preparatory representations.

Poster

315. Brain-Computer Interface: Intracranial

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

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Topic: E.05. Brain-Machine Interface

Support: NIH Grant 5R01EY01554512
T&C Chen Brain-machine Interface Center at Caltech
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Swartz Foundation

Title: Comparing human primary motor cortex (M1) and posterior parietal cortex (PPC) neuron population activity using a brain-machine interface

Authors: *S. SAKELLARIDI¹, T. AFLALO¹, V. N. CHRISTOPOULOS¹, K. W. PEJSA¹, E. ROSARIO², D. S. OUELLETTE², N. POURATIAN³, R. A. ANDERSEN¹;
¹BBE, Caltech, Pasadena, CA; ²Casa Colina Hosp. and Centers for Healthcare, Pomona, CA;³Neurosurg., UCLA, Los Angeles, CA

Abstract: A brain-machine interface (BMI) enables people with severe paralysis to communicate with the external world by creating a direct communication pathway between the brain and external prosthetic devices. One of the central questions in the field of intracortical BMI is which are the most suitable areas to record neural activity to drive prosthetic devices. Although the primary motor cortex (M1) and the posterior parietal cortex (PPC) are two of the most popular implanted areas in previous studies, there is no a systematic, direct comparison between them. In the current study, a patient with tetraplegia (C5-C6 lesion, 2 years post injury) was implanted with two 96 channel microelectrode arrays in M1 and PPC. The participant learned to modulate the activity of the recorded neurons in M1 and/or PPC to control a 2-dimensional computer cursor in a center-out-center task to eight radially arranged stimuli. The only instruction given was to attempt intended movements using only the right thumb and to not switch effectors during the task. Each session started with an initial observation-training phase (O), in which the participant observed the cursor moving from the center to the cued target and back to the center, and was instructed to follow the cursor with his right thumb. Neural activity was recorded from both M1 and PPC. Then, two separate linear decoders were trained; one trained on the units recorded from M1 (DM1), and the other trained on units recorded from PPC (DPCC). Finally, in the BMI control phase, the subject controlled the cursor using only one area (M1 or PPC) at a time as determined by the corresponding decoder (DM1 or DPCC). Our goal was to understand how each brain area behaved during brain control depending on which area was causally involved in producing movement of the cursor. To do that, we compared offline the neural behavior of each brain area when it causally controlled cursor motion versus when activity
was recorded but had no causal influence on cursor motion. In our decoding approach, units highly predictive of the subject’s intent are assigned weights of larger magnitude than neurons with lower predictability and consequently have a larger impact on decoder output. For each unit we calculated the magnitude of the decoder weight assigned to it after normalizing for firing rate differences. Preliminary results suggest that the behavior of the individual units as measured by the decoder weights did not significantly vary depending on which brain area played a direct causal role in controlling the cursor. This result suggests that M1 and PPC are part of an interconnected network that translates plans into actions with limited short-term plasticity.


Poster

315. Brain-Computer Interface: Intracranial

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 315.06/P13

Topic: E.05. Brain-Machine Interface

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MGH-Deane Institute
The Executive Committee on Research (ECOR) of Massachusetts General Hospital

Title: Identifying changes in volitional state and BCI task engagement based on the intrinsic structure of neural ensemble activity patterns in motor cortex of people with tetraplegia

Authors: *T. K. PUN\textsuperscript{1,2}, A. J. CATOYA\textsuperscript{3}, C. E. VARGAS-IRWIN\textsuperscript{4,2,5}, S. S. CASH\textsuperscript{6,8}, J. D. SIMERAL\textsuperscript{5,1,7,2}, L. R. HOCHBERG\textsuperscript{5,1,7,8,2};
\textsuperscript{1}Sch. of Engin., \textsuperscript{2}Carney Inst. for Brain Sci., \textsuperscript{3}Dept. of Mol. Pharmacology, Physiology, and Biotech., \textsuperscript{4}Dept. of Neurosci., Brown Univ., Providence, RI; \textsuperscript{5}VA RR&D Ctr. for Neurorestoration and Neurotechnology, Dept. of VA Med. Ctr., Providence, RI; \textsuperscript{6}Dept. of Neurol., \textsuperscript{7}Ctr. for Neurotechnology and Neurorecovery, Dept. of Neurol., Massachusetts Gen. Hosp., Boston, MA; \textsuperscript{8}Dept. of Neurol., Harvard Med. Sch., Boston, MA

Abstract: Neuromotor deficits resulting from conditions such as amyotrophic lateral sclerosis (ALS), brainstem stroke, and spinal cord injury (SCI) result in loss of volitional movement
reducing independence. Brain-computer interfaces (BCI) bypass damaged motor pathways and provide new links to assistive technologies. Voluntary action engages vast networks of neurons performing complex calculations which are still not fully understood. It is widely accepted that motor cortex incorporates a mix of incoming sensory, cognitive, and motor planning information, reflecting latent variables that are not directly related to kinematic motor output. Reliably identifying neural activity patterns indicative of a set of latent factors impacted by task and cognitive context is a challenge for developing BCI systems that support continuous, multi-effector use.

Here we present a new approach to generate state spaces based solely on the intrinsic properties of single unit ensemble recordings. We used Spike train SIMilarity Space (SSIMS) analysis to map neural activity patterns into low dimensional state spaces (based on spike train metrics combined with dimensionality reduction). These state space projections can be used to identify clusters of similar, recurring activity patterns, without the need to define task-related tuning models for individual neurons. We applied this method to data collected from two participants enrolled in the BrainGate2 clinical trial - T10, an adult male with tetraplegia due to SCI (C4 AIS-A), and T9, an adult male with tetraplegia due to ALS (ALSFRS-R of 8). T10 had two 96 microelectrode arrays (Blackrock Microsystems, Inc) implanted on the left middle frontal gyrus (MFG) and precentral gyrus (PCG), and T9 on the left PCG. We collected data during sessions where T10 & T9 controlled either a computer cursor or a JACO robotic arm to perform a 2D target acquisition task via intracortical BCI. We demonstrate that our approach can be used to generate state spaces that differentiate between effectors (cursor vs. robotic arm) in both T10 & T9 (81.4±8.0%, 99.5±0.58% accuracy). Additionally, we recorded data from T10 over a 24 hour period; we can identify 5 partially overlapping states related to session tasks, interaction with caretakers and others, engagement with a sip-and-puff computer system, eating, and sleeping (Nearest Neighbor Classifier: 46.5±0.6%, p<.01; against chance classification: 23.5±0.8%). Our findings suggest that state space models based on intrinsic similarity can be used to detect context-dependent changes in volitional state, providing a useful indicator of when BCI control modes should be adjusted.

Title: Coding of intent in M1 and PPC: Simultaneous array recordings in a human tetraplegic subject for neural prosthetic applications

Authors: *T. AFLALO*¹, S. SAKELLARIDI¹, K. KADLEC¹, E. ROSARIO², N. POURATIAN³, R. A. ANDERSEN⁴;
¹Caltech, Pasadena, CA; ²Res. Inst., Casa Colina Hosp. and Centers For Healthcare, Pomona, CA; ³UCLA, Los Angeles, CA; ⁴BBE, Calif Inst. of Technol., Pasadena, CA

Abstract: Individuals with spinal cord injuries resulting in paralysis are still able plan and imagine or attempt movements. The major cortical sensorimotor circuit responsible for planning and producing movement involves the posterior parietal cortex (PPC) and motor areas of the frontal lobe. Cortical implants in both primary motor cortex (M1) and PPC have been used to record movement intention signals in tetraplegic humans in order to control external devices such as robotic limbs and computers. One outstanding question is how the functional properties of these areas differ when used for direct brain control. We have recently initiated a clinical trial in which neural activity is recorded from the hand-knob region of M1 and the superior parietal lobule of PPC in the same patient in order to make direct comparisons. The neural populations from both arrays were used together or individually to perform a variety of tasks in which the participant guided a cursor under brain control. These tasks included reaction time and delayed memory tasks with different sensory motor contexts (Pro-, Anti-, Symbolic, and NoGo trials) to better understand the underlying factors driving neural responses in the two areas. We used off-line analysis at both the single-unit and population levels to understand the functional properties of the >200 units simultaneously recorded from M1 and PPC during each brain-control session. M1 neurons were found to code the desired movement almost exclusively during the execution of cursor movements whereas PPC neurons were active during both the planning and execution epochs. Direction selective signals in M1 and PPC were largely invariant to sensory-motor context, reflecting the intention of the subject invariant to the visuomotor context used to instruct movement. Intriguingly, the relative timing of intention related signals was context dependent: intention signals were coded about 100ms earlier in PPC than M1 when instructed with simple intuitive visual stimuli, but the pattern reversed, with M1 leading PPC (~20ms), when an abstract rule was needed to remap a spatial cue to inform movement. These results show that the network architecture for sensory-motor planning and execution are largely preserved post-injury, even under novel functional demands (closed-loop cortical control). Further, our results suggest a more complicated network architecture for how movement emerges from combining information from our external world with internally stored contextual information.

Poster

315. Brain-Computer Interface: Intracranial

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Topic: E.05. Brain-Machine Interface

Support: NIH Grant U01NS108922
NSF GRFP

Title: The influence of object presence on M1 activity during movement planning and execution

Authors: *A. J. HERRERA*1,4, R. A. GAUNT2,4,1, M. L. BONINGER2,1,5, A. P. BATISTA1,3,4, B. M. YU6,7,4, S. M. CHASE7,4, J. L. COLLINGER2,1,4,5,

Abstract: Brain-computer interfaces (BCIs) can restore upper limb function by providing control of various end effectors using neural signals recorded from motor cortex (M1). Previous studies in our lab have found that during BCI control, a majority of recorded neurons show an increase in firing rate as an object is approached relative to when the same movement is performed in the absence of an object. Here we quantify how population-level neural dynamics during planning and movement are impacted by object presence.

A 32-year old man with tetraplegia who had intracortical microelectrode arrays implanted in M1 participated in this study. Neural data were collected while the participant used a BCI to move a robotic arm and hand to a specified location. He then either grasped an object or closed the fingers at that location. Data were recorded over 5 sessions.

To uncover the latent dynamics underlying the population neural activity during the task, we used factor analysis to identify a low-dimensional subspace summarizing neural co-activation patterns. In this subspace, we observed clear separation in the time course of population activity between the conditions across all sessions. Even prior to movement onset, the neural activity occupied a different area of the neural subspace for trials with and without an object. Since the object-present and object-absent trials were collected in blocks rather than randomized from trial to trial, this may suggest a change in strategy or preparation state. The separation between the neural trajectories tended to increase as the hand approached the target. To understand the dynamics of the trajectories, we defined an object context axis (OCA) as the line connecting the average object-present and object-absent neural trajectories at each time point. The angle of the OCA remained stable during the reach phase and then rotated (by ~60 degrees in 8D space) when the subject was instructed to grasp. The angle remained constant during the grasp phase.

These findings demonstrate the existence of a clear separation of motor cortical activity patterns
when reaching to an object rather than an empty location in space. The difference in neural activity during preparation may be driven by visual feedback or a change in motor control strategy that occurs before movement onset. The substantial rotation of the OCA between the reach and grasp phases may reflect a dynamic change in neural states used to control these different movement types. However, the parallel nature of the neural trajectories within each movement phase may allow the movements to be driven through a constant downstream linear readout without being impacted by the presence or absence of an object.

mirror neurons have been established in premotor and parietal areas [e.g. Mukamel et al. 2010], their existence in primary motor cortex (M1) is a matter of debate: evidence has been shown directly in monkeys using intracortical electrodes [Tkach et al. 2007, Dushanova and Donoghue 2010], but only indirectly in humans using noninvasive methods such as electroencephalography [Eaves et al, 2016].

For the purpose of a study to achieve BMI-based simultaneous control of two robotic arms, we implanted 6 microelectrode arrays (Blackrock Microsystems, Salt Lake City, UT; 2 arrays in M1 and 2 in S1 in left/dominant hemisphere; 1 in M1 and 1 in S1 of right/non-dominant), comprising 384 neural recording channels in a human participant affected by a high spinal cord injury. As an assessment of microarray placement and recordings, we showed videos of a person performing shoulder abductions and wrist extensions on both sides of the body, and the participant was prompted to either passively observe, attempt (imagine) without initiating, or execute the movement along with the video. During each experimental block, one type of movement (randomized) on a side of the body (also randomized) was repeated at a base period of 2 s (plus jittered delay).

Peristimulus activity across electrodes revealed a subset of neurons that modulated across observation, imagery, and execution trials, providing direct evidence of a mirror-like activity in human primary motor and sensory cortices. Increases in activity relative to baseline were detected bilaterally, suggesting these networks may exist in parallel in both ipsi- and contralateral brain hemispheres to the controlled arm. These results provide novel biological support for the use of motor imagery and passive observation to construct and train decoders for BMI prostheses.


Poster

315. Brain-Computer Interface: Intracranial

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 315.10/P17

Topic: E.05. Brain-Machine Interface

Support:  N66001-16-C-4051
          N66001-10-C-4056
          1U3NS107714-01
          1U01NS108922-01
**Title:** Intracortical microstimulation feedback improves grasp force accuracy for high forces when using a brain-computer interface

**Authors:** *K. M. QUICK*¹⁻³, J. M. WEISS¹, R. A. GAUNT¹⁻³,², J. L. COLLINGER¹⁻³,²,⁴; ¹Physical Med. and Rehabil., ²Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA; ³Ctr. for the Neural Basis of Cognition, Pittsburgh, PA; ⁴DVA, Pittsburgh, PA

**Abstract:** After a spinal cord injury, a person may grasp objects using a brain-computer interface (BCI) to control a robot arm. With this approach however, people lack somatosensory feedback on their grasp force. Intracortical microstimulation (ICMS) of sensory cortex can evoke tactile sensations and may therefore offer a viable solution for grasp force feedback. We investigated whether a bidirectional BCI could improve grasp force control over a motor-only BCI. When evaluating the BCI force error during a force matching task, we found that ICMS feedback improved BCI grasp force accuracy when the participant attempted to grasp at a high force, but not for low or medium forces.

We recorded neural activity from motor cortex (M1) using intracortical arrays in a 31-year-old man with tetraplegia. While collecting BCI training data, the participant attempted to mimic a virtual reality hand (“VRH”) as it closed around an object, applied a cued force level, and released the object. From this data, we trained a BCI to decode intended grasp force and grasp velocity. During BCI control of the VRH, we provided feedback on the VRH’s applied force via single-channel amplitude-modulated ICMS. We then evaluated the accuracy of the BCI’s applied force under various ICMS feedback conditions (sham stimulation or actual stimulation) and visual feedback conditions (no visual “NoVis” feedback, VRH feedback, or VRH + applied force trace “VRH + F” feedback).

In addition to success rate on the force matching task, we quantified performance two seconds after grasp onset by evaluating the error between the cued force and the BCI’s applied force. This time period was chosen in order to capture the participant’s first attempt to match the force target. For high-force targets, ICMS feedback improved the BCI’s applied force accuracy during the NoVis and VRH feedback conditions. For medium and low-force targets, ICMS feedback did not affect BCI grasp force accuracy during any visual feedback condition. For medium-force targets, the participant already had an accurate implicit representation of the cued force such that ICMS did not provide added benefit. For low-force targets, the subject had difficulty concurrently closing the gripper and applying a small force; the BCI frequently overshot the low-force target regardless of the provided feedback. Some of the performance hurdles encountered resulted from the dual velocity-force decoding scheme which made it difficult for the participant to keep the VRH closed for low-grasp forces or for long duration grasps. To better realize the potential for increased grasp force accuracy with ICMS feedback, new decoders for maintaining grasp force will need to be developed.

**Disclosures:** K.M. Quick: None. J.M. Weiss: None. R.A. Gaunt: None. J.L. Collinger: None.
Poster

315. Brain-Computer Interface: Intracranial

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 315.11/P18

Topic: E.05. Brain-Machine Interface

Title: Restoring the sense of touch using a sensorimotor demultiplexing brain-computer interface

Authors: *P. D. GANZER1, S. COLACHIS, IV1, M. A. SCHWEMMER2, D. FRIEDENBERG1, C. SWIFTNEY3, A. JACOBOWITZ4, C. DUNLAP5, D. J. WEBER6, M. A. BOCKBRADER7, G. SHARMA1;
1Battelle Mem. Inst., Columbus, OH; 2Battelle Mem. Institute, Columbus, OH; 3GE, Wayne, MI; 4Ohio State Univ., Columbus, OH; 5The Ohio State Univ., Columbus, OH; 6Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA; 7Physical Med. & Rehabil., Ohio State Univ. Col. of Med., Columbus, OH

Abstract: The sense of touch is a key component of motor function. Several efforts have successfully used a brain-computer interface (BCI) to restore motor function alone. Importantly, sensory information relevant for BCI control is potentially represented in the brain, even following severe spinal cord injury (SCI). Unfortunately, severe SCI should essentially eliminate sensory information transmission to the brain, that originates from skin innervated from below the lesion. We assessed the hypothesis that, following SCI, residual touch-related hand sensory information is transmitted to the brain, can be decoded amongst competing sensorimotor signals, and used to enhance the sense of touch via an intracortically controlled closed-loop BCI. Experiments were performed with a participant who has an AIS-A C5 SCI and an intracortical recording array implanted in left primary motor cortex (M1). Recordings of evoked M1 activity from hand tactile stimulation, neural decoding, and standard clinical assessments of sensorimotor functions were used throughout a series of experiments. Our results demonstrate that residual afferent hand sensory signals surprisingly reach M1 following SCI, and can be simultaneously demultiplexed from ongoing efferent motor intention (~85% accuracy, using non-linear support vector machines), enabling intracortically controlled closed-loop sensory feedback during BCI operation. The closed-loop sensory feedback system was able to detect residual sensory signals from up to the C8 spinal level. Using the closed-loop sensory feedback system enabled significantly enhanced object touch detection, sense of agency, movement speed, and other sensorimotor functions. To our knowledge, this is the first demonstration of simultaneously decoding multiplexed afferent and efferent activity from human cortex to control multiple assistive devices, constituting a ‘sensorimotor demultiplexing’ BCI. Overall, our results support the hypothesis that sub-perceptual neural signals can be decoded reliably and transformed to conscious perception, significantly augmenting function. Additional experiments using deep learning architectures also demonstrate the ability to demultiplex touch, movement intention, and
proprioception simultaneously as well as decode discrete pressure levels during object grip. We hope this new set of findings will enable patients with an implanted BCI to maximize the information encoded in the recorded neural activity for new functional gains.


Poster

315. Brain-Computer Interface: Intracranial

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 315.12/P19

Topic: E.05. Brain-Machine Interface

Support: NIH UH3

Title: Habitation of perceived intensity with intracortical microstimulation

Authors: *C. L. HUGHES¹, S. N. FLESHER³, M. BONINGER², R. A. GAUNT²; ¹Bioengineering, ²Physical Med. and Rehabil., Univ. of Pittsburgh, Pittsburgh, PA; ³Dept. of Neurosurg., Stanford Univ., Stanford, CA

Abstract: Somatosensation is crucial for motor control and is an important part of the human experience. 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. For ICMS to be practically useful, the percepts should be consistent and reliable. However, we have observed that continuous stimulation can alter perceived intensity, as has been observed with peripheral stimulation. We aimed to systematically document habituation to ICMS and understand how this might affect clinical BCI applications. To quantify how continuous stimulation alters perceived intensity, we delivered stimulation on single electrodes at 80 uA for 60 seconds at 100 Hz. The participant used an analog slider to indicate how the intensity changed throughout the stimulation interval. We found that on 25 out of 27 trials tested across 9 electrodes, the perceived intensity decreased to be imperceptible within the 60 second stimulation period. Interestingly, the change in intensity was not gradual but rather happened in steps, often shifting quickly from baseline intensity to imperceptible. To quantify how intermittent stimulation alters perceived intensity, we delivered a 1-second pulse train at 60 uA and 100 Hz on single electrodes every four seconds for 50 pulse trains and asked the participant to report the perceived intensity on a self-selected scale following each pulse train. To measure recovery, we then presented a 1-second pulse train every 60 seconds for 5 pulse trains. We found that the perceived intensity decreased with each additional stimulus on all but one electrode, with the most significant changes occurring within
the first 10 pulses, although the degree and time course of habituation varied by electrode. During the recovery period, the perceived intensity partially recovered in the first minute following stimulation with no significant changes after this recovery. Furthermore, intensity would rarely recover to the original reported intensity within test days on any tested electrode. We found that continuous stimulation could result in complete loss of perception, typically within 15 seconds. Intermittent stimulation also decreased intensity, but over three minutes, the sensations never became imperceptible. In clinical application of BCIs, intermittent stimulation should be used to drive consistent percepts. This supports a stimulation approach in which ICMS is delivered transiently based on changes in force rather than tonically, as is the case when ICMS is mapped to the absolute value of force.

Disclosures: C.L. Hughes: None. S.N. Flesher: None. R.A. Gaunt: None. M. Boninger: None.

Poster

315. Brain-Computer Interface: Intracranial

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 315.13/P20

Topic: E.05. Brain-Machine Interface

Support: DARPA, Contract #N66001-10-C-4056

Title: Human brain-machine interface using bilateral sensorimotor intracortical implants

Authors: *M. C. THOMPSON¹, R. W. NICKL², T. M. THOMAS³, M. A. ANAYA², D. N. CANDREA³, D. P. MCMULLEN⁵, W. S. ANDERSON⁴, E. A. POHLMEYER¹, M. S. FIFER¹, F. V. TENORE¹, G. L. CANTARERO², N. E. CRONE², P. A. CELNIK², B. A. WESTER¹; ¹Johns Hopkins Univ. Applied Physics Lab., Laurel, MD; ²Physical Med. & Rehab, ³Biomed. Engin., ⁴Neurosurg., ⁵Neurol., Johns Hopkins Univ., Baltimore, MD; ⁶NIH, Bethesda, MD

Abstract: Intracortical brain-machine interfaces (BMIs) have been used to effectively control multidimensional manipulators such as cursors and robotic arms. Prior intracortical BMIs in humans have relied on neural inputs from sensorimotor regions in a single hemisphere. Recording neural activity simultaneously from both hemispheres may improve on these prior demonstrations by: (1) enabling additional dimensions of control via unique information in each hemisphere and (2) improving on the reliability of existing dimensions of control via information that is related or shared between hemispheres. This work represents the first demonstration of an intracortical BMI leveraging input signals from bilateral sensorimotor implants in a human participant. A volunteer with incomplete quadriplegia was implanted with the Bidirectional Cortical Neuroprosthetic System (BiCNS) [1] consisting of 3 pairs of NeuroPort arrays (Blackrock
Each array pair included one 96-channel array implanted in primary motor cortex (M1) and one 32-channel array implanted in primary sensory cortex (S1). Two array pairs were implanted in the dominant hemisphere and one array pair was implanted in the non-dominant hemisphere.

Beginning 4 weeks after implantation, the participant practiced controlling a multidimensional manipulator in up to 3 sessions per week. At the start of each session, spike sorting to isolate multi- and single-unit neural inputs from one or more arrays was performed. Calibration was then performed with an imagery task in which the participant observed a series of automated center-out movements by the virtual Modular Prosthetic Limb (vMPL) and imagined that he was performing the movements. Given the participants’s novel combination of bilateral arrays, separate decoders for velocity outputs were trained for two vMPLs: the right vMPL used inputs from dominant (left) M1 and the left vMPL used inputs from non-dominant (right) M1. Decoders were trained using indirect optimal linear estimation and then used for online control of the vMPLs. Initially an assistive training scheme was used in which control inputs for an ideal trajectory were added to the participant’s own inputs. The assistive input was progressively reduced as the participant learned to generate his own control.

Performance for different decoder configurations is assessed using a combination of online and offline metrics including overall accuracy during real-time control tasks and $R^2$ values on data withheld from decoder training. Additionally, stability of neural recordings and decoder performance are discussed.


Title: Similar low dimensional neural population dynamics in dorsal motor cortex during human speech and hand movements

Authors: *S. D. STAVISKY\textsuperscript{1,2,3}, F. R. WILLET\textsuperscript{1,2,3}, P. REZAI\textsuperscript{1}, D. T. AVANSINO\textsuperscript{1,2,3}, L. R. HOCHBERG\textsuperscript{8,9,11,12,10}, K. V. SHENOY\textsuperscript{2,4,5,6,3}, J. M. HENDERSON\textsuperscript{1,3,7},
\textsuperscript{1}Neurosurg., \textsuperscript{2}Electrical Engin., \textsuperscript{3}Wu Tsai Neuro. Inst., \textsuperscript{4}Dept. of Bioengin., \textsuperscript{5}Dept. of Neurobio., \textsuperscript{6}Howard Hughes Med. Inst., \textsuperscript{7}Bio-X Program, Stanford Univ., Stanford, CA; \textsuperscript{8}VA RR&D Ctr. for Neurorestoration and Neurotechnology, Dept. of VA Med. Ctr., Providence, RI; \textsuperscript{9}Sch. of Engin., \textsuperscript{10}Carney Inst. for Brain Sci., Brown Univ., Providence, RI; \textsuperscript{11}Ctr. for Neurotechnology and Neurorecovery, Dept. of Neurol., Massachusetts Gen. Hosp., Boston, MA; \textsuperscript{12}Dept. of Neurol., Harvard Med. Sch., Boston, MA

Abstract: A growing body of research suggests that cortical neural ensemble activity during motor behaviors is well-described by orderly dynamics in a low-dimensional underlying neural state space. Most of this prior work has examined neural activity during limb movements, in animals. Is there similar neural population structure during different types of movements, such as the uniquely human behavior of speaking?

We recorded neuronal spiking activity from two 96-electrode arrays implanted in the ‘hand knob’ area of dorsal motor cortex of a participant in the BrainGate2 brain-computer interface clinical trial. This individual has tetraplegia, but can still speak, allowing us to record neural activity leading up to and during speech production.

Individual neurons in this area modulated strongly and distinctly while speaking different words. This allowed us to examine neural population dynamics at the resolution of action potentials, complementing previous speech neurophysiology studies which have been primarily conducted at the coarser resolution of electrocorticography (e.g., Anumanchipalli et al. 2019).

We found that two motor cortex dynamics motifs previously reported during arm/hand movements were also strongly present when speaking. When the person was beginning to produce speech, the largest component of the population activity (found via dPCA) was largely invariant across speaking different words. This population-level structure is similar to Kaufman et al. 2016’s finding of a condition-invariant motor cortical signal when monkeys initiated a variety of reaching movements.

During the subsequent speech production (i.e., a time epoch centered on acoustic onset), neural population firing rates largely followed rotatory dynamics (found via jPCA) as previously described for monkey reaches (Churchland et al. 2012) and human hand movements (Pandarinath et al. 2015).

These conserved motor cortical dynamics patterns across reaching and speaking could indicate that this is a widely-deployed computational strategy for producing different kinds of movements. In particular, such neural trajectories may be an effective way to transition the neural state from a preparatory regime to a different regime in which time-varying motor outputs are assembled from an oscillatory basis set (Sussillo et al. 2015).

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is a consultant to Neuralink Corp. and on the Scientific Advisory Boards of CTRL-Labs Inc., MIND-X Inc., Inscopix Inc. and Heal Inc. These entities did not support this work. J.M. Henderson: F. Consulting Fees (e.g., advisory boards); J.M.H. serves on Medical / Scientific Advisory Boards of Circuit Therapeutics, Enspire DBS, and Neuropace. These entities did not support this work.

**Poster**

**315. Brain-Computer Interface: Intracranial**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 315.15/P22

**Topic:** E.05. Brain-Machine Interface

**Title:** Classification and fitting of phonemes and words are the underlying basis of an invasive speech prosthetic that uses chronically recorded single units

**Authors:** P. R. KENNEDY¹, *M. GEARING²;
¹Neural Prosthetics, Neural Signals Inc, Duluth, GA; ²Emory Univ., Atlanta, GA

**Abstract:** Data will be presented on the classification and fitting of phonemes and words using single units recorded from a person speaking audibly and silently. Phonemes showed no difference in correlations between audible and silent speech, but both modalities demonstrated a significant difference to control periods (P< 0.05, 2 tailed ‘t’ test) using the Fitting app from Matlab. These results indicate that silent speech can be detected with a correlation coefficient (R) that is not significantly different from the R for audible speech, and both can be distinguished from control periods of rest. Twenty words were classified using a support vector machine paradigm with accuracy of 85.3% using 3 words, falling to 59.7% with 20 words. These results provide confidence that classification and fitting paradigms can detect silent speech.
Disclosures: P.R. Kennedy: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 98% ownership of Neural Signals. M. Gearing: None.

Poster

315. Brain-Computer Interface: Intracranial

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 315.16/P23

Topic: E.05. Brain-Machine Interface

Support: NIH Grant K99 NS101127

Title: Changes in target-dependent neuromodulation during BCI learning

Authors: F. B. MCAFEE¹, *A. G. ROUSE²;
¹Biomed. Engin., ²Neurosci., Univ. of Rochester, Rochester, NY

Abstract: Brain-computer interface (BCI) research provides a unique opportunity to study neural adaptation and learning. In this analysis, we investigate the degree to which the subjects identified and selectively modulated the four neural dimensions used for control of a BCI. We analyze whether the dimensions used for BCI control have (i) larger magnitude and/or (ii) more consistent modulation than non-BCI dimensions. In the BCI experiment, a hybrid controller that included both active dimension selection and velocity control along the selected dimension was used. The BCI task involved the direct control of a virtual hand by 16 neurons to attain a target hand position. The 90 session experiment started with only two control dimensions with a set of four targets, then adding a new dimension each time the monkey was competent in the previous set until four control dimensions with eight targets were included. For each BCI session, the target independent, target dependent, and noise portions of the recorded firing rates were analyzed. The neural dimensions were analyzed separately to compare the four orthogonal BCI dimensions used for control with the 12 non-BCI dimensions. We analyzed both the magnitude and consistency of the neural firing rates. We defined magnitude as the sum of the target dependent variance across the target hand postures. We defined consistency as the sum of the target independent and dependent variance divided by the total variance. With the addition of each new BCI dimension the magnitude increased rapidly during the first couple sessions and then later decreased as the subject became more practiced with the new task. There was a steady increase in consistency throughout the experiment, which tracked the constantly improving performance of the subject. The neural activity in the BCI control dimensions increased slowly in both consistency and magnitude over the entire experiment relative to the non-BCI dimensions. Indicating that with practice, the subject learned to selectively modulate neural activity more in the BCI-specific dimensions than in the null dimensions.
Our analysis of the magnitude and consistency of neural modulation suggested three distinct processes with different time courses (i) initial large changes in magnitude, (ii) increases in consistency that lead to increased performance, and (iii) finally selective modulation in only BCI-specific dimensions for increased efficiency. These techniques identifying the magnitude and consistency of neural signals could be translated to future studies of learning to better characterize the time courses of such neuromodulation under different conditions.

Disclosures:  F.B. McAfee: None. A.G. Rouse: None.

Poster

315. Brain-Computer Interface: Intracranial

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 315.17/P24

Topic: E.05. Brain-Machine Interface

Support: Brain Research Program, Ministry of Science and ICT (MSIT), 2016M3C7A1904986

Title: Robot arm and hand control by neural activities from primary motor cortex in rhesus monkey

Authors: *S.-M. KIM1,2, H. PARK1,2, J.-W. SOHN1,2;

Abstract: Brain-machine interface (BMI) is a direct communication channels between the brain and external devices. It enables to control external device using neural activities. BMI is often used to restore function for patients with paralysis. It is important that external device in BMI provide high-precision movement like actual arm. To develop high-precision BMI technique, we designed a platform that external device (in this research, robot arm and hand) is controlled by neural activities from M1 area in rhesus monkey, at the same time, kinematic information of robot is encoded to the brain directly using intracortical microstimulation (ICMS). Reaching to grasp is natural movement and it happens frequently for serving daily functions in human life. For this reason, we designed reaching to grasp task and trained rhesus monkey so that he could perform task using his own arm and hand. For 11 days, the monkey achieved 86% of success rate (3845/4450 trials). After training, we implanted two 96-channel intracortical microelectrodes in the primary motor cortex (M1) and posterior parietal cortex, respectively. After two weeks of the surgery, we confirmed neural activities and started recording. We set up the task in which a monkey only observed reaching to grasp motion of robot arm and hand. We recorded neural activities from M1 and determined that neural activities respond to movement by observations. We developed decoder based on this result for real-time BMI platform. Using developed
decoder, the monkey was trained to control robot arm and hand using real-time population vector of neural activities. As a result of the monkey training, the success rate was gradually improved during 10 days from 50.0% of success rate on first day (9/15 trials) to 84.8% of success rate on 10th day (28/35 trials). The success rate is 83.8% (62/74 trials) on average for 10 days. This result enables us to study the effect of bi-directional BMI combining with ICMS method to encode kinematic information of robot arm directly to the brain in the future.

Disclosures:  S. Kim: None.  H. Park: None.  J. Sohn: None.

Poster

315. Brain-Computer Interface: Intracranial

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 315.18/P25

Topic: E.05. Brain-Machine Interface

Support: NIH Grant 1 DP2 HD087955

Title: Improving decoding from high-density ECoG recordings by optimizing single-trial latent factor dynamics

Authors: *R. ABIRI¹, D. B. SILVERSMITH², N. NATRAJ¹, E. F. CHANG³, K. GANGULY⁴; ¹Univ. of California, San Francisco, San Francisco, CA; ²Bioengineering, Univ. of California San Francisco, San Francisco, CA; ³Neurosurg., ⁴UCSF, San Francisco, CA

Abstract: Electrocorticography (ECoG) is a promising method to allow direct neural control over Brain-Machine Interfaces; it is less invasive and may allow for the recording of stable signals over long periods of time. However, it remains unclear how best to optimally capture single trial dynamics using mesoscale ECoG measurements. In contrast, there is a rapidly growing body of work showing that latent factor estimation can allow more robust single-trial decoding and control using spike-based recordings. Here, we hypothesized that the activities of motor skill can be represented using a low dimensional manifold of single-trial ECoG signals recorded from high-density grids. ECoG signals were collected from 4 subjects with implanted ECoG grids (with 256 channels) while they were instructed to perform individual finger movement. The subjects performed around 20 trials of individual finger movement (flexion & extension) while the kinematics of fingers was captured by a Leap Motion device. ECoG signals and kinematics of fingers were synced and recorded for further analysis.

Among various dimensionality reduction methods, Principal Component Analysis (PCA) was applied to generate latent factors of cortical dynamics from high-density ECoG grids. For each individual finger movement, PCA was applied to concatenated trials or averaged trials of ECoG data for raw data as well as different bands including delta, theta, alpha, beta, low gamma and
high gamma band. The first 10 extracted principal components were used to project single-trial ECoG data and decode the corresponding kinematics of fine movements using multiple linear regression. The results showed that PCA of averaged trials of ECoG demonstrated better performance than using PCA of concatenated trials. Furthermore, the accuracy of kinematic decoding was higher when using the delta band. Our results suggest that ECoG processing using latent factor dynamics can improve decoding. We anticipate that the generated low dimensional manifold can facilitate the development of a robust and real-time controller for a ECoG-based control of neurprosthetic devices.


Poster

315. Brain-Computer Interface: Intracranial

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 315.19/P26

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

Support: JST ERATO (JPMJER1801)
JSPS Grants-in-Aid for Scientific Research (18H05525)
the Human Frontier Science Program (RGP0019/2016)

Title: Deep learning-based classification for rat visual cortical electrocorticogram

Authors: *D. KONNO¹, N. MATSUMOTO¹, T. SUZUKI², Y. IKEGAYA¹;
¹Grad Sch. Pharma Sci, Univ. Tokyo, Tokyo, Japan; ²Natl. Inst. of Information and Communications Technol., Osaka, Japan

Abstract: Machine-learning techniques have been used to extract information from electrophysiological signals of neural activity. Recently, deep learning techniques have been used to classify neural signals. Specifically, convolutional neural networks (CNNs), one type of deep learning techniques, have shown good performance in classification of electroencephalogram (EEG) signals. However, it is well known that EEG has a low signal-to-noise ratio. To achieve better classification accuracy, we introduced electrocorticogram (ECoG) rather than EEG because in general, ECoG has a better signal-to-noise ratio than EEG. In this study, we first designed a novel ECoG probe which has 32 electrodes and mesh structure for stable electrode contact to the curved brain surface. Next, we recorded ECoG signals from the visual cortex of rats while presenting either of two distinct visual stimuli, vertical or horizontal grating. Rats were head-fixed, and a virtual cylinder comprising a vertical or horizontal sinusoidal grating was displayed in the three-dimensional coordinate space on four 24-inch
monitors that were arranged in a quadrangular arena. The images on the monitors were extended using two mirrors on the top and bottom of the arena. Then we tried to construct a CNN model that can classify which visual stimuli rats watch using raw ECoG signals as inputs. We collected 2,400 training data and 600 test data, reaching an accuracy of 58% in the test data. Although this accuracy is not so high, it seems that this CNN model learned some features of ECoG signals between vertical and horizontal stimuli. In the future, we aim to improve the accuracy by increasing the number of data.

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Poster

315. Brain-Computer Interface: Intracranial

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 315.20/P27

Topic: E.05. Brain-Machine Interface

Title: Improved classification of individual finger movements using temporal dynamics of ECoG

Authors: L. YAO, *M. SHOARAN;
ECE, Cornell Univ., Ithaca, NY

Abstract: Decoding of individual finger movements is a fundamental step for advanced prosthetic control. In this work, we exploit the temporal dynamics of the multi-channel electrocorticography (ECoG) signal in the feature space to improve the movement decoding accuracy compared to prior work, through classification of five finger movements and a resting state.

The ECoG recordings were segmented into 200ms epochs with 40ms step size. Nine features were subsequently extracted from each epoch to quantify the movement state, including alpha (8-13Hz), beta (13-30Hz), low-gamma (30-60Hz), gamma (60-100Hz) and high-gamma (100-200Hz) power, local motor potential (LMP) calculated as running average of the raw time-domain signal, the Hjorth activity, mobility, and complexity (Hjorth 1970). In order to precisely infer the current movement state, we concatenated the ECoG features extracted from the previous 15 epochs to form an expanded feature vector.

We evaluated the performance of our supervised machine learning model using a five-fold chronological cross-validation on the BCI competition IV dataset (3 subjects) and a similar paradigm ECoG dataset (9 subjects). By using the extreme gradient-boosted decision trees (XGB) as the classifier and the concatenated features of ECoG as input, we achieved an average accuracy of 72.3%±6.5%, about 8% higher than conventional XGB without sequential features (P<0.0001), as shown in Fig. 1. Moreover, the XGB classifier with concatenated features obtained a mean accuracy of 73.5% on the BCI competition IV testing set, outperforming the
state-of-the-art by 7.3% on the same dataset (Saa et al., 2016). Our approach offers the potential for high-performance and minimally invasive ECoG-based BMI control.

**Disclosures:** L. Yao: None. M. Shoaran: None.

**Poster**

315. Brain-Computer Interface: Intracranial

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 315.21/P28

**Topic:** E.05. Brain-Machine Interface

**Support:** Miami Project to Cure Paralysis for financial support

**Title:** A fully implantable brain machine interface for restoration of functional hand grasp in cervical SCI

**Authors:** *N. W. PRINS*¹, *I. CAJIGAS*², S. GALLO¹, J. A. NAEEM¹, A. E. PALERMO³, A. WILSON⁴, L. FISHER⁴, S. VANNI², M. E. IVAN², J. R. JAGID², A. PRASAD¹;

¹Biomed. Engin., ²Dept. of Neurolog. Surgery, ³Physical Therapy, ⁴Miami Project to Cure Paralysis, Univ. of Miami, Miami, FL

**Abstract:** Spinal cord injury (SCI) is a devastating disease, which exerts a disproportionate medical, social, and economic toll on both the affected individuals and society at large. However, to date, no therapeutic intervention has been demonstrated to definitively improve neurological...
outcomes or mitigate the effects of secondary neural injury. The objective of our study was to assess the feasibility of using the Medtronic Activa PC+S as a therapeutic system to restore movement to paralyzed muscles by: (1) evaluating the ability of the system to sense electrocorticographic (ECoG) signals in subjects living with quadriplegia (C5 or C6 level), and (2) assessing the feasibility of activating upper extremity muscles via functional electrical stimulation to reproduce hand grasp. We recruited a research participant (ClinicalTrials.gov NCT02564419) with a cervical SCI (C5 ASIA A) as a result of a motor vehicle accident to undergo implantation of the PC+S device with cortical electrodes placed stereotactically in hand motor region of the dominant hemisphere which was identified using pre-operative diffusion tensor imaging and fMRI while the subject performed motor imagery of his dominant hand. Intraoperative electrical stimulation and electromyograms (EMG) were used to further isolate the implant location. Currently, the subject is able to modulate his ECoG signals by following cues on a screen to trigger an external orthosis (Bioness H200) to cause opening and closing of his hand, with an online decoding accuracy of 88.2%. The subject is also able to grasp, transfer, and release objects of varying sizes by using brain signals to trigger the orthosis and write simple words. Our results demonstrate that a fully implantable brain machine interface can be safely implanted and used to reliably decode movement intent allowing for volitional control of hand grasp in a laboratory setting.

**Disclosures:** N.W. Prins: None. *I. Cajigas: None. S. Gallo: None. J.A. Naeem: None. A.E. Palermo: None. A. Wilson: None. L. Fisher: None. S. Vanni: None. M.E. Ivan: None. J.R. Jagid: F. Consulting Fees (e.g., advisory boards); Medtronic. A. Prasad: None.

**Poster**

**315. Brain-Computer Interface: Intracranial**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 315.22/P29

**Topic:** I.06. Computation/Modeling/and Simulation

**Support:** LBNL-internal LDRD “Neural Systems and Engineering lab” (KEB)

**Title:** Laminar origin of distinct high-frequency components of cortical surface electrical potentials revealed with biophysically detailed simulations

**Authors:** *V. L. BARATHAM*\(^1\)\(^2\), K. E. BOUCHARD\(^1\)\(^2\);
\(^1\)UC Berkeley, Berkeley, CA; \(^2\)Lawrence Berkeley Natl. Lab., Berkeley, CA

**Abstract:** Electrocorticography (ECoG) is a powerful neural recording technique which allows for high temporal resolution monitoring of distributed brain circuits at mesoscale spatial resolution in both humans and model organisms. However, its use as a basic research tool is hindered by an incomplete understanding of the biophysical mechanism generating the signals.
The primary goal of this research is to determine where, within the laminar structure of cortex, different frequency components of surface potentials (e.g., ECoG) are generated. Here, we use a large scale, anatomically detailed simulation of a cortical column, where our ability to precisely control the biophysics allows us to refine our understanding of ECoG signals. We first employ the Line Source Approximation (LSA) to compute cortical surface electrical potentials (CSEPs) from simulations. Simulated CSEPs show qualitative agreement of the frequency content with experimental CSEPs obtained from rat auditory cortex. Under the LSA, we can compute exact contributions to CSEPs from subsets of neurons, such as those located in specific layers: initial results suggest layer dependent contributions to different CSEP frequencies.

The LSA models extracellular space as homogenous and purely ohmic, thus electrical signals propagate with no frequency dependence. However, our previous simulation efforts to understand ECoG found that a spatially varying low-pass filter was needed to reproduce the experimentally observed surface frequency spectrum. We are particularly interested in frequency filtering properties of brain tissue, as neuroscientists often extract information from frequency-resolved analyses of neural data. Extending recent theoretical work showing that non-neuronal cells in the brain can become polarized and thus imbue the extracellular medium with nonzero capacitance, we are implementing such filters in the simulation via biophysically realistic mechanisms.

A parallel experimental effort to determine the laminar origins of ECoG using simultaneously recorded surface and depth data is currently ongoing. Future work will directly integrate experimental data with simulations: using a large volume of simulated data with different input magnitudes, spatial distributions, etc., it may be possible to use the surface signal to predict current sources through the cortical depth (or from specific neuron types). With an accurate forward model of CSEP generation, we hope to enable inference of laminar specific processing from experimental cortical surface recordings.

**Disclosures:** V.L. Baratham: None. K.E. Bouchard: None.

**Poster**

315. Brain-Computer Interface: Intracranial

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 315.23/P30

**Topic:** E.05. Brain-Machine Interface

**Support:** Eurostars RapidMaps

**Title:** Brain-computer interface for finger movement decoding

**Authors:** J. GRÜNWALD\(^1\), A. ZNOBISHCHEV\(^2\), C. KAPELLER\(^3\), K. KAMADA\(^4\), J. SCHARINGER\(^5\), A. CICHOCKI\(^2\), *C. GUGER\(^1\);

\(^1\)G. Tec Med. Engin. GmbH, Schiedlberg, Austria; \(^2\)Skolkovo Inst. of Sci. and Technol.,
Abstract: Linear discriminant analysis (LDA) is the de-facto standard for classification in non-invasive and invasive brain-computer interfaces. It is however static in nature and not suited to exploit transient information in features. We therefore developed a time-variant extension of linear discriminant analysis, termed TVLDA. Besides its time-variance, TVLDA also features an internal feature reduction stage. This makes manual channel selection or spatial projection approaches (such as common spatial patterns) obsolete in the given context. TVLDA is easy to implement, fully automatic and deterministic. It is suitable for real-time applications. We assessed the performance of TVLDA on experiments involving motor tasks for invasive brain-computer interfaces. Our study comprises six epilepsy patients with temporarily implanted subdural grids, who volunteered to participate in additional research experiments besides clinical treatment. We conducted two types of experiments involving three high-level gestures (rock, paper, scissors) and individual finger movement. We used log-transformed band-power features from the high-gamma band (50 Hz - 300 Hz). We evaluated our processing pipeline by 20 repetitions of a full 10-by-10 cross-validation. TVLDA outperformed LDA by 11.3% on average and yielded more stable results, even if only few trials were available. For the experiment involving three high-level gestures, TVLDA achieved 88% accuracy on average for standard-sized grids, and 99% on average for high-density grids. In the finger-movement experiment, the average accuracy over all subjects was 97% (chance level 20%). To our knowledge, this is the highest ever reported brain-computer interface performance for three-class gesture control and five-class finger control. The convincing offline classification performance of TVLDA opens the door to high-performance online applications. For example, a real-time and asynchronous TVLDA classification framework will enable online prosthetics control at very high accuracies. As well, TVLDA produces a reliable output metric for trial-based high-gamma mapping. And lastly, first evaluations have shown that the results from invasive data extend to the non-invasive domain, where TVLDA reliably outperforms CSP and LDA in motor-imagery experiments. This may have a considerable impact for motor-imagery based rehabilitation systems, such as for stroke recovery.


Poster

315. Brain-Computer Interface: Intracranial

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 315.24/P31
Abstract: Deep neural networks (DNNs) have recently been used to synthesize speech directly from electrocorticographic (ECoG) signals during speech production and speech perception. Recently, we developed an online keyword spotting system for spoken syllable stimuli with near-perfect speech detection and a level of accuracy determined by the quality of electrode coverage. Here, we extend these results to demonstrate, using pseudo-online streaming data, a system that synthesizes and recognizes speech with high quality and accuracy, and with latency under 1 s. Our system is composed of a modified DenseNet architecture that regresses from the raw ECoG spectrum to 18 Bark cepstral coefficients and 2 pitch parameters that may then be used for synthesis with a pre-trained LPCNet vocoder or for recognition triggered by neural voice activity detection (VAD). We show that the duration of the utterance can be precisely estimated and that our network is highly sensitive to speech- vs-non-speech discrimination and to self-generated speech vs perceived speech. Additionally, we demonstrate that these results extend to silently spoken speech in the absence of any auditory feedback, a necessary step for proving the feasibility of such a system for locked-in patients, who are otherwise unable to speak.

**Title:** Semantic vector based decoding of natural scenes from electrocorticogram

**Authors:** *R. FUKUMA*¹,², T. YANAGISAWA¹,²,³, S. NISHIMOTO⁴,⁵,⁶, M. TANAKA¹, S. YAMAMOTO¹, S. OSHINO¹, Y. KAMITANI⁷,², H. KISHIMA¹,³;
¹Dept. of Neurosurgery, Osaka Univ. Grad. Sch. of Med., Suita, Japan; ²ATR Computat. Neurosci. Labs., Seika-cho, Japan; ³Osaka Univ. Hosp. Epilepsy Ctr., Suita, Japan; ⁴Ctr. for Information and Neural Networks (CiNet), Natl. Inst. of Information and Communications Technol. (NICT), Suita, Japan; ⁵Osaka Univ. Grad. Sch. of Frontier Biosci., Suita, Japan; ⁶Ctr. for Twin Research, Osaka Univ. Grad. Sch. of Med., Suita, Japan; ⁷Grad. Sch. of Informatics, Kyoto Univ., Kyoto, Japan

**Abstract:** Recent brain decoding studies have utilized semantic space as a medium for modeling and decoding brain activity. A prior study has shown that a semantic space, derived from a natural language processing model word2vec, could be used to build a brain decoder that could infer perceptual contents in words (Nishida and Nishimoto, 2018). This study explores the potential use of the semantic modeling approach to realize practical brain-machine interface (BMI) by applying it with electrocorticography (ECoG), which has a higher temporal sampling rate than the functional magnetic resonance imaging used in the prior study. Seven patients under clinical monitoring for epileptic seizures participated in this study. During ECoG recording, the patients were shown a 60-min training video and a 10-min evaluation video (four times repetitions of 2.5-min video), both composed of short films or animation clips, with no overlapping scenes between the two videos. Movie scenes were extracted as still images once per second from the videos, and annotated manually using a natural language. Based on vector representations learned by word2vec using a Wikipedia dump, scene vectors were constructed by averaging 1,000-dimensional vector representations of the words in the annotation to each scene. The scene vectors were then decoded using ridge regression, and powers of the ECoG signals in four frequency bands (α, β, low-γ, high-γ). Nested cross-validation was applied to optimize the penalty term in order to decode the training scene vectors; on the other hand, evaluation scene vectors were decoded using a decoder trained with the training scenes vectors. Decoding performance was measured by two-alternative forced choice. For each scene in a test dataset, predicted vector of the tested scene was paired with another predicted vector randomly selected within the test dataset; for all possible pairs, binary test to distinguish predicted vector of the tested scene from that of the randomly selected one, solely based on the correlation coefficient with true scene vector of the tested scene. The averaged classification accuracy of the two-alternative forced choice was 61.6% (range: 58.2 - 65.3%) for the training video, and 58.9% (56.7 - 61.7%) for the evaluation video. This study demonstrated that the vector representation of movie scenes could be decoded from the ECoG signals in the semantic space of the word2vec model. The decoder could also infer scenes that were new to both the patients and the decoder. The combination of the semantic space and ECoG could be a promising approach to realizing practical BMI.

Poster

316. Posture and Gait I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 316.01/P33

Topic: E.06. Posture and Gait

Support: University of Sydney Early Career Researcher Grant

Title: Small amounts of involuntary muscle activity reduce passive joint range of motion

Authors: *J. Diong*¹, S. C. Gandevia², D. Nguyen², Y. Foo¹, C. Kastre³, K. Andersson³, J. E. Butler², M. E. Héroux²;
¹The Univ. of Sydney, Sydney, Australia; ²Neurosci. Res. Australia (NeuRA), Randwick, Australia; ³Linköping Univ., Linköping, Sweden

Abstract: Contracture is the loss of passive joint range of motion common in people with neurological conditions. When passive joint range of motion is assessed, any concomitant involuntary muscle activity is generally regarded small enough to ignore. This assumption is untested. If false, many clinical and laboratory studies that rely on these assessments may be in error. We determined to what extent small amounts of involuntary muscle activity limit passive range of motion in thirty able-bodied adults. Subjects were seated with the knee flexed 90° and the ankle in neutral, and predicted maximal plantarflexion torque was determined using twitch interpolation. Next, with the knee flexed 90° or fully extended, the soleus muscle was continuously electrically stimulated to generate 1, 2.5, 5, 7.5 and 10% of predicted maximal torque, in random order, while the ankle was passively dorsiflexed to a torque of 9 Nm by an investigator blinded to stimulation intensity. A trial without stimulation was also performed. Ankle dorsiflexion torque-angle curves were obtained at each percent of predicted maximal torque. On average (mean, 95% CI), each 1% increase in plantarflexion torque decreases ankle range of motion by 2.4° (2.0 to 2.7°; knee flexed 90°) and 2.3° (2.0 to 2.5°; knee fully extended). Thus, 5% of involuntary plantarflexion torque, the amount usually considered small enough to ignore, decreases dorsiflexion range of motion by ~12°. Our results indicate that even small amounts of involuntary muscle activity will bias measures of passive range, and hinder the differential diagnosis and treatment of neural and non-neural mechanisms of contracture.

Disclosures: J. Diong: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); This study is supported by a Kickstart Early Career Researcher Grant from the University of Sydney. S.C. Gandevia: A. Employment/Salary (full or part-time); Supported by the National Health and Medical Research Council (NHMRC) of Australia. D.
**Title:** A novel robot for separate body weight supported treadmill training to modulate gait patterns in patients with hemiparetic stroke

**Authors:** *H. FUJIMOTO*¹, T. TERAMAE³, T. NODA³, A. TAKAI³, N. FUJITA², M. HATAKENAKA¹, Y. HIRAMATSU¹, A. JINO², J.-I. FURUKAWA³, H. YAGURA¹, T. KAWANO¹, H. OTOMUNE¹, J. MORIMOTO³, I. MIYAI¹;
¹Neurorehabilitation Res. Inst., ²Dept. of Rehabil., Morinomiya Hosp., Osaka, Japan; ³Dept. of Brain Robot Interface, ATR Computat. Neurosci. Labs, Soraku-gun, Kyoto, Japan; ⁴Dept. of Neurology, Graduate. School. of Med., Osaka Univ., Suita, Japan

**Abstract:** Background: Body weight-supported treadmill training (BWSTT) is used as an effective therapy to improve post-stroke gait disorder. However, because conventional body weight supported (BWS) training continuously supports the body with identical forces to both paretic and non-paretic sides, the temporal feature of each leg support force coordinated with the gait cycle cannot be optimized. To challenge this problem, we started clinical trials of a novel split-force BWS robot that two Pneumatic Artificial Muscles (PAMs) provide the vertical support forces to the right and left sides of the body separately to induce natural gait patterns.

**Objective:** The aim of this study is to assess whether the split-force BWS can modulate gait patterns of patients with hemiparetic stroke.

**Methods:** Five patients with stroke (M:F = 4:1, 39.0 ± 23.3 years-of-age, 90.6 ± 60.8 days after onset) with mild to moderate hemiplegia (Lower-extremity Fugl-Meyer assessment score: 28.4± 3.3) admitted for inpatient intensive rehabilitation participated in this study. Participants wore custom-made shoes with load cells and a harness. The magnitude of split-force BWS support forces changes according to the walking movement, while the total BWS was kept at constant.
The lateral distribution of support forces was set to proportional to the ratio of ground reaction forces. Before experimental measurement, comfortable walking speed (1.3 to 2 km/hour) and degree of total BWS (10% to 33%) were set for each patient. In the experimental sessions, patients walked on the treadmill without holding handrails according to the following control mode: 1) split-force BWS mode; 2) total BWS mode; and 3) no BWS mode. We measured swing and stance time for each leg. The study protocol was approved by the local ethical committee.

**Results:**
There was no adverse event. The single stance time (SST) of the paretic leg increased under split-force BWS compared to no BWS and total BWS. The SST of the non-paretic leg increased under total BWS compared to no BWS, while the time varied under split-force BWS. The symmetry of the SST as assessed by the mean SST ratio (SST paretic/SST non-paretic) tended to be improved under split-force BWS than the other protocols.

**Conclusion:**
Our preliminary results suggest that split-force BWS treadmill training will be a potential tool to modulate gait patterns of stroke patients with mild to moderate hemiplegia. Further studies are needed to optimize its parameters to increase efficacy for gait training of stroke patients with various severity.

**Disclosures:**

**Poster**

316. Posture and Gait I

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 316.03/P35

**Topic:** E.06. Posture and Gait

**Title:** Effects of aging on muscle activation patterns and postural control during a sit-to-stand with different initial foot positions

**Authors:** *W. JEON*¹, H.-Y. HSIAO², B. WREFORD², C. CORONADO², L. GRIFFIN²; ¹The Univ. of Texas At Austin, Austin, TX; ²Kinesiology and Hlth. Educ., The Univ. of Texas at Austin, Austin, TX

**Abstract:** *Introduction:* The ability to perform a sit-to-stand (STS) is particularly challenging for elderly individuals because of age-related declines in muscle strength and neuromuscular coordination. The initial foot position (IFP) is an important strategy determinant of successful STS performance. The purpose of this study is (1) to investigate the effect of IFP on kinematics and muscles activation patterns during a STS and (2) to identify differences between healthy
younger (18-45 years) and older (>65 years) individuals during a STS. **Methods:** Two initial foot positions (IFPs) were tested: 1) reference; the feet were placed shoulder-width apart with the knee angle at 100 degrees of flexion, and the feet placed in parallel on separate force plates. 2) Toe-Out; toes turned out symmetrically with a toe-out angle of 20° from the reference IFP. EMG activity of the rectus femoris (RF), adductors (Add), tibialis anterior (TA), and soleus (Sol) were recorded along with trunk flexion angle during the STS and center of pressure (CoP) sway area during the stabilization phase (time interval between completion of uprising and the start of quiet standing). **Results:** Compared to the reference IFP, EMG activity of Add was greater for the Toe-Out IFP (reference: 25.76 ± 5.96%, Toe-Out: 32.57 ± 6.83% EMGmax, p < 0.01, respectively), whereas the TA EMG was smaller for the Toe-Out IFP (reference: 39.81 ± 10.23%, Toe-Out: 31.89 ± 8.86% EMGmax, p = 0.03). There was no difference in RF EMG amplitude between the two IFPs. The normalized CoP sway area (mm²/s) during the stabilization phase was smaller for Toe-Out IFP than for the reference IFP (Toe-Out: 16.01 ± 4.26 mm²/s, reference: 22.39 ± 5.20 mm²/s, p < 0.01). The EMG activity of the RF and Add was greater for older adults (RF: reference IFP; 45.51 ± 3.91%, 31.87 ± 3.33%, Toe-Out IFP; 39.43 ± 4.33%, 31.50 ± 4.45% EMGmax, for older and young adults respectively, p < 0.01). The trunk forward tilt-angle was also greater for older adults (reference: 31.54 ± 12.25° and 18.44° ± 3.45°, Toe-Out: reference: 35.24 ± 4.32° and 17.60° ± 6.61, for older and younger adults respectively, p < 0.01). **Conclusion:** The RF and Add participate more in generating vertical force in older adults during rising from a seated position. Older adults initiate a STS by flexing their trunk further forward to prevent possible backward displacement of the body’s center of mass. A Toe-Out IFP is an efficient foot position to engage the adductor muscles to assist the knee extensors during uprising while it decreases TA activation. The Toe-Out IFP also improves balance during the stabilization phase possibly by increasing the medio-lateral margin of stability for both younger and older adults.

**Disclosures:**  W. Jeon: None. H. Hsiao: None. B. Wreford: None. C. Coronado: None. L. Griffin: None.

**Poster**

**316. Posture and Gait I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 316.04/P36

**Topic:** E.06. Posture and Gait

**Support:** NIH R01- HD091184
AHA 16POST29610000
SC CTSI KL2TR001854
Title: Prolonged exposure to split-belt walking promotes energetic optimization during locomotor adaptation

Authors: *N. SANCHEZ*1, S. N. SIMHA2, J. M. DONELAN2, J. M. FINLEY1;
1Div. of Biokinesiology and Physical Therapy, USC, Los Angeles, CA; 2Dept of Biomed. Physiol. & Kinesiology, Simon Fraser Univ., Burnaby, BC, Canada

Abstract: Introduction: The dominant paradigm for studying locomotor adaptation involves having people walk on a dual belt treadmill with one belt moving faster than the other. During the first 15 minutes of adaptation, people transition from taking longer steps with the leg on the slow belt to taking steps of equal lengths. Researchers hypothesize that adaptation toward symmetric step lengths occurs because this pattern minimizes the energetic cost of walking. However, we demonstrated that when participants use visual feedback of their steps to take longer steps with the leg on the fast belt, which is not typically seen during adaptation, they reduce energetic cost below the cost of symmetric step lengths. What remains to be seen is whether adoption of this more economical pattern requires explicit instruction, or if participants converge to this asymmetric behaviour when given sufficient exposure to walking on the split belt treadmill. We hypothesized that after prolonged exposure to split-belt walking in the absence of instruction, participants will take longer steps on the fast belt, supporting the theory that locomotor adaptation is driven by energetic optimization. Methods: 15 participants walked on a split-belt treadmill for 45 minutes at a 3:1 ratio and belt speeds of 1.5 and 0.5 m/s. We measured step lengths as the distance between the feet at foot strike and we measured metabolic cost using expired gas analysis. We defined step length asymmetry as the difference between the fast and slow step lengths, normalized by their sum, such that negative asymmetries refer to longer steps on the slow belt and positive asymmetries refer to longer steps of the fast belt. We calculated metabolic cost and step length asymmetry at the 15th and 45th minute, to determine if people continue to modulate step length asymmetry in a manner that reduces energetic cost. Results and Discussion: Although participants took steps of approximately equal length after 15 minutes (not Normal, median ± IQR, -0.013 ± 0.050), they adopted positive step length asymmetries after 45 minutes (mean ± standard deviation, 0.030 ± 0.037, paired Wilcoxon p=0.083), in the same range as those we identified previously to minimize energetic cost. There was also a reduction in metabolic cost of 0.14 ± 0.21 W/kg from minute 15 to minute 45 (median 4.46, IQR 8.6%, sign rank p=0.021). These results suggest that adaptation involves a process that continuously adjusts step length asymmetry to minimize energetic cost and that taking steps of equal length is not the goal of adaptation but a point along the path toward energetic optimization.

Changes in the common synaptic drive to the ankle dorsiflexor muscle by adaptation during split-belt walking in humans

Authors: *A. OSHIMA, H. MURAI, N. TSUJIUCHI, K. KAMIBAYASHI;
Doshisha Univ., Kyoto, Japan

Abstract: Humans can maintain physical activity by adapting their motor program flexibly to varied environmental conditions. These adaptations in physical activity have been studied using a split-belt treadmill, on the basis of changes in electromyography (EMG) activities and ground reaction force (GRF). To clarify the neural adaptation to environmental changes, few studies have focused on changes in the common synaptic drive to a muscle. The purpose of the present study was to investigate changes of the common synaptic drive to adaptation during a novel physical activity using a split-belt treadmill by an EMG-EMG coherence analysis. Twelve adult subjects walked on a split-belt treadmill with two belts controlled separately. The treadmill was operated moving together "tied" or at difference speeds "split." The walking speeds were 0.5 and 1.25 m/s for the slow and fast conditions, respectively. The experimental paradigm consisted of three baseline phases ("tied" slow, "tied" fast, and then "tied" slow speeds for 2 min each) for 6 min, an adaptation phase ("split" condition of slow and fast speed) for 10 min, and a washout phase ("tied" slow speed) for 6 min. Each leg on the slow or fast belt speed during the adaptation phase was defined as the "slow leg" or "fast leg" respectively. A wireless surface EMG measurement system was used to investigate the EMG-EMG coherence. EMG electrodes were placed on the proximal and distal ends of the tibialis anterior (TA-p and TA-d), soleus, medial gastrocnemius, rectus femoris, and biceps femoris muscles. The intramuscular coherence in the 300 ms before heel strike was evaluated at the TA-p and TA-d pair. For the index of coherence strength, values by frequency domain analyses were calculated in 1 min (0.5-1.5 min) of each baseline phase; in the first and last minutes of the adaptation phase as early and late adaptations, respectively; and in the first and last minutes of the washout phase as early and late washouts, respectively. During the adaptation phase, the anterior component of the GRF during the stance phase and the amount of TA-TA coherence at the gamma band (35-60 Hz) in the slow leg decreased at late adaptation as compared with early adaptation. By contrast, the amount of TA-TA coherence at that band in the fast leg was not changed between the early and late adaptation phases. During the washout phase, the amount of TA-TA coherence at that band in the slow leg
significantly deceased at late washout as compared with early washout. These results suggest that the common synaptic drive to the ankle dorsiflexor muscle might be changed during the adaptation and washout phases.


Poster

316. Posture and Gait I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 316.06/P38

Topic: E.06. Posture and Gait

Title: Effect of cognitive fatigue on static and dynamic postural control in healthy older adults and stroke

Authors: *G. VARAS-DIAZ, S. WANG, T. BHATT;
Univ. of Illinois, Chicago, IL

Abstract: Background: Mental fatigue is a psychobiological state induced by sustained periods of demanding cognitive activity and characterized by feelings of tiredness. It has been well described that cognition plays an important role in movement and balance control, however the effect of cognitive fatigue on balance changes with aging and with a neurological insult remain unclear. The aim of this study was to examine the effect of cognitive fatigue on balance under different sensory conditions in healthy older adults and stroke population.

Methods: 10 healthy older adults (>65yrs) and 10 older adults with chronic stroke were asked to stand on a force platform while performing 2 cognitive condition (no cognitive task and serial subtractions (SS) task) across the 6 sensory conditions of the sensory organization test of the Balance Master before and after a cognitive fatigue task (stop-signal task for 60 min). The conditions were: eyes open, fixed surface (EO/FS); eyes closed, fixed surface (EC/FS), eyes open, sway referenced vision (EO/SRV); eyes open, sway referenced surface (EO/SRS); eyes close, sway referenced surface (EC/SRS); eyes open, sway referenced surface and vision (EO/SRVS)). Center of mass (COM) acceleration changes were recorded using an inertial sensor (Xsens Inc.). Jerk, as an indicator of the smoothness of postural sway and the root mean square (RMS) of the COM acceleration signal were analyzed for all the experimental conditions.

Results: Jerk and RMS were higher in the stroke group compared to the older adults group for all the 6 sensory conditions (p<0.05). Both groups showed a significant increase of Jerk and RMS after the cognitive fatigue task for all the sensory conditions (p<0.05). Differences in balance, assessed by jerk, between single and dual-task condition were observed only during EO/SRVS after the cognitive fatigue task for older adults group, and during EO/SRS, EC/SRS, and EO/SRVS after the cognitive fatigue task for stroke group. In addition, a loss of balance
incidence of 10% in EC/SRS and 35% in EO/SRS, and 30% in EC/SRS and 10% in EO/SRSV, and 30% in EC/SRS and 10% in EO/SRSV, which increased post cognitive task to 45% in EC/SRS and 55% in EO/SRSV for older adults, and to 55% in EC/SRS and to 65% in EO/SRSV in stroke.

**Conclusion:** Our results indicate that mental fatigue, induced by sustained cognitive activity, can impair balance during single and dual-task in older adults and stroke population. Cognitive fatigue could be considered as an intrinsic risk factor for falls in older people and stroke population, and should be taken into account for preventive and therapeutic strategies.

**Disclosures:** G. Varas-Diaz: None. T. Bhatt: None. S. Wang: None.

**Poster**

316. Posture and Gait I

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 316.07/P39

**Topic:** E.06. Posture and Gait

**Support:** NIH 1R01HD088543-01A1

**Title:** Fall risk predictors in community dwelling ambulatory stroke survivors

**Authors:** *R. GANGWANI*¹, S. DUSANE², T. S. BHATT³;

¹Dept. of Physical Therapy, Univ. of Illinois At Chicago, Chicago, IL; ²Dept. of Physical Therapy, ³Physical Therapy, Movt Sci., Univ. of Illinois at Chicago, Chicago, IL

**Abstract:** **Background:** Given the diversity of stroke specific impairments and the multifactorial nature of falls in stroke, identification of measurements that best predicts fall risk is important. Thus, the purpose of this study was to determine clinical and laboratory measures that best predict fall risk among community dwelling ambulatory chronic stroke survivors.

**Methods:** 36 chronic stroke survivors were subjected to a battery of tests to assess balance and other potential risk factors. Balance was assessed using clinical performance-based tests (Berg Balance Scale (BBS), Timed Up and Go (TUG)) and instrumented measures (Sensory Organization Test (SOT) and dynamic gait stability) as well as balance confidence (Activities specific Balance Confidence (ABC)). Other risk factors assessed were gait speed (10-meter walk time (10MWT)), severity of impairment (Fugl-Meyer Assessment (FMA)), Chedoke McMaster Scale (CMSA)), disability status (Modified Rankin Scale (MRS)), muscle strength, physical activity level (Physical Activity Scale for Elderly (PASE)) and social integration (Community Integration Questionnaire (CIQ)). Participants were subjected to an unannounced slip perturbation on the paretic side while walking on an over ground instrumented walkway during which perturbation outcome (fall or recovery) was identified. Accuracy of each measure was examined for prediction of perturbation outcome based on its sensitivity (accurate identification
of fallers) and specificity (accurate identification of non-fallers). Univariate logistic regression was performed for all the variables. Variables with a significance of p<0.3 were selected for further analysis with multivariate modeling. **Results:** On the slip perturbation, 17 participants fell while 19 recovered their balance. Univariate logistic regression analysis identified dynamic stability as the only variable to predict fall perturbation outcome with a sensitivity of 64.7% and specificity of 73.7%. The multivariate regression analysis model identified a combination of dynamic stability and SOT score successfully predicted falls with a sensitivity of 75% and specificity of 85.7%. **Conclusion:** Assessment of dynamic stability and posture control during fall risk assessment might help in distinguishing fallers from non-fallers. Moreover, fall prevention interventions specifically designed to improve dynamic stability and SOT score might help in reducing fall risk in chronic stroke survivors.

**Disclosures:** R. Gangwani: None. T.S. Bhatt: None. S. Dusane: None.

**Poster**

316. Posture and Gait I

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 316.08/P40

**Topic:** E.06. Posture and Gait

**Support:** NIH 1R01HD088543-01A1

**Title:** Motor adaptation and transfer of fall resisting skills to overground slips in chronic stroke survivors- A randomized control trial

**Authors:** *S. DUSANE*¹, T. S. BHATT²;
⁰¹Physical Therapy, Univ. of Illinois At Chicago, Chicago, IL; ²Physical Therapy, Movt Sci., Univ. of Illinois at Chicago, Chicago, IL

**Abstract:** Background: Chronic stroke survivors have demonstrated motor adaptation and transfer of the acquired fall resisting skills with repeated treadmill-based, stance slip perturbations. Evidence indicates an increased preference for compensatory stepping with the non-paretic limb signifying failed recovery with paretic stepping. Given the evidence of inter-limb transfer in healthy adults, if non-paretic overground gait slips could prime and improve paretic stepping it might ensure better patient safety and training tolerability than directly initiating paretic slip training. Considering the unexpected demands placed on either of the limbs during real life gait slips, reducing slip-induced fall-risk on both paretic and non-paretic limbs is equally important. Thus, this study aimed to determine whether chronic stroke survivors can acquire motor adaptation to repeated overground gait slips under the non-paretic limb and further demonstrate immediate inter-limb transfer of the acquired fall resisting skills to the untrained paretic limb. **Methods:** 45 community-dwelling chronic stroke survivors were randomly
assigned to either the training group (n=24), which received 8 unannounced, non-paretic slips prior to the novel paretic slip or the control group (n=21), which received a single novel paretic slip only. Participants were asked to walk on an 8-meter instrumented overground walkway while the computer controlled moveable platforms were used to induce slips with a maximum slip distance of 45cms. Slip outcome (falls and backward loss of balance (BLOB)), recovery strategies, pre-slip and post-slip COM state stability and step length were analyzed. **Results:**

With repeated non-paretic slips, the training group demonstrated significant falls reduction, from 33% to no falls and reduced BLOB from 67% to 0% (p<0.05); along with decrease in aborted stepping strategy from 42% to 0% and increase in recovery stepping strategy from 58% to 100%. There was also significant improvement in pre and post-slip COM stability at TD and step length (p<0.05). Although the training group demonstrated better control of COM state stability at TD on the novel paretic slip and reduced falls (63%) than the control (76%), the differences were not significant (p>0.05). **Conclusion:** The results indicate that the chronic stroke survivors can acquire immediate proactive (pre-slip) and reactive (post-slip) adaptation following repeated slip training under non-paretic limb leading to reduced falls and improved recovery strategies. However, there was no significant inter-limb transfer of acquired fall resisting skills from trained non-paretic limb to untrained paretic limb.

**Disclosures:** S. Dusane: None. T.S. Bhatt: None.

**Poster**

316. Posture and Gait I

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 316.09/P41

**Topic:** E.06. Posture and Gait

**Support:** NSF-CRCNS-1723998

**Title:** Interactive perceptual support can improve postural balance

**Authors:** *M. RUSSO*¹, A. KROTOV², D. STERNAD³;
¹Biol., ²Bioengineering, ³Departments of Biology, Electrical & Computer Engineering, and Physics, Northeastern Univ., Boston, MA

**Abstract:** Frail individuals often use a walker or a cane to reduce the risk of falling. In contrast, trained humans, like ballet dancers or gymnasts, can achieve astonishing balance abilities. For example, during a *pas de deux* a ballerina can hold very unstable position, like a pose on the tip of one foot, by only lightly touching the hand of her partner. What is the role of the hand in this challenging balancing task? While numerous studies already showed that not only mechanical but also perceptual support can reduce sway, we hypothesize that active support from another human provides further compensation or guidance for stabilization of posture. However, is the
support provided by a human supportive enough? How stable does support have to be to increase postural stability? This study examined the effect of mechanical and human support on postural sway with different levels of stability. Participants were asked to balance in a tandem stance on a narrow beam to create postural instability. While maintaining balance, participants used their dominant hand to hold the end effector of a robotic arm, measuring the force applied at the interaction. The nature of the support could be of two types: mechanical and human. For each of these two categories the participant experienced 4 increasing levels of stability. The handle of the robotic arm could be either fixed (stable) or simulating a cane of different lengths (2 unstable conditions). For control a set of trials was included in which the robotic arm was fully compliant. For the human support, a second participant held the handle of the robotic arm together with the target participant. The two performed the same 4 conditions presented before. Interaction force was measured together with EMG activity of 13 muscles from the active arm, torso and both legs. Postural sway was quantified by the center of pressure on a force platform below the beam. In addition, whole-body kinematics of both subjects was recorded via motion capture system. Participants included 16 healthy students and 16 professional ballet dancers. Preliminary results showed that mechanical support decreased the sway more than active human support. In contrast, ballet dancers significantly improved their balance when supported by another dancer over and above mechanical support. Ongoing analyses examine the nature of this active information transmission in its relation to postural sway. Further insights into active perception and control will inform the design of a robot controller that assists humans with impaired postural control.

Disclosures: M. Russo: None. A. Krotov: None. D. Sternad: None.

Poster

316. Posture and Gait I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 316.10/P42

Topic: E.06. Posture and Gait

Support: German Research Foundation (DFG) Grant 414705959
NSERC Grant RGPIN-2015-03871
CIRH Grant 407217

Title: Muscle synergies for murine and human locomotion

Authors: *A. SANTUZ1,2, O. D. LAFLAMME1, T. L. WELLS1, A. EKIZOS2, L. BRÜLL3,2, M. SCHWENK4, A. ARAMPATZIS2, T. AKAY1;
Abstract: The coordination of vertebrate locomotion results from the intercorrelation of an enormous number of variables and is computationally challenging. It has been suggested that the central nervous system might overcome the overabundance of degrees of freedom by synchronously activating different muscle groups in patterns called muscle synergies. From human experiments, we found that four synergies are enough to describe locomotion when considering 13 muscle activities (Santuz et al. 2018, Sci. Rep. 8, 2740). Our more recent study showed that three synergies can sufficiently account for the patterns observed in walking mice (Santuz et al. 2019, J. Physiol. JP277515). However, the results from mouse experiments were obtained by pooling the data recorded in animals implanted with a variable amount of electromyogram (EMG) recording electrodes. In this study, we aimed to compare human and murine synergies for walking when a) the number of recorded muscles is reduced from 13 to eight in humans and b) recording from eight functionally-matched muscles simultaneously in mice. We extracted synergies using non-negative matrix factorization from eight ipsilateral muscle activities of the right lower limb recorded during treadmill walking (1.3 ± 0.1 m/s) in 94 young and healthy humans (53 males, 41 females, age 28 ± 5 years). In addition, we extracted synergies from eight functionally-matched ipsilateral muscle activities of the right hindlimb in one wild-type adult mouse (male, age 67 days) walking on a treadmill at 0.2 m/s. Despite lowering the number of considered muscles from 13 to eight, humans still showed four synergies (3.7 ± 0.5) related to as many phases of the walking gait cycle (i.e. weight acceptance, propulsion, early swing, and late swing). Four synergies with similar function were enough to describe the walking patterns of a mouse implanted with eight EMG electrodes, in contrast with what we recently found pooling data from different animals. The number and function of these synergies were similar to those found in humans. We do not exclude the possibility that our current efforts to increase the sample size could lead to different conclusions. Yet, we show for the first time that human and mice share the same number of synergies extracted from eight functionally-matched muscles of the lower/hindlimb.


Poster

316. Posture and Gait I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 316.11/Q1

Topic: E.06. Posture and Gait

Support: NIH grant NS086973

Title: Coordination between quadriceps muscles activity in rats suggests neural regulation of joint stresses and strains
Abstract: How does the central nervous system (CNS) determine motor commands? One obviously important criterion is to generate motor commands that result in the desired movements. However, muscle activity also produces mechanical stresses to structures such as bones, ligaments, and articular cartilage. Poor regulation of such internal joint stresses could lead to pain and degeneration. We therefore hypothesize that the CNS generates motor commands that, while producing the desired movements, minimize stresses and strains within the joints. To evaluate this hypothesis, we exploited the biomechanical properties of the rat knee joint. As we previously showed, in the rats the quadriceps muscles vastus medialis (VM) and vastus lateralis (VL) produce very similar knee extension torque, but opposite mediolateral forces on the patella. To avoid excessive mechanical loading between the patella and femur, the activity of VL and VM should therefore be closely coordinated in order to reduce the net mediolateral force on the patella.

We recorded quadriceps muscles activity in rats (n=10) during treadmill locomotion at different speeds and inclines, and evaluated their coordination patterns. The activation profiles (i.e. time course of electromyographic signals) of VL and VM during each locomotor cycle were strikingly similar, and were clearly different from those of the other quadriceps muscles vastus intermedius and rectus femoris. Similarly, the activation intensities (i.e. integral of activation profiles across each locomotor cycle) of VL and VM covaried consistently across locomotor cycles, while those of the other muscles varied more independently to one another. Accordingly, both the activation profiles and the step-to-step activation intensities of VL and VM were highly correlated (r>0.8), and significantly more correlated than those of the other muscle pairs.

These results suggest that the CNS tightly coordinates the activity of VL and VM both within and across gait cycles, hence minimizing the net mediolateral force on the patella, consistent with the regulation of internal joint stresses and strains. We are currently investigating the neural underpinnings of these phenomena, evaluating in particular the role of joint afferent activity.

Title: Recognition of leg muscle motion in children with cerebral palsy using off-line EMG signals

Authors: *R. TANG, C. LI, X. XIE, Z. LI;
Hainan Univ., Haikou, China

Abstract: Cerebral palsy (CP) is a very common physical defect, which is caused by brain injury, and 90% of children with cerebral palsy have difficulty in walking. Therefore, the method of improving the walking ability of children with CP has been widely concerned. Gait training for children with CP is a common method to improve their walking ability in clinic. But previous studies on gait training based on angle and pressure sensors, which cannot follow the user's intention because it use constant gait speed and motion. In recent years, Surface electromyography (sEMG) signals plays an important role in the control of modern electric prosthesis and rehabilitation robotics. Due to the difference SEMG signal between subjects with CP and normal people, it is difficult to apply the results of EMG signals of normal people directly to patients with CP. Therefore, in this study we propose a method to recognize the muscle movement of CP children based on off-line SEMG signal. In this experiment, five girls and six boys with spastic cerebral palsy participated, and their age span was 7-16. Each participant walked for one minute at the speed chosen by the participants. The experiment was repeated in three groups, with a five-minute break between groups. At the same time, Data acquisition mainly divided into two categories, one is EMG signal, the other is angle information. The function of angle signal is to divide EMG signal into five stages. But one patient's data, so we totally got ten patients' data. All obtained SEMG signals are processed using our proposed method. Wavelet processing is used to denoise, elliptic filters are used for filtering and Derivative processing is used to change the expression of data. Feature extraction using SPA combine RMS. Finally, five classifications are established by SVM, and in order to evaluate the performance of the proposed method, two traditional feature extraction methods which are MVA and ZC are used for comparison. From the above, the results of the model we learned are shown in the table below. The average accuracy of the proposed model is 92.8% (±4.3%), and we also find that the modeling effect of two commonly used feature extraction methods are 71.7% (±13.3%) of MVA and 77.1% (±9.0%) of ZC respectively. We find traditional methods have limited performance in extracting the characteristics of SEMG signals of CP children. And the proposed method can extract the information of SEMG signals of CP children effectively, which means we find an effective way to use off-line SEMG signal to recognize leg muscle movements in CP children, i.e. We can use EMG signals to identify the CP children’s motional intentions, which can be used in future rehabilitation control strategies.

Disclosures: R. Tang: None. C. Li: None. X. Xie: None. Z. Li: None.
Poster

316. Posture and Gait I

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Topic: E.06. Posture and Gait

Support: JSPS Grant 18K17894

Title: Detecting task-dependent modulation of spatiotemporal module via tensor decomposition: Application to kinematics and EMG data in locomotion and running at various speed

Authors: *K. TAKIYAMA*¹, H. YOKOYAMA¹, N. KANEKO², K. NAKAZAWA²; ¹Tokyo Univ. of Agr. and Technol., Tokyo, Japan; ²The Univ. of Tokyo, Tokyo, Japan

Abstract: How the CNS controls our body is a fundamental question. The spatiotemporal module is an influential hypothesis for how the CNS manages a tremendous amount of degree of freedom (DoF) inherent in our body (Bizzi+, 1991). In the theory, the CNS controls a group of joints or muscles, referred to as spatial module, rather than a single joint or a single muscle separately to reduce the DoF. The time-varying recruitment pattern of each spatial module is referred to as a temporal module.

How the brain attains various repertoire of motions is another fundamental question. A possible solution is to modulate the spatiotemporal modules depending on the task. The temporal module rather than the spatial module can be modulated (Ivanenko+, 2005; Chvatal+, 2012). On the other hand, the spatial module rather than the temporal module can be modulated (Torres-Oviedo+, 2010). In our findings, the number of modules can change (Yokoyama+, 2016). In sum, there are seemingly different perspectives on the task-dependent modulation of the spatiotemporal modules.

Here, we propose a novel method to discuss the task-dependent modulation of the spatiotemporal modules flexibly. We apply tensor decomposition (Kolda+, 2009) to joint angle and EMG data. Although conventional methods or matrix decompositions (i.e., PCA and NNMF) are suitable for discussing two factors (i.e., spatial and temporal modules), there are limitations to consider more than three factors. For example, with the matrix decomposition, the task-dependent modulation of the temporal module between two tasks was discussed under the constraint of the same spatial module, or the modulation of the spatial module was discussed without considering temporal module. Without those constraints, the tensor decomposition enables to examine the task-dependent modulation of the spatiotemporal modules.

The tensor decomposition extracted the three types of spatiotemporal modules inherent in joint angle and EMG data in walking and running at various speed: 1) the modules recruited more significantly in a higher speed, 2) those mainly recruited in walking, and 3) those recruited primarily on the running. Further, the tensor decomposition extended a previous perspective on
switching walking and running: the CNS switches the two types of motions by modulating not only the temporal but also spatial modules via recruiting proximal muscles larger in the running than in walking.

Disclosures: K. Takiyama: None. H. Yokoyama: None. N. Kaneko: None. K. Nakazawa: None.

Poster

316. Posture and Gait I

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Program #:/Poster #: 316.14/Q4

Topic: E.06. Posture and Gait

Support: JSPS KAKENHI 17K01479

Title: Relationship between multi-joint dynamics and rambling/trembling movements of center of foot pressure during quiet standing

Authors: *S. SASAGAWA¹, A. IMURA²;
¹Dept. of Human Sciences, Kanagawa Univ., Yokohama, Japan; ²Tokyo Metropolitan Univ., Tokyo, Japan

Abstract: The trajectory of center of foot pressure (CoP) during quiet standing can be decomposed into rambling and trembling components (Zatsiorsky and Duarte, 2000). The former corresponds to slower migration of reference point with respect to which balance is controlled and the latter to faster deviation of the CoP from the reference equilibrium point. The purpose of this study was to investigate the relationship between multi-joint dynamics of the body and the rambling/trembling movements of the CoP during quiet standing. Healthy young participants (n=11) were required to stand quietly for 60 s on a force platform. At the same time, angular motions of the ankle and hip joints were measured by an optical motion capture system. We obtained the rambling component by low-pass filtering the CoP time-series with a cut-off frequency of 0.5 Hz. We then obtained the trembling component by subtracting the rambling component from the original CoP time-series. By using time-locked averaging technique with respect to unidirectional rambling or trembling movements, we investigated spatio-temporal relationship between the multi-joint dynamics of the body (i.e., joint kinetics and kinematics) and the rambling/trembling movements of the CoP. We first found that a forward shift of the CoP in the rambling trajectory was associated with increases in both the ankle plantarflexion and hip extension torques and was paralleled with increases in inclination angles of the leg and trunk segments. These slow increases in the joint torques along with the rambling trajectory tonically counteracted the effects of gravity. We next found that a forward shift of the CoP in the trembling trajectory was associated with rapid increases in both the ankle and hip joint torques.
These rapid increases in joint torques along with the trembling trajectory induced mutually opposite kinematic patterns in the ankle and hip joints (i.e., ankle plantarflexion and hip flexion) due to the inter-joint dynamic interaction and accelerated the body back to the reference equilibrium point. Our findings indicate that changes in joint torques in different time scales along with the rambling and trembling trajectories induce different kinematic patterns at the ankle and hip joints and play different roles in postural control.

**Disclosures:** S. Sasagawa: None. A. Imura: None.

**Poster**

**316. Posture and Gait I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 316.15/Q5

**Topic:** E.06. Posture and Gait

**Support:**

EU: PLATYPUS
DFG: IRTG-1901
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**Title:** Human body sway echoes induced by visual optic flow in VR

**Authors:** *D. ENGEL*¹², A. SCHUETZ¹², J. SCHWENK¹², A. MORRIS³, F. BREMMER¹²;
¹Dept. of Neurophysics, Philipps-Universität Marburg, Marburg, Germany; ²Ctr. for Mind, Brain and Behavior, Universities of Marburg and Gießen, Marburg, Germany; ³Dept. of Physiology, Biomedicine Discovery Inst., Monash Univ., Melbourne, Australia

**Abstract:** To uphold our bipedal stance, amongst other senses, we rely on vision. When humans perceive their body as moving relative to the environment, they trigger compensatory movements, resulting in their center of mass swaying around its point of stability. This sway can be found in unperturbed standing as well as elicited by external perturbations. Body sway is traditionally investigated in a real, moving-room by monitoring the body’s center of pressure (COP). Typically utilized patterns of room-movements describe linear displacements or (pseudo-)periodical oscillations. In our explorative study, we were interested in the body-sway response to a visual scene showing a random and thereby unpredictable succession of movements. For this purpose, we induced body sway by visually simulated self-motion in virtual reality, using 3-D random optic flow patterns. Subjects wore a head-mounted display while standing on a force plate to record the displacement of their COP. VR simulated subjects standing inside a 3-D tunnel, its walls comprised of random dots. During trials, the tunnel shifted its position along a world-fixed anterior-posterior axis according to a sequence of random displacements. The sequence contained frequencies at identical amplitudes (flattened white noise) ranging from 0 to 2 Hz. For each trial, COP trajectory in the anterior-posterior direction was cross-correlated with
the respecting presented sequence and its derivative, to obtain impulse responses to spatial displacement and velocity of self-motion, respectively. The cross-correlations revealed subjects to systematically adjust their sway to the stimulus. In all subjects, the impulse response was dominated by a transient oscillatory component at a specific frequency (echo). The oscillation exhibited a consistent phase-lag relative to the stimulus sequence. This component remained stable, even across different days of measurement, albeit the system, i.e. stable stance, being disturbed in an incoherent, unpredictable way. Considering all frequencies to be equally present in the stimulus, this oscillatory component could be interpreted as the outcome of an internal frequency filter, idiosyncratic to the subject. Our results demonstrate that human body sway responses can be characterized by the use of a random stimulus input along with reverse correlation techniques. These findings could be used to estimate an “eigenfrequency” of subjects, conceivably even allowing for future clinical applications.

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**Poster**

316. Posture and Gait I

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 316.16/Q6

**Topic:** E.06. Posture and Gait

**Title:** Analysis of hindlimbs locomotion kinematics in a mouse model of penetrating brain injury

**Authors:** *L. C. LEON-MORENO¹, R. CASTAÑEDA-ARELLANO¹, J. RIVAS-CARRILLO², G. MENDIZABAL-RUIZ³, S. DUEÑAS-JIMÉNEZ¹; ¹Lab. of Neurophysiol., Univ. De Guadalajara, Guadalajara, Mexico; ². Dept. of Physiology, Lab. of Tissue Engin. and Transplant, ³Dept. of Computer Sci., Univ. de Guadalajara, Guadalajara, Mexico

**Abstract:** There is evidence suggesting that hippocampus plays an important role in locomotion control because of its connections with locomotor areas such as motor cortex, cerebellum, thalamus and diencephalon. An injury in the hippocampus produce theta wave alterations that result in kinematic dysfunction. Previously, our workgroup described that a penetrating injury in the rat hippocampus generates neuron loss, the interruption of the sensory-motor circuitry that resulted in kinematic speed changes. Regard the continuous development of genetic modified mice to study the effects of neurological diseases and treatments, it is important to know that these connection between hippocampus and locomotion system is conserved among different species and further analyze this mechanism. To confirm the connection previously mentioned, we positioned a 0.5 mm in diameter stainless steel cannula 1 mm to the left, 2 mm behind bregma and lowered to a 2-mm depth to injure the hippocampus of five mice. Kinematic patterns
were obtained before the PI and 7 days after de injury (DAI). Five joints of hindlimbs were studied (iliac crest, hip, knee, ankle and fifth metatarsal) plus the displacement movement. A control pattern was calculated by averaging three steps from each subject. Statistical analysis was carried out in the SPSS software using a Kruskal–Wallis test with a Dunn’s test for multiple comparisons. A statistically significant value was taken when p<0.05. We found a shortening in the stride duration in the lesion group and a decrease of the distance traveled by those animals in comparison to the control pattern. Stride quality in both ipsilateral and contralateral hindlimbs remains uniform in the two groups, but locomotion speed was increased in injury animals. These results are comparable with the ones obtained in rat. Therefore, we can assume that locomotion kinematics can be used as a parameter to evaluate new treatments for neurodegenerative diseases or to further investigate the mechanisms of the connection between hippocampus and movement.


Poster

316. Posture and Gait I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 316.17/Q7

Topic: E.06. Posture and Gait

Support: STRATEGMED3/306011/1/NCBR/2017

Title: Sample entropy analysis of postural sway in early Parkinson’s disease patients

Authors: *G. JURAS¹, A. KAMIENIARZ¹, J. MICHALSKA¹, W. MARSZAJEK¹, K. SŁOMKA¹, M. RUDZIŃSKA-BAR²;
¹The Jerzy Kukuczka Acad. of Physical Educ., Katowice, Poland; ²Dept. of Neurol., Andrzej Frycz Modrzewski Krakow Univ., Kraków, Poland

Abstract: Introduction: Parkinson’s disease (PD) is a neurodegenerative disease, which may affect the body balance. According to standard clinical tests the balance disorders may occur only in advanced PD, however it is crucial to detect such disorders in the earlier stages of disease. For this purpose computerized posturography is successfully used (Nardone et al. 2006). It was found that the amount of attention invested in postural control is related to the dynamic structure of postural sway, specifically to COP regularity (Stins et al., 2009). It was quantified with the use of sample entropy method (Richman et al., 2000) based on which it was proved that the automatic postural control processes increase the sample entropy, while volitional control decreases it (Roerdink et al., 2011). Therefore, the aim of this study was to assess the COP signal regularity using sample entropy in patients with early Parkinson's disease. Methods: The research was conducted on 15 PD patients at stage II H&Y (PD-II) (age: 61.9±8.6 years; body
mass: 75.7±9.0 kg; height: 170.4±6.7 cm) and 15 healthy elderly (age: 63.5±4.3 years; body mass: 70.2±7.9 kg; height: 167.3±4.3 cm). The procedure consisted of three quiet standing trials with eyes open (EO) and eyes closed (EC). Each trial lasted 30s. Further analysis was conducted for COP velocity [cm/s], COP range [cm], COP root mean square [cm] and sample entropy.

**Results:** The values of sample entropy both in EO and EC was significantly lower in sagittal (EO: p < 0.001, EC: p = 0.001) and frontal planes (EO: p < 0.001, EC: p = 0.005) in the PD-II group compared to the elderly. In both, eyes open and eyes closed trials, the data showed no significant differences between PD-II and control group in all of standard measures of postural sway in sagittal plane (p > 0.05). The only significantly higher values were registered for frontal plane in the EO trials for COP range (p = 0.009) and COP root mean square (p = 0.003) in the PD-II compared to the elderly. **Conclusion:** The registered lower values of sample entropy in the PD suggest a more regular COP signal, which indicates the less automatic postural control. The PD patients need to invest more attention in postural control than healthy subjects. Sample entropy is a better measure compared to standard posturographic measures to detect early signs of postural control deficiencies.


**Poster**

316. Posture and Gait I

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 316.18/Q8

**Topic:** E.06. Posture and Gait

**Support:** 107-2221-E-182-009-MY3

107-2218-E-182-003

**Title:** Anticipatory postural adjustment (APA) in different targeted weight shifting tasks

**Authors:** *G.-S. Li*1, Y.-J. Chang1, J.-W. Lia1, J.-K. Yu1, M.-J. HSU2;

1Chang Gung Univ., Taoyuan, Taiwan; 2Kaohsiung Med. Univ., Kaohsiung, Taiwan

**Abstract:** Anticipatory postural adjustment (APA) is important to maintain postural stability during a self-induced perturbation. For example, during a self initiated arm forward reaching task, the COP would move backward before the actual perturbation of COP by the arm movement occurs. APA is not easily to be standardized due to the perturbation is varied according to individual’s movement speed and/or amplitude. It is not clear if APA is existed in self-initiated targeted weight shifting tasks in which the amplitude of weight shifting can be quantified. The purpose of this study was to investigate whether the APA component could be identified in targeted weight shifting tasks and to evaluate if the APA is influenced by the target
distance and direction. Seven healthy subjects performed two target distances (60% and 80% maximal) and five directions (0, 45, 90, 135, and 180 degrees) weight shifting tasks guided by a custom designed software. An APA component is defined if the COP moves to the opposite direction to the target. The results showed that the APA was detected in every trial. The duration and amplitude of APA was invaried to the target distance in all weight-shifting directions except the forward (90 degree) direction. This study supports the feasibility of evaluating APA during targeted weight shifting tasks. The underlying mechanism of APA control for forward weight shifting might be different from other directions. Future studies with patient groups are suggested.

Disclosures:  G. Li: None. Y. Chang: None. J. Liaw: None. J. Yu: None. M. Hsu: None.

Poster

316. Posture and Gait I

Location: Hall A

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Program #/Poster #: 316.19/Q9

Topic: E.06. Posture and Gait

Support: Gatsby Charitable Foundation
          Wellcome Trust

Title: Characterisation and circuit analysis of anticipatory postural adjustments in mice

Authors: *E. X. MORINA, A. J. MURRAY;
          Sainsbury Wellcome Ctr., Univ. Col. London, London, United Kingdom

Abstract: Whether it be lifting your leg to take a step or reaching to pick up an object, voluntary motor actions have the potential to disrupt postural equilibrium, destabilise the body and result in a fall. To prevent this destabilisation, purposeful movements are preceded by anticipatory postural adjustments (APAs), a subtle shift in the centre of mass (CoM) which prepares the body for the upcoming disturbance. A simple example of an APA is the movement of the CoM away from the initial swing leg during the initiation of locomotion. However, despite their key role in the initiation of movement, little is known about the neural circuits that generate and control APAs.

To allow us to probe the neural circuits involved in the generation of APAs we have developed a mouse behavioural assay to assess the APA associated with the locomotor initiation. The apparatus consists of linear track with four force sensors, used to measure the ground-reactive forces on each limb, embedded in the floor of a start chamber. Mice are trained to stand on the force sensors and perform a nose poke in order to open a door to receive a reward at the end of a linear corridor. This behavioural apparatus combined with high speed video allowed us to characterise in detail the APA associated with mouse gait initiation.
Previous studies have indicated that reticulospinal neurons in the pontine reticular nucleus are active during the generation of APAs. To probe whether pontine reticulospinal neurons are required for APA generation we have used archaerhodopsin to inhibit their firing at different phases of APA generation. Furthermore, we have carried out monosynaptic rabies tracing selectively from reticulospinal neurons and identified over 70 areas that provide input to these neurons. Overall these studies will give us a clearer understanding of the neural circuits that generate APAs.

Disclosures: E.X. Morina: None. A.J. Murray: None.

Poster

316. Posture and Gait I

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Program #/Poster #: 316.20/Q10

Topic: E.06. Posture and Gait

Support: NIH Grant R01HD083314

Title: Effects of anodal transcranial spinal cord direct current stimulation on locomotor adaptation to lateral pelvis perturbation in people with spinal cord injury

Authors: *J.-T. LIN¹, C.-J. HSU², W. DEE², M. WU³;
¹Shirley Ryan Abilitylab, Chicago, IL; ²Shirley Ryan AbilityLab, Chicago, IL; ³Dept Physical Med. & Rehabil, Northwestern Univ. - Chicago, Chicago, IL

Abstract: Dynamic balance during walking is usually impaired in people with spinal cord injury (SCI). However, effective training in improving dynamic balance is still lacking. The less efficacy of neuroplastic changes following training may be due to the reduced excitability of spinal circuitries, where most people with SCI were affected because of the reduced descending motor signals. Anodal transcranial spinal cord direct current stimulation (tsDCS) has been used to modulate the excitability of motoneurone synapse, which is beneficial to forming a motor memory. The purpose of this study is to evaluate the effects of anodal tsDCS on the motor learning of dynamic balance during walking in people with SCI. We hypothesized that anodal tsDCS could increase the motor learning of dynamic balance because improved excitability of spinal circuitries. Five subjects with SCI participated in this 2-day study. Each day of testing consisted 1-minute baseline, 10-minutes with either sham or anodal tsDCS paired with pelvis perturbation, and 1-minute with no perturbation. The anodal electrode was placed over the 7th thoracic vertebra, and the reference was placed over the shoulder blade. The intensity of current stimulation was set at 2mA. For sham tsDCS, the stimulation was turned off after 10s. A controlled pelvis perturbation was provided from heel strike to mid-stance phase of gait in the medial-lateral direction. The magnitude of force was randomly altered between 30% and 100%
of the determined force, which was set at 8-12% of body weight depending on subjects’
tolerance. The outcome measure for dynamic balance was the minimal margin of stability
(MoS). The learning outcome was assessed using the rate of adaptation within adaptation and the
amount of improvement after adaptation. Cohen’s d (d) was used to indicate the standardized
difference between conditions. Subjects tended to adapt faster to the pelvis perturbation with
tsDCS than without tsDCS (d= .506). Subjects reached stable MoS after 17±7 steps with tsDCS
vs. 35±17 steps without tsDCS. Compared to baseline, subjects improved their dynamic balance,
which is shown on the reduced MoS after locomotor adaptation to the pelvis perturbation either
with or without tsDCS (d= 1.039 and 0.788, respectively). However, the amount of improvement
was not different between conditions (d=.144). Our results suggested that tsDCS may improve
the rate of motor learning in controlling dynamic balance in people with SCI, although the
amount of improvement was determined by pelvis perturbation. Results from this study may be
used to develop a balance training paradigm paired with tsDCS to accelerate the learning of
dynamic balance in people with SCI.


Poster

316. Posture and Gait I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 316.21/Q11

Topic: E.06. Posture and Gait

Title: Segmental coordination changes during walking with and without haptic forces

Authors: *G. U. SORRENTO, P. S. ARCHAMBAULT, J. FUNG;
Sch. of Phys & Occ Therapy, McGill Univ., Montreal, QC, Canada

Abstract: Background: We have simulated walking with a dog leash by combining robot-
controlled haptic tensile forces with a self-paced treadmill synchronized to a virtual environment.
We have reported gait adaptation and post-adaptation effects, respectively during and after haptic
force exposures, in healthy young, older, and chronic stroke individuals. The adaptations were,
however, mainly in postural and gait spatiotemporal outcomes, most notably gait speed. This
study focuses on the intersegmental coordination of the thigh, leg and foot segments during and
after the tensile force exposure. Both planar and phase diagram methods are proposed for a 3D
comparison of coordination. Methods: One chronic stroke participant (73 y.o., 8 months post-
stroke) and one age-matched control participant (71 y.o.) walked on a self-paced treadmill in a
virtual environment holding a robot-controlled haptic leash. Both participants walked an initial
30s baseline pre-force epoch with a slack leash. This was instantly followed by a 60s force epoch
where the robotic arm applied and maintained a 15N tensile force to the hand via a leash handle.
The force was then released, and the participants continued to walk an additional 60s in the post-
force epoch. Results: Both the post-stroke and control participant displayed changes in bilateral lower limb intersegmental coordination as gait speed increased during force and post-force epochs. For example, both subjects increased dorsiflexion in the non-dominant leg during and after the 15N force exposure. Specifically, the post-stroke individual increased dorsiflexion in the paretic leg by ~7 degrees in both epochs. Changes in limb segment coordination also corresponded to bilateral increases in 3D intersegmental trajectory areas and slight increases in angular velocity, just prior to, and during the swing phase of the paretic leg during force and post-force epochs. Conclusion: The use of haptic tensile forces elicited proportional increases in both the kinematic and dynamic profiles when haptic forces were induced and released. Further investigation on a wider range of chronic stroke functional levels will ascertain if tensile forces improve both segmental coordination and gait symmetry.


Poster

316. Posture and Gait I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 316.22/Q12

Topic: E.06. Posture and Gait

Support: NIA Grant AG048262
        AHA postdoctoral fellowship 19POST34390411

Title: Knee extensor fatigability during high- and low-load resistance exercise protocols in young and old adults

Authors: *J. D. DELGADILLO, C. W. SUNDBERG, M. KWON, S. K. HUNTER;
        Physical Therapy, Marquette Univ., Milwaukee, WI

Abstract: Resistance exercise training is a cornerstone in the prevention of the age-related decline in muscle mass and strength, and fatigability of limb muscle may be an important parameter for this adaptive response. It is unknown, however, whether fatigability and the underlying mechanisms differ between different types of resistance exercise protocols in young and old men and women. The purpose was to determine the fatigability of the knee extensors and identify the involved mechanisms in 20 young (19-24, 22.2 ± 1.3 yr, 10 men) and 20 old adults (64-85, 73.8 ± 5.4 yr, 10 men) elicited by a high- and low-load resistance exercise. Participants performed 4 sets of 8 repetitions with 3 min rest between sets for each exercise protocol. One leg performed a high-load protocol (HLP, 80% 1 Repetition Max, 1RM) and the contralateral leg a low-load protocol (LLP, 30% 1RM). Participants were instructed to kick as fast as possible for the HLP and perform a slow 12 s contraction (6-s shortening, 6-s lengthening) for the LLP. Voluntary activation and contractile properties were quantified with electrical stimulation before
and immediately following each set of exercise. The LLP induced greater reductions in the maximal voluntary isometric force (MVC) after the fourth set compared with the HLP (21% vs. 12%, P=0.003) with no age or sex differences in either protocol. There was a trend towards a greater reduction in the involuntary twitch amplitude in the LLP than the HLP (13% vs. 8% reduction, P=0.057), which was correlated with the reduction in MVC (r=0.500, P < 0.001). In contrast, the change in voluntary activation did not differ between protocols (5% vs. 2% reduction, P=0.329). The low-load with slow contractions induced greater knee extensor fatigability than a high-load with fast contractions due to greater disruptions in contractile function occurring within the muscle.

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Poster

316. Posture and Gait I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 316.23/Q13

Topic: E.06. Posture and Gait

Support: U.S. Army’s Telemedicine and Advanced Technology Research Center Grant 81XWH-09-2-0020
the Department of Defense Joint Warfighter Project Grant W81XWH-14-C-0105

Title: Employing impedance to simplify the neural control of movement

Authors: *D. LUDVIG1,2, M. W. WHITMORE1, E. J. PERREAULT1,2;
1Northwestern Univ., Evanston, IL; 2Shirley Ryan AbilityLab, Chicago, IL

Abstract: Completing motor tasks that require contact is dependent on an ability to regulate the relationship between limb motions and interaction forces with the environment. This can be achieved by exploiting the mechanical properties of a limb or through active regulation of joint torques through changes in muscle activation. Since the mechanical properties of a joint are not infinitely malleable, reliance on joint mechanics is only feasible for certain tasks. For example, healthy joints are stable, implying a positive joint stiffness that generates torques opposing externally imposed displacements. Hence, relying on joint stiffness would not be a feasible strategy for tasks that require torque to decrease with increasing angular excursions. In contrast, leveraging the mechanical properties of a joint might simplify neural control when they are matched to the functional requirements of a task. The purpose of this study was to determine if humans change their control strategy, relying on limb mechanics rather than regulated muscle
activation, when feasible. This was accomplished by measuring ankle impedance and muscle activation strategies in three tasks requiring joint torques to: oppose movements, assist movement, or remain constant during movement. Experiments were conducted on 10 subjects. The right foot of each was attached to a rotary motor that controlled motion and measured torques in the sagittal plane. Subjects were instructed to match a target torque aided by visual feedback, while an imposed sinusoidal movement (0.5 Hz) was applied. Three torque targets were used: torque opposing motion (TO; mechanically feasible), constant torque (TC), and torque assisting motion (TA; mechanically infeasible). The motor applied small, pseudo-random angular perturbations to estimate impedance during the tracking tasks. EMGs were measured from the tibialis anterior and triceps surae to assess neural control strategies across the tasks. We found that subjects leveraged ankle impedance to control torque during the TO task. The measured impedance accounted for 88 ± 5% of the measured torque variance during the TO task, but ~ 0% of the torque variance for the TC and TA tasks even though the accuracy with which the torque was tracked was similar across conditions. The reliance on ankle impedance simplified the neural control. There was significantly less muscle activation modulation in the TO task than in the TC (p = 0.006) or the TA (p < 0.001) tasks. These results demonstrate how leveraging the mechanical properties of a joint can greatly simplify the neural control needed to complete a task. The extent to which natural behaviors exploit mechanics remains to be determined.


Poster
316. Posture and Gait I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 316.24/Q14

Topic: E.06. Posture and Gait

Support: NIH T32 4T32HD007418-25
VA Merit 1 I01 RX001979-01

Title: Inverted pendulum-like stance behavior during lateral walking maneuvers in persons with and without incomplete spinal cord injury

Authors: *W. L. OCHS1, J. L. WOODWARD2, K. E. GORDON3;
1Northwestern Univ., Chicago, IL; 2Shirley Ryan Ability Lab., Chicago, IL; 3Northwestern Univ.
- Chicago, Chicago, IL

Abstract: Inverted pendulum models have been widely applied to describe the stance phase of human walking. This simple model captures a substantial portion of the body’s center of mass (CM) dynamics and has provided insight on how the nervous system controls straight-ahead
walking. It is unknown how well an inverted pendulum model captures the challenging task of laterally maneuvering, where physical task requirements change from step-to-step. Pendulum model parameters, such as step width and time, can be manipulated to produce near pendulum-like behavior sufficient for maneuvering and favorable for control simplicity, but humans have the capacity to use alternative joint torque coordination patterns in ways that may yield maneuverability, stability, or energetic advantages. We hypothesized that during lateral maneuvers, non-impaired individuals would use more distinct frontal-plane behaviors step-to-step during maneuvers that deviate from pendulum-like behavior compared to ambulatory individuals with an incomplete spinal cord injury (iSCI). We assessed frontal plane stepping behavior during lateral walking maneuvers performed at preferred speed on an extra-wide treadmill and during overground walking across force plates. Degree of pendulum-like behavior was determined by comparison of CM acceleration predicted from a pendulum model to the measured CM acceleration during single-limb stance. Motion capture markers on the feet and pelvis were used to characterize stepping, estimate CM location, and in the case of treadmill walking, estimate center of pressure (CP) location. Treadmill results showed individuals with iSCI tended to use less step-to-step variation in foot placement within a maneuver compared to non-impaired participants. This trend had a positive correlation with decreased performance in clinical metrics of impairment. This behavior supports our hypothesis of using more consistent, simplified control strategy with impairment. A subgroup of individuals with iSCI showed larger lateral CM accelerations on all steps compared to non-impaired individual, which may reflect a deficit in control. The pendulum-like nature of this behavior will be reported once the treadmill model using a fixed CP is validated using overground data. The degree to which humans behave like an inverted-pendulum and modify parameters within that model during maneuvers will provide insight on control priorities and how they may change with impairment. This information aids in our understanding of how iSCI impacts neuromechanical control of an important walking task.


Poster

316. Posture and Gait I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 316.25/Q15

Topic: E.06. Posture and Gait

Support: CONACYT Grant 243247
CONACYT Grant 243333
VIEP-BUAP to Cuerpo Académico en Neuroendocrinología BUAP-CA-288.

Title: Effects of pramipexole on the organization of gait in the myelin mutant: The taiep rat
Abstract: The demyelinating diseases of the central nervous system (CNS) has a high prevalence worldwide, and one of the most important signs of disability caused by these diseases is walking disability which affects the coordination and the stepping sequence between anterior and posterior extremities. The taiep rat is a myelin mutant whose name is the acronym of the motor signs that characterize it: tremor, ataxia, immobility, epilepsy, and paralysis. Pathologically is an homologue model for demyelinating diseases. Importantly, CNS taiep increase proinflamatory mechanisms with age. The objective of this study was to evaluate the effects of systemic administration of a D2-like dopaminergic pramipexole on walking pattern. We used six three month old male taiep rats. All subject were recorded under control (NaCl 0.9%) and after intraperitoneal injection of 0.5 and 1 mg/Kg of pramipexole. Walking was analyzed using CatWalk™ system (Noldus, The Netherlands), measuring support base, speed, stride length, duration of support, swing phase duration, footprint width and step sequences. Pramipexole 0.5 mg/Kg administration decreased the duration of the swing phase (P<0.05), increased the cadence (P<0.05), and the footprint width (P <0.01). With pramipexole 1 mg/Kg increase only the step sequence (P <0.05) with respect to the control group. In conclusion, pramipexole improved the walking coordination and gait cycle. There results showed that D2-like receptors are capable to modulate the walking and ataxia in this myelin mutant of TUBB4 mutations in humans.

Disclosures: J.C. Ahumada-Juárez: None. J. Eguibar: None. C. Cortes: None.

Poster

317. Afferent Control of Posture and Gait

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 317.01/Q16

Topic: E.06. Posture and Gait

Title: Effects of Achilles tendon vibration and foot noise on postural control

Authors: *C. S. LAYNE¹, B. C. LEE¹, P. LEUNG², D. R. TEMPLE¹;
¹Ctr. for Neuromotor and Biomechanics Res., ²Grad. Col. of Social Work, Univ. of Houston, Houston, TX

Abstract: Stochastic resonance (SR) is a phenomenon where response of nonlinear systems to weak input signals, such as in sensory neurons, may be enhanced with noise added to the system. Small levels of noise can increase signal detection; however, too much noise degrades weak signals. Studies note improved balance when adding slight mechanical foot noise (FN) to the
plantar surface, presumably by increased sensory feedback through SR. The SR induced improvements in sensory feedback may lead to up-weighting of plantar mechanoreceptor afferents, resulting in improved balance. We aimed to determine if FN could modulate the postural effects of Achilles vibration (AV). Twenty-four young healthy adults stood with their eyes closed, under 30 second conditions of AV (on or off) and FN (on or off). Anterior-posterior (AP) center of pressure (CoP) was used to compute mean position, speed, and approximate entropy (ApEn). To assess body segment coordination, Anchoring Index (AI) was computed of three AP body segment angles (head, torso, and thigh) in relation to their inferior segment angles. Repeated-measures ANOVAs were used to analyze effects of AV, FN, and their interactions. An alpha level of 0.05 was adopted, and Bonferroni adjustments were made to correct for multiple comparisons. A significant interaction effect of FN and AV for CoP speed indicated FN modulated AV by producing significantly faster CoP speeds. CoP ApEn was significantly reduced by AV, regardless of FN condition, suggesting AV produces a more regular CoP motion. ApEn was also significantly increased by FN when no AV was present, suggesting it can produce more regular CoP motion in the absence of AV. The thigh AI was significantly increased by AV, regardless of FN condition, indicating AV modifies the coordination between the thigh and shank. Overall the findings signify that FN is capable of modulating the typical postural response produced by AV by increasing CoP speed, while AV reduces thigh stabilization on the shank. It is unclear if these findings are the result of SR leading to increased upweighting of foot plantar mechanoreceptors, as FN would have then likely led to decreased CoP speed. The study does however reveal novel findings of how AV and FN stimuli can interact to influence postural control.


Poster

317. Afferent Control of Posture and Gait

Location:  Hall A

Time:  Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #:  317.02/Q17

Topic:  E.06. Posture and Gait

Title:  Effects of Achilles tendon vibration, fingertip light touch, and fingertip noise on postural control

Authors:  *D. R. Temple*, B.-C. Lee, P. Leung, C. S. Layne;  
1Hlth. and Human Performance, 2Grad. Col. of Social Work, Univ. of Houston, Houston, TX

Abstract:  Several studies note improved balance when sensory feedback is increased by fingertip light touch (FLT), despite not providing mechanical support. During FLT with slight mechanical noise added to the touch surface (FLT+N), postural stability is increased beyond effects of FLT alone. This presumably occurs if the noise elicits stochastic resonance (SR), a
A phenomenon where response of nonlinear systems to weak input signals may be enhanced by adding noise. FLT also mitigates the destabilizing effect of Achilles vibration (AV) on postural control, but this effect has not been tested under FLT+N conditions. We explored whether FLT+N modulates the postural effects of AV, beyond that of FLT alone. Young healthy adults (n = 22) stood with their eyes closed, under 30 second conditions of AV (on or off) and touch (no FLT, FLT, or FLT+N). Anterior-posterior (AP) center of pressure (CoP) was used to compute mean position, speed, and approximate entropy (ApEn). To assess body segment coordination, Anchoring Index (AI) was computed of three AP body segment angles (head, torso, and thigh) in relation to their inferior segment angles. Repeated-measures ANOVAs were used to analyze effects of AV, touch, and their interactions. An alpha level of 0.05 was adopted, and Bonferroni adjustments were made to correct for multiple comparisons. FLT and FLT+N mitigated typical postural responses of AV with CoP mean position significantly less posterior and reduced CoP speed. FLT and FLT+N significantly increased CoP ApEn, regardless of AV condition (on or off), indicating FLT reduces the regularity of CoP motion. AV itself significantly reduced CoP ApEn under all touch conditions, indicating it increases CoP motion regularity. Torso AI was significantly increased by FLT+N under both AV conditions (on and off). FLT significantly increased torso AI when AV was off, but it did not when AV was on. This suggests FLT+N further modifies coordination between the torso and thigh beyond FLT alone, when AV is present. AV also significantly increased Torso AI during the no FLT and FLT+N conditions. The findings suggest that the stabilizing effect of FLT during AV is generally enhanced by FLT+N, which may occur as a result of SR further increasing sensory feedback beyond FLT alone.


Poster

317. Afferent Control of Posture and Gait

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 317.03/Q18

Topic: E.06. Posture and Gait

Title: Effects of ankle tendon vibration on lean after-effect

Authors: *D. R. YOUNG, C. S. LAYNE;
Hlth. and Human Performance, Univ. of Houston, Houston, TX

Abstract: Previous research has identified deficits in postural adaptation due to inadequate sensory integration to be among the chief reasons for falls. Postural adaptation studies typically involve a change in environmental context to bias or decrease the reliability of the vestibular, visual, and/or proprioceptive systems. Lean after-effect (LAE) studies utilize an incline-intervention (Inc_I), a bout of quiet stance on an inclined surface for several minutes before returning to a flat surface post-test. Most subjects display a forward lean during the post-test
which may persist up to several minutes, known as LAE. LAE studies have been used as an effective proprioception-based illusion which leads to global alterations of the body schema and postural adaptation without a coinciding decrease in proprioceptive reliability. Previous LAE experiments have shown that stable, trait weighting of the vestibular and proprioceptive system effects the magnitude and duration of forward lean. This investigation sought to identify the effects of a concurrent proprioceptive illusion, tendon vibration of the Achilles (AT) or tibialis anterior (TA) tendon, on LAE. By adding tendon vibration to an Inc_I or post-test, we were able to identify if changes in state/dynamic sensory weighting affected LAE, and if those changes were directionally specific. Subjects were tasked with performing five bouts of an Inc_I, one baseline condition with no vibration, and bouts with tendon vibration of the AT or TA during the Inc_I or post-test. We compared LAE outcomes between these conditions using integrated area under the curve during the 5-minute post-test. AT vibration during the post-test led to a decrease in LAE. However, this posterior shift was not as great as when subjects were exposed to AT vibration alone, suggesting the Inc_I mediated the effect of tendon vibration. Conversely, TA vibration during the post-test led to an increased LAE compared to the baseline and compared to TA vibration alone, again suggesting the Inc_I served to mediate the effects of tendon vibration. While vibration during the post-test had direction specific effects, when presented with either TA or AT vibration during an Inc_I, subjects had an increase in LAE compared to the baseline. These results show that shank tendon vibration influences the development of LAE if presented concurrently with an Inc_I and moderates the expression of LAE if presented afterwards. This suggests a role of dynamic sensory reweighting as well as an effect of local proprioceptive inputs on the development and expression of LAE.

Disclosures: D.R. Young: None. C.S. Layne: None.

Poster

317. Afferent Control of Posture and Gait

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 317.04/R1

Topic: E.06. Posture and Gait

Support: HMRF Grant 14150211

Title: Effects of levels of illumination and visual tasks on postural stability in older adults

Authors: *A. CHEONG¹, H.-Y. LAM¹, V. SUEN¹, C. TSANG¹, G. WOO¹, J. CHO⁴, J. KWAN⁵, L. A. ABEL⁶, P. LEE², W. TSANG³;
¹Sch. of Optometry, ²Sch. of Nursing, ³Dept. of Rehabil. Sci., The Hong Kong Polytechnic Univ., Hong Kong, China; ⁴Hong Kong Society for the Blind, Hong Kong, China; ⁵UDA Consultants Ltd., Hong Kong, China; ⁶Dept. of Optometry & Vision Sci., Univ. of Melbourne, Melbourne, Australia
Abstract: Background: Glare induced by excessive illumination affects our visual integration. Such interrupted visual input may affect balance control, especially in people with compromised balance function. Our study was aimed to investigate the impact of illumination and visual search on postural stability in community-dwelling older adults.

Method: 13 older adults (mean age: 61.8±1.4) without known pathology and normal vision were recruited. Static balance on a stationary surface was measured by a computerized dynamic posturography (SMART EquiTest). Subjects were asked to undergo either 1) a pure fixation task for 18 seconds, or 2) a combined task with visual search in the first 6 seconds followed by fixation for 12 seconds. To simulate visual search in daily life, subjects had to identify the target presenting on one of the 6 monitors which were located at 3 metres away in an arc shape, covering a visual field of 120 degree. Balance measures were conducted under 3 different illumination levels (100, 520 and 2100 lux). Postural sway in terms of sway area (mm2), maximum sway (mm) in anterior-posterior (A-P) and medio-lateral (M-L) directions were analyzed across 3 time-points (6 seconds each) to study the effect of illumination and visual tasks.

Results: Results from three-way repeated measures ANOVA showed that postural sway significantly reduced across time (p<0.001), with significantly larger sway area and displacement in the first time-point compared with others. Postural sway in combined task was significantly greater than pure fixation task (p<0.001). Significant interaction effect was found between time and task (p<0.001). In the combined task, visual search in the first 6 seconds impacted the postural sway, where subjects presented larger sway area, maximum A-P and M-L sway (p<0.001). However, when the task changed from searching to fixation, the postural sway substantially but gradually reduced (p<0.01), compared with pure-fixation task. Postural sway was similar among the 3 different levels of illumination (p>0.10), with no interaction effect of illumination and time or illumination and task (p>0.05).

Conclusion: Opposing to our hypothesis, our study did not find illumination imposing significant impact on postural stability among healthy older adults. Instead, visual search significantly altered their postural stability, which might be explained by the shift of attention contributing to postural stability to search task. Once the postural stability was interrupted, time was required to reacquire postural stability. Further study on older adults with comprised balance control will be conducted.


Poster

317. Afferent Control of Posture and Gait

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 317.05/R2

Topic: E.06. Posture and Gait
Support: University of Miami Provost's Research Award

Title: Electrocortical activation in patients with an acute lateral ankle sprain during balance task

Authors: *J. KIM¹, Y. AN³, B. ARWARI¹, N. PATEL², K.-M. KIM¹;
¹Kinesiology and Sport Sci., ²Psychology, Univ. of Miami, Coral Gables, FL; ³Kinesiology and Dance, New Mexico State Univ., Las Cruces, NM

Abstract: Lateral ankle sprains have been reported as the most common injury in physically active populations. Poor postural control following the lateral ankle sprain is a key factor for chronic ankle instability, which is characterized by the feeling of the ankle giving way along with residual signs and symptoms for months to years. Up to 74% of acute lateral ankle sprain (ALAS) patients suffer chronic ankle instability with persistent postural control deficits. Recently, neuroimaging studies have shown that ligamentous injuries cause neural adaptation in the central nervous system (CNS), and such altered cortical activation may be underlying mechanisms for chronic ankle instability. However, to date, there is a limited study that identifies the centrally mediated neural adaptation in ALAS patients during a postural control task. Thus, the purpose of this case-control study was to identify the cortical activation pattern in ALAS patients compared with healthy controls during the bipedal balance task. A total of 10 subjects participated (5 ALAS patients, 5 healthy controls). Electrocortical activations over the frontal (Fz), parietal (Pz) and occipital (Oz) cortices were recorded using a 64-channel Electroencephalograph (EEG) during a total of 12 trials of 10 second-bipedal balance task with eyes open. Theta (4-8 Hz) and alpha-2 (10-12 Hz) frequency bands power and total center of pressure path length (tCOPlength) were calculated for electrocortical activation and balance, respectively. We found that there was no significant difference in tCOPlength between groups during the bipedal-balance (p=.099, ALAS: 13.73 ± 3.66, control: 10.23 ± .58cm). However, the ALAS group showed greater parietal theta (Pz, p=0.003, ALAS: 0.28±0.03, control: 0.20±0.03) and less frontal alpha-2 (Fz, p=0.038, ALAS: 0.15±0.06, control: 0.24±0.05) compared to the control group. Theta frequency in the parietal cortex plays a key role in situational awareness to external stimuli, while less frontal alpha-2 power indicates more excitatory responses in the frontal cortex, which is the area for the cognitive decision-making process for motor tasks. These preliminary results suggest that ALAS patients were able to maintain postural stability similar to the healthy individual but different electrocortical activation patterns, indicating that ALAS patients demand more neural recruitments than the healthy individuals to process and integrate internalized attention and proprioceptive deafferented input.

Title: Development of an optogenetic method to stimulate gamma motor neurons in vitro

Abstract: The muscle spindle is a sensory organ located in skeletal muscle that is critical for motor control and proprioception, or the sense of body position in space. It is innervated by the Group Ia and II muscle spindle afferents, which respond to changes in the length of the muscle. The gamma motor neurons control the length of the intrafusal fibres, and therefore the sensitivity of the muscle spindle afferents. However, it has been challenging to study the gamma motor neurons since it is hard to specifically stimulate the gamma but not the alpha motor neurons that control the force generating extrafusal fibers. A previous study showed that alpha motor neurons were recruited from small diameter to large diameter with increasing optical intensities in mice expressing the blue light gated Channel rhodopsin 2 (ChR2) in motor neurons (Llewellyn, et al., 2010). This is the reverse recruitment pattern of electrical stimulation. We hypothesized that gamma motor neurons, which are smaller than even the smallest alpha motor neurons, will be recruited first using low optical stimuli and that this will allow us to characterize their effects on muscle spindle afferent sensitivity. The extensor digitorum longus muscle and peroneal branch of the sciatic nerve from adult mice expressing ChR2 in choline acetyltransferase (ChAT) positive motor neurons were dissected and placed in an in vitro tissue bath with oxygenated synthetic interstitial fluid. The sciatic nerve was attached to an extracellular suction electrode that recorded muscle spindle afferent activity. We used a light guide to deliver blue LED light (470nm; 0.5mW-5 mW ) to the end of the nerve. We found that, as expected, the lowest optical intensities recruited the more slowly conducting gamma motor neurons. Higher optical intensities recruited the more slowly conducting gamma motor neurons, as evidenced by the presence of twitch contraction. At the lowest optical intensities we observed an increase in muscle spindle afferent firing rate in the absence of twitch contraction, confirming that gamma motor neurons alone had been recruited. Further, increasing the gamma motor neuron firing frequency led to a greater increase in muscle spindle afferent firing rate. We are currently using this technique to characterize gamma motor neuron control of muscle spindle afferent tone. In summary, we have
developed an optogenetic technique to recruit gamma motor neurons in vitro that can be used to study fusimotor control of the muscle spindle. Future studies will develop this approach for use in vivo to better study gamma motor neurons and their contribution to motor control under normal and disease conditions.


Poster

317. Afferent Control of Posture and Gait

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 317.07/R4

Topic: E.06. Posture and Gait

Support: University of New Mexico RAC Grant

Title: Frequency analysis of the static postural stability after a self-mobilization exercise program

Authors: *D. SHIBATA;
Dept. of Health, Exercise and Sports Sciences, Univ. of New Mexico, Albuquerque, NM

Abstract: Central processing of multi-sensory feedback and motor commands responsible for force production is critical for postural control. A self-mobilization exercise program was developed to realign spinal curvature, and immediate and sustained effects on balance control were documented. However, it is unknown to what extent the exercise program influences central processing for postural control. This study addressed this question through the use of spectral analysis of center of pressure (CoP) displacements in standing before and after the intervention of exercise program. Sixteen subjects were randomly assigned into Exercise (n = 8) and Control group (n = 8). The Exercise group performed the exercise program while lying supine on a cylinder-shaped tube (98-cm length, 15-cm diameter), consisting of three preparatory positions and seven small motions. The duration was approximately 15 min. The Control group rested on a flat surface for 15 min. Center of pressure (CoP) was measured while subjects were asked to stand quietly on a pressure mat (Tekscan, Inc. USA) for 3 trials of 10 sec each with eyes open and closed (EO and EC, respectively). CoP displacements in anteroposterior (AP) and mediolateral (ML) directions were used to calculate power spectrum by fast Fourier transformation. The power spectrum was analyzed in three frequency bands: low (LF: 0-0.3 Hz), medium (MF: 0.3-1 Hz), and high (HF: 1-3 Hz) reflecting contributions from the visual, vestibular, and proprioceptive systems, respectively. The total power spectrum of each band was normalized by the sum of the three bands and is expressed as percentage in this study. Repeated measures ANOVAs were used to examine changes in percent power spectrum for each of three...
frequency bands in the AP and ML directions for the EO and EC conditions (Pre vs. Post) and
group differences (Exercise vs. Control). There was no significant change in percent power
spectrum of the three frequency bands in the ML direction for the EO and EC conditions. In the
AP direction, percent power spectrum of LF band significantly increased while that of MF band
decreased for the EO and EC conditions in the Exercise group ($p < 0.05$), but not in the Control
group. There was no change in percent power spectrum of HF band in the AP direction for the
both groups. These findings may indicate that the contributions of visual and vestibular systems
for postural control became greater and lesser, respectively, while a proprioceptive contribution
remained the same after performing the exercise program. In sum, the exercise program altered
central processing of sensory inputs for postural control in the AP direction regardless of visual
feedback.

Disclosures: D. Shibata: None.

Poster

317. Afferent Control of Posture and Gait

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 317.08/R5

Topic: E.06. Posture and Gait

Title: A new system to objectively measure ankle proprioception

Authors: *J. HOLST-WOLF, J. PIEKARSKI, J. KONCZAK;
Human Sensorimotor Control Lab., Univ. of Minnesota, Minneapolis, MN

Abstract: Proprioceptive afferents of the ankle are essential feedback signals for the
maintenance of balance and posture. While the importance of ankle proprioception for balance
control has been recognized, there is no widely accepted test or measurement system available.
We here present newly developed hardware and a psychometric testing protocol suitable to
measure ankle proprioceptive acuity. The aims of this study were: 1) to establish system
reliability and validity, 2) to obtain normative values of ankle proprioceptive acuity in healthy
adults, and 3) to determine, if ankle proprioceptive acuity correlates with kinetic measures of
balance. METHODS: While sitting, participants placed their foot on a footrest of the device,
which could tilt in the sagittal plane allowing for foot dorsiflexion/plantarflexion. An examiner
rotated the footrest to two different ankle positions (standard and comparison). Then participants
judged which position was furthest from the neutral starting position. Using a Bayesian inference
based adaptive algorithm, 30 pairs of ankle positions were presented and the just-noticeable-
difference (JND) threshold was determined as a measure of ankle proprioceptive acuity. A subset
of healthy adult participants completed the assessment three times on different days to establish
the system reliability. Using a force platform, participants underwent a balance assessment,
which consisted of a series of 30-second double- and single-leg stance in an eyes open and eyes
closed sensory condition. The center-of-pressure sway path length and sway area were obtained as balance measures. **RESULTS:** We show exemplar and group data of ankle JND thresholds for both the dominant and non-dominant leg and present the relationship between ankle JND threshold and the balance measures. **DISCUSSION:** Here we introduce a novel, objective measure of proprioceptive acuity for the ankle and document the instrument reliability. This assessment has potential to become a simple tool for clinicians to identify proprioceptive impairment at the ankle and measure the efficacy of sensorimotor interventions for balance.

**Disclosures:** J. Holst-Wolf: None. J. Konczak: None. J. Piekarski: None.

**Poster**

**317. Afferent Control of Posture and Gait**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 317.09/R6

**Topic:** E.06. Posture and Gait

**Support:** Dutch Technology Foundation TTW, part of the Netherlands Organization for Scientific Research (NWO): STW grant 14094

**Title:** What triggers modulation of proprioceptive reflexes: Environment dynamics or perturbation properties?

**Authors:** *W. MUGGE*¹, A. DEL VALLE HIDALGO¹, P. A. FORBES²;
¹Delft Univ. of Technol., Delft, Netherlands; ²Dept. of Neurosci., Erasmus Med. Ctr., Rotterdam, Netherlands

**Abstract:** Effective upper limb control in the face of unpredictable disturbances relies on our ability to vary limb resistance, which can be achieved by regulating both intrinsic muscle visco-elasticity (i.e. cocontraction) and reflexive feedback from sensory organs (i.e. proprioceptors). There is considerable controversy surrounding the adaptability of reflexive feedback mechanisms in upper limb motor control. Notably, one view is that reflex modulation is governed by stability constraints such that reflexive feedback is tempered to prevent oscillations under conditions with reduced stability margins (De Vlugt et al. 2002). This view requires that humans assess the stability margins when interacting with environments and any accompanying perturbations. On the other hand, since the influence of the perturbations themselves depend on the properties of the environment, it may be possible that we sense changes in the characteristics of the perturbation and modulate reflexive feedback accordingly.

This study aims to identify whether the trigger for reflex modulation is driven to a greater extent by environmental dynamics (i.e. damping and stability margin) or the resultant perturbation properties. Subjects were instructed to minimize arm displacements caused by continuous force perturbations applied to the hand. Joint admittance (i.e. arm displacement due to the imposed
force) and combined admittance (i.e., human and environment) were estimated as a function of frequency to quantify dynamics of the resultant motor control behaviour. Admittance is known to modulate with environmental damping, but in conventional damping conditions both the stability margin of the combined system and the perturbation properties change. Therefore, we applied sham conditions where the perturbations were prefiltered to mimic or compensate for the filtering effect of the added environmental damping. As a result, the velocity profiles that the subjects experienced were held constant despite the underlying changes in the stability margin across different damping conditions.

The results validated that the force and displacement properties of the perturbations in the sham conditions were indeed similar to the conventional damping conditions they mimicked. The change in admittance in the sham conditions was less than what was observed in the conventional damping conditions, suggesting that stability constraints are not solely responsible for the admittance modulations.

References


Poster

317. Afferent Control of Posture and Gait

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 317.10/R7

Topic: E.06. Posture and Gait

Support: DARPA N66001-15-C-4038

Title: Evaluating the role of electrically-evoked plantar sensation in an ambulatory searching task

Authors: *B. P. CHRISTIE*¹,², H. CHARKHKAR², C. E. SHELL³,², D. J. TYLER¹,², R. J. TRIOLO²,¹;
¹Biomed. Engin., Case Western Reserve Univ., Cleveland, OH; ²Louis Stokes Cleveland Dept. of Veterans Affairs Med. Ctr., Cleveland, OH; ³Biomed. Engin., Cleveland Clinic, Lerner Res. Inst., Cleveland, OH

Abstract: Plantar cutaneous inputs and vision contribute to correct foot positioning in response to obstacles and while walking over uneven terrain [Rossignol 2006]. When visual resources are not available, such as while walking in the dark, somatosensory cortex activation increases [Oliveira 2017]. Yet, it is still unclear how plantar cutaneous sensation aids in searching tasks when vision is obstructed. We hypothesized that plantar sensation is used to acquire action-
relevant information during locomotion, and that this information improves task performance and modulates foot placement strategy.

The horizontal ladder walking test has been used to evaluate cutaneous sensation in animal models [Bouyer & Rossignol 2003]. In the present study, two trans-tibial amputees (LL01 and LL02) performed a version of this test adapted for clinical trials and while blindfolded. Both participants had nerve cuff electrodes installed around their residual sciatic and tibial nerves. In half of the trials, electrical stimulation was delivered directly to the nerves to evoke somatosensory percepts referred to the missing feet. Closed-loop stimulation was triggered and modulated by readings from pressure insoles placed underneath the prostheses. Stimulation parameters were selected to elicit sensations referred to a region of the missing foot that matched the activated insole region: forefoot, midfoot, and/or rearfoot. We measured completion time, number of foot placement errors, and ground reaction forces. Errors included missing a rung, slipping off a rung, and placing the foot on two rungs at once.

Preliminary results indicate that task performance improves when sensation is restored, but foot placement strategy is not affected. During trials with sensation, task performance improved in different ways for the two participants: for LL01, completion time decreased (t-test, p=0.01). For LL02, the number of errors decreased (p<0.001). LL02 performed the task faster than LL01 but had a higher error rate (p<0.001), which could explain why restoring somatosensation affected each participant differently. Foot placement strategy, i.e. the region of the foot that stepped onto a ladder rung, did not change during trials with feedback.

In summary, our results suggest that restoring plantar cutaneous sensation in lower-limb amputees could improve their performance in searching tasks, but its mechanism may be subject-dependent. Overall, findings from this study demonstrate how sensory-enabled prostheses could aid lower-limb amputees in searching tasks when vision is compromised.


Poster

317. Afferent Control of Posture and Gait

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 317.11/R8

Topic: E.06. Posture and Gait

Support: NIH NINDS R01 NS095366
NIH NINDS R01 NS104194
NIH NINDS F30 NS110199
Drexel University Dean's Fellowship for Excellence in Collaborative or Themed Research
Title: Response patterns of mouse spinal central pattern generator neurons to stimulation of low-threshold hindlimb afferents


Abstract: The basic hindlimb locomotor pattern is generated in the thoracolumbar spinal cord by neural circuits known as central pattern generators (CPGs). The locomotor CPG integrates descending commands and ascending sensory information to activate, modulate and halt the rhythmic program. In spinal cord injury and stroke, descending control is impaired but afferent pathways to the CPG remain comparatively intact. Thus, understanding the specific structure of CPG afferent processing circuits may have therapeutic relevance for gait recovery in these disease states. Modeling experiments suggest a two-layer architecture in which rhythm-generating (RG) neurons produce the basic motor program and indirectly recruit motoneurons through pattern-forming (PF) neuron populations. Activation of ankle afferents has been shown to strongly modulate locomotor phase transitions, which is predicted to be mediated by synapses at the RG level with possible involvement of the PF layer. Several genetically-labeled neuronal populations have been shown to participate in CPG circuits based on locomotor alterations following ablation or inhibition and have hypothesized connectivity in this two-layer structure. The transcription factor Shox2 marks a heterogeneous population of CPG neurons that can be subdivided into neurons contributing to rhythm generation (Shox2\(^{RG}\)) and neurons thought to be pre-motor PF neurons (Shox2\(^{PF}\)) by presence/absence of the transcription factor Chx10. To directly test integration of afferent signaling by CPG neurons, we developed a lumbar-hemisected isolated spinal cord preparation with preserved peripheral nerves in neonatal Shox2::cre;Rosa26-lsl-tdTomato;Chx10GFP mice. Shox2\(^{RG}\) and Shox2\(^{PF}\) neuron responses to activation of functionally-specific hindlimb afferent pathways were measured using whole-cell patch clamp. Both Shox2\(^{RG}\) and Shox2\(^{PF}\) neurons displayed postsynaptic currents following afferent activation. Surprisingly, many Shox2 neurons displayed long-lasting inhibitory responses following stimulation of both flexor- and extensor-related ankle afferents. These results are consistent with data from neonatal mouse preparations showing that stimulation of either flexor- and extensor-related afferents during fictive locomotion resets the phase to flexion. Developmental changes in afferent effects on ongoing locomotion have been shown in the rat and investigation of Shox2 response patterns in older animals may reveal functional maturation of these pathways.

Disclosures: E.Z. Li: None. D.L. Garcia-Ramirez: None. L. Yao: None. K.J. Dougherty: None.
Plantar tactile augmentation improves lateral balance with and without cognitive load, but palmar tactile augmentation improves lateral balance only without cognitive load.

Authors: *J. S. AZBELL*, J. K. PARK, S.-H. CHANG, M. ENGELEN, H. PARK;  
1Electrical and Computer Engin., 2Hlth. and Kinesiology, Texas A&M Univ., College Station, TX; 3Dept. of Physical Med. and Rehabilitation, UTHealth, Neurorecovery Res. Ctr. At TIRR Mem. Her, Houston, TX

Abstract: Peripheral neuropathy (PN) is common among individuals with diabetes; about 50% of them develop the condition within 10-15 years. PN with reduced plantar cutaneous feedback increases the risk of slips, trips, and falls. To mitigate these risks, several sensory augmentation approaches have been proposed. However, the majority of these methods provide sensory cues via visual, audio, or vibrotactile feedback rather than addressing the original plantar sensory deficiency. The efficacy of such methods can be limited by the cognitive load involved in processing these sensory cues, because cognitive capability varies upon the situation and cognitive load increases response time and fatigue. The objective of this study is to test our hypothesis that tactile augmentation, by stimulating the sensory nerves on the foot sole, is more effective than indirect sensory cues in improving lateral balance for individuals with reduced plantar cutaneous feedback. In our study, four healthy human subjects repetitively stood on a lateral balance board and maintained balance for as long as possible. Balance time was defined as the total time the subject remained on the board without touching the ground on either side. Subjects were instructed to close their eyes during the experiments to increase dependency on plantar cutaneous feedback for balancing. A layer of foam was placed on top of the board to replicate the reduced plantar cutaneous feedback of PN. For tactile augmentation on the foot sole or palm, low-intensity electrical stimulation was transcutaneously applied on either the calcaneal or ulnar nerve, respectively. To test the effect of cognitive load (CL), subjects were asked to count backward from a given random number. Experiments were performed with five conditions: control with no augmentation, plantar augmentation with and without CL, and palmar augmentation with and without CL, in a random order to remove the motor learning effect. Experimental data shows that both plantar and palmar augmentation increase average balance time. However, with a CL, palmar augmentation has no effect on average balance time, while the increase in average balance time remains with plantar augmentation. This result suggests that plantar augmentation may be more effective than providing indirect sensory cues (i.e., palmar) to treat balance deficit associated with reduced plantar cutaneous feedback in persons with PN.

Poster

318. Neuromodulation of Motor Pattern Generation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 318.01/R10

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSFC 31671097
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Title: Molecular and circuit analysis of functions of a neuropeptide family, Aplysia CCK

Authors: *G. ZHANG¹, J. W. CHECCO², S.-Q. GUO¹, Y.-Y. XUE¹, E. V. ROMANOVA², D. H. MAST², S.-C. QIAN¹, W.-D. YUAN¹, K. YU¹, Z. YANG¹, F. S. VILIM³, E. C. CROPPER³, K. R. WEISS³, J. V. SWEEDLER², J. JING¹;
¹Nanjing Univ., Nanjing, China; ²Dept. of Chem., Univ. of Illinois at Chicago, Urbana, IL; ³Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: All behaviors are implemented by specific neural circuits, but the initiation and termination of a behavior, and the frequency and form of a behavior are often mediated by actions of neuromodulators. Neuropeptides represent one of most diverse class of neuromodulators, and many of them have been shown previously to have a profound impact on behaviors and the underlying neural circuits. Despite this progress, the functions of some peptide families remain to be elucidated. We sought to use an experimentally advantageous model system, the marine mollusc Aplysia to study a neuropeptide family, cholecystokinin (CCK), that appears to be present in both invertebrates and vertebrates. CCKs are well studied in mammals, and undergo a rare form of posttranslational modification, i.e., sulfation of the tyrosine residue. Sulfated and nonsulfated forms of CCKs make CCKs diverse, and they may act on at least two types of G protein-coupled receptors. Interestingly, there is evidence suggesting that CCKs may act in both feeding and locomotor networks in Drosophila, but the neural mechanisms are poorly understood. Here, we cloned the Aplysia CCK precursor, which generates two forms of CCK, i.e., CCK1 and CCK2. Both CCK1 and CCK2 have two tyrosine residues that may be sulfated. We obtained mass spectrometry evidence of unmodified and mono-sulfated CCKs in the Aplysia central nervous system (CNS) via liquid chromatography-tandem mass spectrometry (LC-MS/MS) and trapped ion mobility spectrometry (IMS). Specific locations of the sulfated tyrosine and the presence of di-sulfated CCKs remain to be determined. Importantly, we also cloned two putative CCK receptors from the Aplysia CNS by RT-PCR. We determined the tissue distribution of both the CCK precursor and receptors using whole mount in situ hybridization. We then found that different forms of CCK peptides have different activity at the two CCK
receptors when recombinantly expressed in CHO-K1 cells. Furthermore, in Aplysia intact ganglia, some of these peptides appeared to be active in the feeding and locomotor networks. Overall, the di-sulfated CCK appears to be the most potent in receptor assay and intact ganglia experiments. Notably, in the feeding network, active CCKs shortened the protraction duration of Aplysia feeding motor programs and made ingestive programs egestive, consistent with a role of the CCKs in satiation. Because of diverse presence of CCKs across phyla, our findings may have broad implications regarding peptide functions in general, and CCK actions in particular.


Poster

318. Neuromodulation of Motor Pattern Generation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 318.02/R11

Topic: E.07. Rhythmic Motor Pattern Generation

Support: National Natural Science Foundation of China Grant 31371104
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Title: Glutamatergic and serotonergic higher order neurons drives exploratory like and avoidance locomotion in alypsia

Authors: K. YU1, Z. YANG1, S.-Y. YIN1, D.-D. LIU1, G. ZHANG1, R.-N. JIA1, S.-Q. GUO1, W.-D. YUAN1, Y.-Y. XUE1, E. C. CROPPER2, K. R. WEISS2, *J. JING1;
1Sch. of Life Sci., Nanjing Univ., Nanjing, China; 2Dept. of Neurosci. and Friedman Brain Inst., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: To deal with different situations, animals generate two forms of locomotion, i.e., exploratory and avoidance. Neural, mechanisms that distinguish between these behaviors are poorly understood. Here, we address this issue in Aplysia. In Aplysia both forms of locomotion are mediated by rhythmic pedal rolling waves propagating from front to back. Previous studies often monitored locomotion using the parapedal commissural nerve (PPCN). We verified the efficacy of this method with implanted electrodes in PPCN and recorded PPCN bursts corresponding to tail contraction in intact animals, establishing PPCN as a monitor of locomotion. Moreover, PPCN during escape locomotion showed higher cycle frequency than


during spontaneous locomotion. Previous work showed that a high-order neuron, cerebral CC9/10 evokes locomotion (Jing et al 2008). We show that another high-order neuron, cerebral-pedal regulator (CPR), also drives locomotor programs. CPR mediates food-induced arousal (Teyke et al 1991). We found that locomotor programs elicited by CC9/10 and CPR are different. With the same stimulation parameters (10 Hz, 2 min), CC9/10-elicited programs had a higher cycle frequency (CC9/10: 4.0/min; CPR: 2.3/min) and higher frequency activity in PPCN (CC9/10: 7.6 Hz; CPR: 4.5 Hz), and also lasted longer (duration post stimulation of CC9/10: 2.8 min; CPR: 0.5 min). We also observed a difference in sensory activation. CC9/10 responded to tail nerve shock, whereas CPR responses were quite weak. In semi-intact preparations, CPR was strongly activated by food prior to consummatory feeding, and by mechanical stimulation of the tentacles. CC9/10 responses to these stimuli were weak. CC9/10 is immunoreactive to serotonin. We found that CPR is immunoreactive to *Aplysia* vesicular glutamate transporter. At 10 μM, glutamate receptor agonist, AMPA activated a relatively slow locomotor program (peak cycle frequency: 4.2/min) that decayed fast (6 min) in the maintained presence of AMPA. AMPA receptor antagonist, CNQX, blocked CPR-elicited programs (cycle frequency from 2.5/min to 0.7/min) but had little effect on CC9/10-elicited programs. Conversely, serotonin at 10 μM activated relatively fast locomotor programs (peak cycle frequency: 5.72/min) that remained active for more than 20 min in the presence of serotonin. The serotonin receptor antagonist, methysergide, slowed CC9/10-elicited programs (cycle frequency from 4.7/min to 2.6/min), but had only a slight effect on CPR-elicited programs. Overall, our data suggest that two higher-order neurons use different transmitters, glutamate and serotonin respectively, to mediate exploratory-like and avoidance locomotion.


**Poster**

**318. Neuromodulation of Motor Pattern Generation**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 318.03/R12

**Topic:** E.07. Rhythmic Motor Pattern Generation

**Support:** R35 NS097343

**Title:** Turned on by blue: Activation of pyloric neurons by light

**Authors:** *S. KEDIA, E. MARDER;
Brandeis Univ., Waltham, MA
Abstract: The earliest reports of photoreceptive behavior independent of visual systems were made in invertebrate systems decades ago. The phenomenon has since been widely encountered in species from annelids to mammals. Many instances of direct photoactivation of neurons are known, and most of these are selective for blue wavelengths of light. Blue light causes depolarization and an increase in firing in neurons in the abdominal ganglion of crayfish, Aplysia, Helix and Onchidium but the function of this response and the underlying photoreceptive molecule remain unidentified. Entrainment of circadian rhythm is an established role of photosensitive neurons in brain structures other than the eye, as in the case of Drosophila, in which the light-responsive molecule has been identified as a cryptochrome and the underlying molecular pathways are well described. Melanopsin is a non-visual photopigment found in vertebrates that plays a similar role in circadian regulation. We describe a blue light (λ460-500nm) dependent activation of pyloric neurons in the Cancer borealis stomatogastric ganglion (STG); a circuit previously not known to exhibit light sensitivity. We find a light-induced increase in oscillatory frequency of the pyloric neurons in an in vitro preparation of the intact circuit; measured intracellularly through sharp electrode recordings. LEDs were used for illumination of the samples. The effect is more pronounced in the absence of the neuromodulatory inputs that are necessary for maintaining the pyloric rhythm, often restoring rhythmicity to a network rendered silent by removal of neuromodulators. The activation is cell intrinsic and independent of synaptic parameters and accompanied by a small depolarization of membrane potential. There is a correlation between intensity of illumination and increase in frequency, time to peak change, and decay period. There is also an interaction with duration of exposure and these factors. Longer wavelengths of light (540-580nm; 620-650nm) do not have the same impact on neuronal activity. We identified putative cryptochrome sequences within the C.borealis transcriptome and have checked the expression of these via PCRs from STG samples. We have further confirmation of the expression of these as opposed to opsin family genes in the STG, based on RNAseq data. We are examining the potential contribution of this protein to the light response in STG neurons.

Disclosures: S. Kedia: None. E. Marder: None.

Poster

318. Neuromodulation of Motor Pattern Generation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 318.04/R13

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant R01-NS029436 (MPN)
         NIH Grant R35-NS097343 (EM)

Title: State-dependent modulation of rhythmic motor activity by a native peptide hormone
Authors: *M. P. NUSBAUM*¹, D. J. POWELL², E. E. MARDER³;
¹Dept. of Neurosci., Univ. of Pennsylvania Perelman Sch. of Med., Philadelphia, PA; ²Dept. of Neurosci., ³Volcn Ctr. and Biol. Dept., Brandeis Univ., Waltham, MA

Abstract: Different circuit states often generate divergent output patterns, but they can also generate convergent patterns. For example, stimulating the modulatory projection neuron MCN1 or bath applying CabPK peptide elicit similar gastric mill (chewing) rhythms (GMRs) in the crab *Cancer borealis* stomatogastric ganglion (Saideman et al, 2007 J Neurosci). CabPK does not activate MCN1 nor is it a MCN1 cotransmitter. We are using this system to test the hypothesis that the same input to different circuit states generating convergent patterns would cause those patterns to diverge. We showed previously, in separate experiments, that the peptide hormone CCAP (1 µm) slows the MCN1-GMR by prolonging the protraction phase, while it causes the CabPK-GMR to cycle faster by shortening the retraction phase (DeLong et al, 2009 J Neurosci; Powell et al, 2017 SfN Abstr). CCAP excites the reciprocally inhibitory rhythm generator neurons LG & Int1, but it modulates the MCN1-GMR by activating the voltage-sensitive current \(I_{\text{MI}}\) in LG (Delong et al, 2009). Here we test whether CCAP-activated \(I_{\text{MI}}\) in LG also mediates its distinct effect on the CabPK-GMR.

We first confirmed our previous results by applying CCAP to both GMRs in the same preparations. As before, CCAP prolonged MCN1-GMR protraction (Control- Pro: 4.6 ± 2.2 s; Ret: 11.4 ± 3.4 s; CCAP- Pro: 6.3 ± 1.8 s, p=0.001, n=6; Ret: 8.8 ± 2 s, p=0.03; Bonferroni correction \(\alpha = 0.017\), while it shortened CabPK-GMR retraction (Control- Pro: 3.7 ± 1.8 s; Ret: 9.2 ± 1.5 s; CCAP- Pro: 4 ± 1.8 s, p=0.6, n=6; Ret: 7.2 ± 1.9 s, p=0.01). We next determined if the CCAP action on the CabPK-GMR was likely to also result from its activating \(I_{\text{MI}}\) in LG by using the dynamic clamp to inject an artificial \(I_{\text{MI}}\) conductance (\(g_{\text{MI}}\)) into LG during the CabPK-GMR. This approach did mimic the CCAP influence on the CabPK-GMR, using up to a ~6-fold range of \(g_{\text{MI}}\) values (n=7/8). For example, during applied CabPK, injecting \(g_{\text{MI}}\) into LG (15 - 100 nS) shortened the retraction phase (Control: Ret, 9.2 ± 1.9 s; \(g_{\text{MI}}\)-CCAP: Ret, 6.6 ± 1.9 s, p=0.009, n=8), thereby reducing cycle period (Control: 13.2 ± 2.6 s, \(g_{\text{MI}}\)-CCAP: 11.0 ± 2.5, p=0.02, n=8).

Also, injecting negative \(g_{\text{MI}}\) into LG during a CabPK-GMR while bath applying CCAP nullified the CCAP effect (Control- Ret: 9.2 ± 1.9 s; CCAP- Ret: 6.6 ± 1.7 s; CCAP plus -\(g_{\text{MI}}\)-CCAP- Ret: 8.7 ± 2.3 s; p=0.003, n=8), thereby returning cycle period to the Control level (Control: 13.2 ± 2.6 s; CCAP: 10.9 ± 3.7 s; CCAP plus -\(g_{\text{MI}}\)-CCAP: 13.5 ± 4.0 s, p=0.001, n=8). These data suggest that the peptide hormone CCAP is using the same mechanism, selective modulation of one of its target neurons, to differently modify the output of the MCN1- and CabPK-GMR circuit states.

Disclosures: M.P. Nusbaum: None. D.J. Powell: None. E.E. Marder: None.

Poster

318. Neuromodulation of Motor Pattern Generation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 318.05/R14
Abstract: Network reconfiguration can include neuronal switching (i.e. changes in neuron participation between networks), in which changes in synaptic strength can recruit a neuron into another network (Dickinson, 1995 Curr Opin Neurobiol). Here, we propose that modulation of intrinsic properties can also underlie neuronal switching, with changes in synaptic strength enabling coordination between the switching and network neurons. We are studying switching mechanisms in the crab Cancer borealis stomatogastric nervous system (STNS), which contains two small well-defined networks (gastric mill [chewing, 10 s cycle period]; pyloric [food filtering, 1 s cycle period]) and identified modulatory inputs. Modulatory projection neuron MCN5 activity decreases pyloric cycle period, inhibits pyloric neuron LP, activates the gastric mill network, and switches LPG neuron participation from pyloric-only to dual pyloric/gastric mill (Norris et al, 1996 J Neurophysiol; Blitz et al, 2019 J Neurophysiol). To study this LPG pattern switch, we model MCN5 activity by bath-applying its peptide co-transmitter Gly₁-SIFamide (SIF; 5x10⁻⁶ M) and photoinactivating the LP neuron (Fahoum & Blitz, 2018 SfN Abstr). Gastric mill neurons are not necessary for LPG to switch to pyloric/gastric mill bursting (Fahoum & Blitz, 2018), but strengthened synaptic input from gastric mill neurons to LPG may underlie its coordination with this network. We identified inhibitory synaptic actions from gastric mill neurons IC, DG, & LG onto LPG in SIF, not evident in control saline (IC:LPG, n=7/7; DG:LPG, n=5/7; LG:LPG, n=5/6), which may enable them to regulate LPG gastric mill activity. Indeed, LG and IC regulated LPG’s gastric mill-timed burst period (p<0.05, n=7-8) and burst duration (LG: p<0.05, n=8). LPG maintains coordination with the pyloric pacemaker neurons likely due to maintained electrical coupling (Blitz et al, 2019). This coupling is not necessary for the LPG gastric mill-timed bursting, as it continued when the pyloric rhythm was eliminated (n=9), but it may underlie the fact that changing pyloric cycle period in SIF evoked a negative correlation between the pyloric and gastric mill cycle periods (r²=0.89, p<0.05, n=7). Thus, although the LPG neuron switch from single (pyloric) to dual (pyloric/gastric mill) bursting appears to occur via intrinsic mechanisms (Fahoum & Blitz, 2018), the LPG bursting pattern is regulated by synaptic inputs from gastric mill and pyloric network neurons. Thus, separate modulatory actions on intrinsic and synaptic properties can enable a neuron to exhibit a dual network bursting pattern and underlie its intra- and inter-network coordination.

Disclosures: S.H. Fahoum: None. D.M. Blitz: None.
318. Neuromodulation of Motor Pattern Generation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 318.06/R15

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant MH060605

Title: Frequency-dependent gating of neuromodulator-activated currents

Authors: *A. C. SCHNEIDER, J. GOLOWASCH, D. BUCHER, F. NADIM; Federated Dept. of Biol. Sciences, Inst. for Brain and Neurosci. Res. (IBNR), NJIT & Rutgers U-Newark, Newark, NJ

Abstract: Neural circuits produce distinct activity patterns, depending on behavioral needs. This flexibility is provided by neuromodulators that reshape the output of neural circuits. The effect of neuromodulators is often state-dependent, so that it depends on the circuit activity pattern, but the mechanisms and function of such state-dependence are not well understood. We explored the state-dependent actions of modulation of network oscillations using the pyloric circuit (freq.: 0.5 - 2 Hz) of the crab stomatogastric ganglion, which is influenced by multiple neuromodulators. Many peptide modulators enhance the circuit activity, but only when the network frequency is below some threshold value. These peptides activate a single ionic current (the modulator activated inward current, $I_{MI}$). Because $I_{MI}$ is fast and non-inactivating, its levels do not depend on the membrane potential frequency, indicating other potential mechanisms for frequency dependence.

We used the identified lateral pyloric (LP) neuron to directly measure the frequency dependence of currents activated by proctolin. Because cycle frequency affects the depolarization and hyperpolarization rates during bursting activity, we examined the dependence of the proctolin-activated current on these rates. We voltage-clamped LP with symmetric triangular ramp or ramp-and-hold (ramp slope ±100 to 400 mV/s), and found that 1 µM bath-applied proctolin activated two kinetically-distinct ionic currents: the previously characterized $I_{MI}$, which was activated by both positive and negative ramps, and whose amplitude did not depend on ramp slope, as well as an inactivating current (here called $I_{MI2}$), which was only activated by positive ramps and whose amplitude increased with ramp slope. We also voltage clamped the same neuron with a pre-recorded (realistic) waveform of the LP bursting activity, applied periodically at different frequencies (0.5-2 Hz). On average, the total steady-state current activated by proctolin did not change with the realistic waveform frequency, although frequency-dependence was present in some individual experiments.

A model fit of the ramp currents showed that the frequency-independence of the proctolin-activated current by realistic waveforms is because they range only from ~60 to -20 mV, well
below voltages where $I_{M2}$ is activated at any significant level. Increasing the amplitude of the realistic waveform or shifting the activation curve of $I_{M2}$ to the left exposed a frequency-dependent effect in the model. Together, these results demonstrate a potential mechanism for a frequency-dependent action of proctolin. Experiments are ongoing to test these model predictions.

**Disclosures:** A.C. Schneider: None. J. Golowasch: None. D. Bucher: None. F. Nadim: None.

**Poster**

318. Neuromodulation of Motor Pattern Generation

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 318.07/R16

**Topic:** E.07. Rhythmic Motor Pattern Generation

**Support:** NIH Grant MH060605

**Title:** Does co-modulation produce consistent circuit activity?

**Authors:** *E. CRONIN, A. C. SCHNEIDER, F. NADIM, D. BUCHER;
Federated Dept. of Biol. Sci., New Jersey Inst. of Technol. & Rutgers University-Newark, Newark, NJ

**Abstract:** Neural circuits are modified by many neuromodulators. Neuromodulation is typically studied with the expectation that the modulator configures the circuit to produce a specific output. In most systems, however, many modulators have overlapping and often convergent effects. Additionally, neural circuits are always subject to actions of multiple modulators at any time. Yet, the behavioral needs of the animal require that circuits produce consistent outputs. If multiple neuromodulators have convergent effects with the same sign, but also diverge on their targets, it is reasonable to assume that co-modulation results in a similar circuit output, even if, in each case, the circuit is not exposed to the same combination of neuromodulators. We therefore propose that convergent co-modulation increases the inter-individual consistency of circuit output.

We examined this hypothesis in the triphasic oscillatory pyloric circuit of the crab stomatogastric ganglion (STG). The STG is modulated by an astounding number of neurotransmitters and hormones. In this system, multiple peptide and muscarinic modulators converge to activate a single ionic current and enhance synaptic interactions. Yet, these modulators target distinct subsets of neurons and have different dose-dependent effects. In our protocol, we used a total of five peptides and a muscarinic agonist. We bath applied the modulators in singlets, doublets or triplets (Cases 1 to 3). In each case, the modulator(s) was applied at three concentrations, low (1 nM), medium (30 nM) and high (1 uM).

We compared circuit activity by comparing these circuit attributes: cycle period, burst onset and
end latencies (and therefore phase=latency/period) for the three pyloric phases, and #spikes/burst (equiv., spike frequency). We made a quantitative assessment of these 8 circuit attributes within each Case. We also examined the (state-)dependence of inter-individual consistency on frequency of the unmodulated circuit. Our expectation is that, from Case 1 to 3, inter-individual consistency of most circuit attributes increases. In addition to addressing the main hypothesis, these data also allow a direct comparison of the effects of individual modulators on circuit output, which has not been previously published.


Poster

318. Neuromodulation of Motor Pattern Generation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 318.08/R17

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Santa Clara University

Title: Proprioceptive feedback and its modulation in a crustacean nervous system

Authors: D. GRININGER, *J. T. BIRMINGHAM;
Santa Clara Univ., Santa Clara, CA

Abstract: The gastropyloric receptor (GPR) neurons in the stomatogastric nervous system of the crab *Cancer borealis* respond to both muscle contraction and passive muscle stretch (Katz et al., 1989). In this exploratory investigation we have found that GPR1 and GPR2 spiking can be measured extracellularly on both the lateral ventricular nerve (*lvn*) and dorsal ventricular nerve (*dvn*). In particular, we have observed that GPR1 spikes are always larger than GPR2 spikes on the *dvn* (*n*=15), that both types of spikes can be observed on the *dvn* in the presence of a pyloric rhythm, that nerve-evoked contraction of the gastric mill 4 (*gm4*) muscle elicits many more GPR2 than GPR1 spikes (*n*=4), that GPR1 spiking can be modulated by some of the same substances (serotonin (*n*=5), GABA (*n*=5) and TNRNFLRFamide (*n*=5)) that modulate GPR2 spiking (Birmingham et al., 1999), and that simultaneous neuromodulation of *gm4* muscle contraction and GPR2 response has implications for how muscle movements shape motor patterns in this system. The quality of the recordings and the possibility of improving them suggest that in vivo recordings of GPR spiking may be possible.

Disclosures: D. Grininger: None. J.T. Birmingham: None.
Title: Differential neuropeptide modulation of cell types in the lobster cardiac ganglion underlies alteration of different characteristics of patterned neuronal output

Authors: E. R. OLEISKY¹, J. J. HULL², A. E. CHRISTIE³, *P. S. DICKINSON¹; ¹Neurosci., Bowdoin Col., Brunswick, ME; ²Arid Land Agr. Res. Ctr., USDA, Maricopa, AZ; ³Békésy Lab. of Neurobiology, PBRC, Univ. of Hawaii, Honolulu, HI

Abstract: Central pattern generators (CPGs) are simple neural networks that generate rhythmic motor patterns. Flexibility in CPG output can be achieved via the actions of neuromodulators. Peptides, the largest class of neuromodulators, are integral for communication within neuronal systems, as well as for altering outputs of individual neurons and neuronal networks. The cardiac neuromuscular system of the American lobster, Homarus americanus, is a model CPG system for understanding the modulatory control of rhythmic motor patterns. The CPG, the cardiac ganglion (CG), consists of nine neurons: five large motor neurons and four smaller pacemaker neurons. The electrical and chemical coupling of these neurons is complex. Here, we show that the pacemaker and motor neurons are capable of establishing independent bursting patterns when physically decoupled by a ligature. Myosuppressin (pQDLDHVFLRFamide), a well-characterized crustacean neuropeptide, has been shown to act both centrally on the CG and peripherally on the cardiac muscle. In this study, we asked if myosuppressin was capable of modulating the decoupled pacemaker and motor neurons of the CG, and how their differential modulation might underlie the role that these neurons play in the motor pattern generated by the CPG. In the intact CG, application of 10⁻⁶ M myosuppressin elicited a significant decrease in burst frequency and a significant increase in burst duration; however, at 10⁻⁷ M, only a significant decrease in burst frequency was observed. In the ligatured CG, myosuppressin elicited a significant decrease in burst frequency in the motor neurons at 10⁻⁶ and 10⁻⁷ M, but in the pacemaker neurons, frequency decreased only at 10⁻⁶ M. Moreover, myosuppressin elicited a significant increase in burst duration in the isolated pacemaker neurons only at 10⁻⁶ M; even at
this concentration, myosuppressin failed to change the duration of bursts in the motor neurons. These data suggest (1) that the threshold for effects of myosuppressin differs between the motor and pacemaker neurons, and (2) that motor neurons are more important in determining the burst frequency of the CG, whereas the pacemaker neurons have a greater impact on the burst duration of the ganglionic output. The distribution of five putative myosuppressin receptors within the CG was simultaneously investigated. Transcriptomic analyses previously predicted five myosuppressin receptors (MSR1-5); PCR profiling revealed expression of only three transcripts (MSR2, MSR3, and MSR4) in the two neuron sets. Differential distribution of these receptors may contribute to the distinct physiological responses of the pacemaker and motor neurons to myosuppressin.


Poster

318. Neuromodulation of Motor Pattern Generation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 318.10/R19

Topic: E.07. Rhythmic Motor Pattern Generation

Support: 1ZIAMH002800-16

Title: Lateral transverse muscles mf21-23 in Drosophila are targets of crustacean cardioactive peptide (CCAP)

Authors: *M. G. HOUPERT, A. J. BERNDT, A. ELLIOTT, B. WHITE; NIMH Section On Neural Function, Bethesda, MD

Abstract: Neuromodulators are important determinants of behavior, but they are also often released into the blood as neurohormones to act on non-neural tissues. Many examples exist of neurohormones acting on skeletal muscles, but in most cases the role of this type of modulation on behavior is unknown. A behavior in which neuromodulators play a critical function is the pupal ecdysis sequence of Drosophila melanogaster. The pupal ecdysis sequence consists of three distinct phases, the second of which (Phase II) requires signaling by CCAP, which has previously been shown to act on a subset of motor neurons. Using a driver (CCAP-R-Gal4) that targets cells that express the CCAP receptor (CCAP-R), we find that in addition to a subset of motor neurons, three lateral transverse muscles, mf21-23, are also targets of CCAP. CCAP-containing type III terminals are found on the neighboring longitudinal muscle mf12, which traverses muscles mf21-23 and could be a source of CCAP acting on mf21-23 via the hemolymph. Bath administration of CCAP to pupal fillets shows that CCAP specifically activates mf21-23 as measured by Ca++ imaging using GcaMP6s. The larval musculature
similarly expresses CCAP-R in mf21-23 and responds to CCAP, however the pattern of activation differs from that seen in the pupa. The activation appears to depend on extracellular calcium, as bath administration of CCAP to pupal fillets in solution lacking Ca++ failed to activate mf21-23. Monitoring muscle contraction in vivo using a UAS-GcaMP6s reporter suggests that, at Phase II, the known time of CCAP release, mf21-23 contract for a longer duration than in other phases. Taken together, our data suggest a role for CCAP in modulating the ec dysis behavioral sequence at the level of muscle as well as motor neurons.


Poster

318. Neuromodulation of Motor Pattern Generation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 318.11/R20

Topic: E.07. Rhythmic Motor Pattern Generation

Support: ZIAMH002800-16

Title: Sites of CCAP neuromodulator activity in the pupal ec dysis behavioral sequence

Authors: *A. J. BERNDT, A. D. ELLIOTT, M. G. HOUPERT, B. H. WHITE;
NIH/NIMH, Bethesda, MD

Abstract: Neuromodulators play important roles in regulating the activity of brain circuits, often to promote changes in behavior. To better understand how they do so, we have focused on a neuromodulator-governed behavioral sequence performed by pupal fruit flies called the “pupal ec dysis sequence.” This sequence consists of three behavioral phases, the last two of which are prevented by silencing a subset of the neurons that express the neuromodulator Crustacean Cardioactive Peptide (CCAP). We have previously shown that the CCAP receptor (CCAP-R) is expressed in a large population of motor neurons, indicating a role for CCAP in neuromuscular control. However, the identities of the CCAP-R-expressing motor neurons and their muscle targets have remained unknown, in part due to the limited characterization of the pupal musculature, which is reduced relative to the well-characterized larval musculature and its stereotyped pattern of innervation across repeating hemisegments. During pupal development many larval muscles undergo histolysis, and we have now shown that at the time of pupal ec dysis, a maximum of 19 muscles remain per hemisegment. However, from anterior to posterior segments, individual muscles stop repeating (mfs 4, 5, 12, 30). Approximately half of all muscles (n=9) are consistently innervated by CCAP-R-expressing motor neurons, as determined by co-expression of CCAP-R and the motor neuron-specific gene VGlut. Muscles in this set were either dorsal (mfs 1-3, 9, 10), or ventral (mfs 15, 28, 13, 30), but not lateral. The remaining set of muscles (i.e. mfs 4, 5, 8, 12, 21-26), were innervated by motor neurons expressing VGlut only
and contained all lateral muscles. Interestingly, we find that although the principal lateral muscles of the pupal bodywall were not innervated by CCAP-R motor neurons, mf21-23 themselves do express CCAP-R, suggesting that CCAP modulates the pupal ecdysis sequence at both the level of identified motor neurons and muscles. To facilitate future analysis of neuromuscular innervation patterns, we are also implementing machine learning algorithms to automate identification of synapses based on their bouton characteristics, beginning with a K-Nearest Neighbors approach. Our results lay the groundwork for future studies to determine exactly how activity in the neuromuscular system is altered by manipulations of CCAP signaling during pupal ecdysis.


Poster

318. Neuromodulation of Motor Pattern Generation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 318.12/S1

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NINDS 1 R21 NS111355

Title: Dynamics of the Na+/K+ pump and dopamine within a locomotor central pattern generator

Authors: *A. VARGAS¹, G. S. CYMBALYUK²;
¹Neurosci. Inst., ²The Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: CPGs are oscillatory neuronal circuits controlling rhythmic movements across vertebrates and invertebrates(1). The Na/K pump contributes to the dynamics of bursting activity across several CPGs in various species such as leech, tadpole, and mouse (2,3,4,5,6). Some rhythms, like locomotion in vertebrates and heartbeat in leeches, must be continually regulated for an animal to meet environmental and behavioral demands(3). In vertebrate CPGs, dopamine has been shown to induce a range of subtle to pronounced effects on diverse motor rhythms. Dopamine neuromodulation affects Na+/K+ pump, GIRK2-, A-, and h-currents through D1 and D2 receptors (7,8); this contributes to stabilization of CPG rhythmic activity. We developed a half-center oscillator (HCO) model of a spinal locomotor CPG, which comprises of four populations, two inhibitory and two excitatory. The neuronal populations are intrinsically bursting supported by a persistent sodium current within a physiologically relevant parameter space. We investigated activity regimes of endogenously bursting neurons either in isolation or incorporated into a HCO. In a range of high modulation level we found stable periodic bursting, while within some range of low dopamine modulation levels, pronounced intermittent intrinsic patterns. We investigated a reduced model with the Na+/K+ pump, h-current, and persistent
sodium only. The dynamics within the reduced model qualitatively represented those of the full scale single cell and network. We also investigated the hypothesis that dopamine affects the network through activation of inward rectifying potassium currents, IGIRK and IA, and opposing changes of h-current all while interacting with pump current. The reduction in modulatory level of dopamine in the spinal locomotor CPG causes the model to transition from normal periodic bursting into intermittent bursting and then to silence. Robust rhythmic output within our locomotor CPG model is a consequence of the co-modulation of the Na/K pump along with GIRK2-, A-, and h-currents.

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Disclosures:  A. Vargas: None. G.S. Cymbalyuk: None.

Poster

318. Neuromodulation of Motor Pattern Generation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 318.13/S2

Topic: E.07. Rhythmic Motor Pattern Generation

Support:  1 R21 NS111355 to GSC and RLC

Title: Bursting mechanisms based on interplay of the Na/K pump and persistent sodium current

Authors: *G. S. CYMBALYUK1, R. ERAZO1, A. VARGAS1, C. ERXLEBEN2, A. WENNING2, R. L. CALABRESE2;
1Neurosci. Inst., Georgia State Univ., Atlanta, GA; 2Dept. of Biol., Emory Univ., Atlanta, GA

Abstract: To maintain vital rhythmic functions like breathing and leech heart beating, specialized oscillatory neural circuits, Central Pattern Generators (CPGs), produce functional bursting activity appropriate to variety of physiological conditions. To ensure such robustness of operation different subsets of inward and outward currents can come into action and dominate the dynamics under different parametric regimes. The Na/K pump partakes in rhythm generation and is targeted by neuromodulators in the leech heartbeat CPG [1, 2]. We have investigated the interplay of the Na/K pump current (IPump) and h-current in rhythm generation by this CPG [3]. In the dynamics of this CPG, persistent Na current (IP), which does not inactivate, plays an influential role in supporting spiking phase of bursting activity. We suggest that interaction of IP and IPump could produce a flexible mechanism supporting bursting. We apply modeling and experimental hybrid system approach to investigate dynamical properties of this mechanism. We developed a hybrid system using the biophysical model from Kueh et al. 2016 [3]. In hybrid-system experiments, we investigate effects of upregulation or downregulation of IP and IPump on bursting activity in single leech heart interneurons neurons that pace the leech heartbeat CPG. We show that interaction of IP and IPump constitutes a mechanism, which is sufficient to support
endogenous bursting activity. Interestingly, this mechanism can reinstate robust bursting regime in heart interneurons, which when recorded intracellularly have increased leak and would otherwise fire tonically. In contrast to typical role of $I_{\text{P}}$ in bursting, according to this mechanism the increase of the maximal conductance of $I_{\text{P}}$ can lead to decrease of the burst duration and increase of the interburst interval. We also developed a simple 2D model describing dynamics of the membrane potential and intracellular $\text{Na}^{+}$ concentration through instantaneous $I_{\text{P}}$ and $I_{\text{pump}}$ and investigated how basic kinematic parameters governing dynamics of $I_{\text{P}}$ and $I_{\text{pump}}$ affect responses of the system to up- and down regulation of these currents.

**References**


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**Poster**

318. Neuromodulation of Motor Pattern Generation

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 318.14/S3

**Topic:** E.07. Rhythmic Motor Pattern Generation

**Title:** Bimodal modulation of short-term motor memory via dynamic sodium pumps in a vertebrate spinal cord

**Authors:** L. HACHOUMI, R. RENSNER, C. RICHMOND, *K. T. SILLAR; Univ. St Andrews, St Andrews, United Kingdom

**Abstract:** Activity-dependent Na+/K+-pumps play an evolutionarily conserved role in regulating locomotor network output (Picton et al. 2017). In the spinal cord of *Xenopus laevis* frog tadpoles, recruitment of these “dynamic” Na+/K+ pumps produces an ultra-slow afterhyperpolarisation (usAHP) following intense locomotor activity (Zhang and Sillar 2012). The usAHP lasts ~1 minute and facilitates a short-term motor memory (STMM) mechanism that attenuates the intensity and frequency of subsequent bouts of locomotor activity. In this study, whole cell current clamp recordings of spinal neurons and ventral root recordings were performed to investigate modulation of dynamic Na+/K+ pumps and its effects on fictive motor activity. Our recordings show that two established modulators of locomotor activity in *Xenopus* tadpoles, serotonin (5-HT) and nitric oxide (NO), differentially regulate the usAHP in spinal neurons.
Perfusion of 5-HT (1.5 µM) alone had no discernible effect on the amplitude or duration of the usAHP. However, selective activation of 5-HT7 receptors with 30 µM AS-19 significantly augmented the usAHP an effect that was reversed by the 5-HT7 receptor antagonist, 20 µM SB-269970. Conversely, 5-HT2A receptor activation with 30 µM 25CN-NBOH or inhibition with 50 µM MDL 11,939 significantly reduced and increased the usAHP, respectively. The NO donor, DEA-NO (100 - 200 µM), suppressed the usAHP, but this effect was negated following exposure to the NO scavenger, PTIO (100 µM). Exclusively inhibiting 5-HT7/2A receptors or scavenging NO significantly altered usAHP properties thus demonstrating endogenous roles for 5-HT and NO in regulating the usAHP. Next, the influence of these neuromodulators on STMM was assessed via ventral root recordings. 5-HT7 receptor activation following 5-HT2A receptor inhibition strengthened the relationship between swim episode duration and inter-swim interval, whilst NO consistently weakened this relationship. Furthermore, in preparations where STMM was weak in control conditions, removing the inhibitory effects of NO with the scavenger PTIO or 5-HT2A receptors with the antagonist MDL 11,939 reinforced STMM. This suggests that the STMM mechanism can be controlled by endogenous 5-HT and NO signalling within the spinal cord. In conclusion, this investigation demonstrates that usAHP dynamics can be differentially modulated by 5-HT receptors (by up to ± 15 mV) as well as by NO (down to 0 mV) and that this consequently alters the strength of the STMM mechanism in tadpoles. References: Picton et al. 2017, J Neurophysiol. 118: 1070; Zhang and Sillar 2012, Curr. Biol. 22, 526.

immediate conclusion was that the locomotor rhythm generation could emerge from excitatory circuits incorporating $I_{NaP}$ as a “pacemaker” current. In a “push-pull” organization of the spinal locomotor network, the present study investigates the role of a persistent potassium current mediated by Kv7.2 (KCNQ2)-channels, also called M-current ($I_M$), into the operation of the locomotor CPG. As a first result, *in vivo* intraperitoneal injection of Kv7.2 channel blocker (XE991) lengthens the step duration of juvenile rats whereas the opener (Retigabine) shortens it. These data are consistent with the drugs effects on the NMA-induced fictive locomotor rhythm recorded from the *in vitro* isolated spinal cord preparations of neonatal rats. The early postnatal expression of Kv7.2 channels in the spinal locomotor network has been confirmed with confocal imaging and electrophysiological recordings. We demonstrated that Kv7.2 channels expressed in the axon initial segment contributes to oscillatory processing in the locomotor CPG. The Kv7.2 pharmacological blockade, as well as the KCNQ2T274M/+ mutation, leads to an increase of the duration of (1) autorhythmic activities in interneurones and (2) NMA-induced voltage oscillations in motoneurones. Opposite effects were obtained with KCNQ enhancers. In conclusion, our study provides evidence that the locomotor rhythm may be modulated by balancing the relative contribution of $I_M$ and $I_{NaP}$.

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**Poster**

**318. Neuromodulation of Motor Pattern Generation**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 318.16/S5

**Topic:** E.07. Rhythmic Motor Pattern Generation

**Support:** NIH R01 NS095366  
NIH R01 NS104194

**Title:** Data driven modeling of the rhythmogenic potential of spinal Shox2 neurons

**Authors:** *N. HA*, N. A. SHEVTSOVA, I. A. RYBAK, K. J. DOUGHERTY; Dept. of Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

**Abstract:** The mammalian spinal cord contains neuronal circuits that can generate the basic locomotor rhythm in the absence of supraspinal and afferent inputs. These spinal circuits include the rhythm-generating kernel consisting of neurons capable of generating the populational rhythmic activity. The mechanisms underlying spinal rhythmogenesis are still unknown, but intrinsic properties and interconnectivity of excitatory interneurons are believed to play a significant role. Though no single genetically identified neuron type has been shown to be solely responsible for rhythm generation, a prominent candidate to participate in locomotor
rhythmogenesis is a subset of Shox2 neurons. Experimental studies of the cellular basis for rhythmic bursting in the Shox2 neurons in neonatal mice have shown evidence for expression of T-type Ca^{2+}, hyperpolarization-activated (I_h), A-type K^+, and persistent inward currents in subsets of these neurons. A small subset of these neurons continues to display rhythmic activity in the presence of NMDA and serotonin following blockade of AMPA receptors. Further, bidirectional gap junctional coupling was detected within a distinct subset of Shox2 neurons, providing a potential mechanism for synchronization of populational activity of Shox2 neurons which may be implicated in locomotor rhythmogenesis. To theoretically investigate a potential role of intrinsic properties and connectivity of Shox2 neurons in generation of rhythmic activity, we developed a series of models of single Shox2 cells incorporating intrinsic currents identified experimentally and a population model of these cells sparsely connected by electrical synapses. We have found that the rhythmic activity emerges in a coupled population of modelled cells, even if no single cell demonstrates the intrinsic bursting. We investigated potential roles of different ionic currents expressed in single cells in population bursting and the dependence of the shape and frequency of population bursts on the probability and weights of mutual connections in the network. The model proposes a mechanistic explanation for emergence of a synchronized rhythmic activity in a subset of Shox2 neurons and provides insights into the mechanisms of the locomotor rhythm generation.

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**Poster**

318. Neuromodulation of Motor Pattern Generation

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 318.17/S6

**Topic:** E.07. Rhythmic Motor Pattern Generation

**Support:** NIH R01 NS104194

NIH R01 NS095366

**Title:** Spinal cord injury-induced plasticity of Shox2 interneurons following treadmill training and epidural stimulation in mouse

**Authors:** *D. GARCIA-RAMIREZ*, N. T. HA, L. YAO, K. A. SCHMIDT, S. F. GISZTER, K. J. DOUGHERTY;
Dept. of Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

**Abstract:** The neuronal circuitry that generates locomotion is located in the thoracolumbar spinal cord. Although the locomotor circuitry is able to produce the rhythm and the patterning of locomotion without descending inputs, these are necessary to initiate and adapt the locomotion. Most spinal cord injuries (SCI) are above level of the spinal locomotor circuitry but a disruption
in the descending control results in cellular and network plasticity. Current clinical therapies to recover motor control after SCI include treadmill training and epidural stimulation (ES), which access the locomotor circuitry by targeting proprioceptive afferents. However, the state of the spinal circuits targeted after SCI and further plasticity following rehabilitation is poorly understood. Interneurons (INs) expressing the transcription factor Shox2 are part of the circuitry that generates the locomotor rhythm and patterning and should be a prime access point for these treatments. Previously, we performed whole cell patch clamp recordings targeting Shox2 INs in lumbar spinal slices from uninjured adult Shox2::Cre;Rosa26-lsl-tdTomato mice and found that Shox2 INs are modulated by serotonin (5-HT) producing inhibitory and excitatory actions depending the concentration. In slices from mice 6 weeks after complete thoracic spinal transection, 5-HT only increased the excitability of Shox2 INs. However, in mice in which ES wires were implanted at lumbar spinal level L2 and received treadmill training and ES after SCI for 5 weeks, 5-HT hyperpolarized Shox2 INs. The aim of this study is to identify the contributions of treadmill training and ES independently on the hyperpolarizing effects of 5-HT on Shox2 INs. We found similar inhibitory modulatory effects of 5-HT in the mice that received ES with no treadmill training, demonstrating that treadmill training is not necessary for the 5-HT receptor plasticity in Shox2 INs that is observed with treadmill training and ES. Our results suggest that ES alone can cause changes in the expression of 5-HT receptors on locomotor-related neurons after SCI, altering pharmacological targets for the improvement of ES efficacy.


Poster

318. Neuromodulation of Motor Pattern Generation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 318.18/S7

Topic: E.07. Rhythmic Motor Pattern Generation

Support: DGAPA-UNAM PAPIIT grant RA105518
        ERC grant FLEXNEURO (716643)

Title: A spinal CPG controller that is compatible with neuromodulation and homeostasis

Authors: A. I. RAMÍREZ-HINCAPIÉ1, T. O'LEARY2, *A. FRANC13;
1Univ. Nacional Autónoma De México, Ciudad De Mexico, Mexico; 2Univ. of Cambridge, Cambridge, United Kingdom; 3Dep. Matematicas, Facultad De Ciencias, UNAM, Mexico City, Mexico

Abstract: Many periodic automatic movements such as breathing, chewing, swimming and walking are controlled outside the brain by autonomous neural circuits called Central Pattern
Generators (CPG). The study of crustacean CPGs for chewing and swallowing revealed that the activation of specific rhythmic patterns and the switch between different patterns is mediated by the modulation of intrinsic and synaptic neural properties [1]. This modulatory signal must cope with ongoing homeostatic control of cellular activity [2]. Spinal CPGs for quadruped locomotion exhibit a higher level of complexity, both in the circuit composition and in the number and types of possible rhythmic patterns, as compared to their invertebrate analogs. Genetic knockdown of specific neural types provided some insights on how quadruped CPGs can switch between different activity pattern [3] but how this control is physiologically achieved remains largely unknown.

We propose a general CPG control mechanism that uses neuromodulation of intrinsic properties as control input and that is compatible with homeostatic regulation. We suggest that inhibitory interneuron modulation can control the phase relation between pairs of excitatory neurons without perturbing the homeostatic set point. The proposed control mechanism is fast enough to be compatible with quadruped motor behavior control and does not require synaptic plasticity. Mathematical modeling provided deep insights into the structure of quadruped CPG circuits, in particular, in terms of their symmetries [4]. In this framework, quadruped gaits and the transitions between them arise as symmetry breaking of the underlying CPG circuit. However, the physiological mechanism underlying gait transition remains obscure. Interneuron neuromodulation provides such physiological and homeostasis-compatible control mechanism. Using a computational model, we show how the same connectome can produce all six primary gaits through Ca-mediated neuromodulation of the inhibitory interneurons in an eight coupled-cell system.


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Poster

318. Neuromodulation of Motor Pattern Generation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 318.19/S8

Topic: E.07. Rhythmic Motor Pattern Generation

Title: Fictive scratching patterns in restricted cat preparations treated with serotonin or serotonin or antagonist WAY100635
Authors:  I. AGUILAR GARCÍA¹, *L. P. OSUNA CARRASCO², B. DE LA TORRE VALDOVINOS², J. R. LOPEZ RUIZ¹, R. CASTAÑEDA ARELLANO³, C. TORO CASTILLO², M. TREVIÑO VILLEGAS⁴, J. M. DUEÑAS JIMÉNEZ¹, S. H. DUEÑAS JIMÉNEZ¹;
¹Univ. of Guadalajara CUCS, Guadalajara, Mexico; ²Univ. of Guadalajara CUCEI, Guadalajara, Mexico; ³Univ. of Guadalajara CUTonalá, Guadalajara, Mexico; ⁴Neurosc. Inst., Univ. of Guadalajara CUCBA, Guadalajara, Mexico

Abstract: Fictive scratching (FS) patterns were evaluated in brain-cortex-ablated cats (BCAC), in spinal cats (SC) and in midcolicular-decerebrated cats (MCC) to dissect pathways comprising the scratching patterns. Additionally, in MCC the effects of electrical stimulation of cutaneous and extensor nerves were also studied. In BCAC, the scratching aiming phase (AP) initiates with the activation of flexor or extensor motoneurons. The application of serotonin during the AP produced simultaneous extensor and flexor bursts. Furthermore, WAY 100635 (5HT1A receptors antagonist) produced a very brief burst in the flexor TA (tibialis anterior) nerve followed by a reduction in its electroneurogram (ENG), whilst the soleus (SOL) ENG remained silent. In SC, rhythmic phase (RF) activity in SOL motoneurons was recorded. Serotonin or WAY produced FS bouts with variability in flexor-extensor activity during the AP. In MCC, FS began with flexor activity, electrical stimulation of either deep peroneous (DP) or superficial peroneous (SP) nerves increased the duration of the TA electroneurogram. DP electrical stimulation during a TA bursts produced a large-amplitude and prolonged TA burst. MG stretching or electrical stimulation produced a reduction in the TA electroneurogram and an initial MG extensor bursts. MG and TA monosynaptic reflexes wax and wane during the scratch cycle. Present experiments support an asymmetrical model for the scratching AP of the BCAC and the symmetrical model in MCC. Serotonin and WAY produce several effects on FS pattern, assembling CPG modules to use disponible segregated motoneurons.


Poster

318. Neuromodulation of Motor Pattern Generation

Location:  Hall A

Time:  Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #:  318.20/S9

Topic:  E.07. Rhythmic Motor Pattern Generation

Support:  AIHS Grant 63025

Title:  The effects of T-type antagonists on the genesis of motor activity in the neonatal mouse spinal cord
Authors: *A. P. LOGNON*¹, S. A. SHARPLES¹, P. J. WHELAN²;
¹Neurosci., ²Comparative Biol. and Exptl. Med., Univ. of Calgary, Calgary, AB, Canada

Abstract: The spinal cord is important for the generation of locomotion as it contains sufficient neuronal circuitry to drive various forms of rhythmic and non-rhythmic motoneuronal output. T-type calcium channels have been previously described as important electrophysiological modulators of central pattern generators used to produce rhythmic spinal motor output. Studies have described the role of T-type channels in the generation of locomotor-like activity from the neonatal spinal cord. Many studies that investigate the role of T-type channels in neuronal function make use of the antagonist nickel. Here we investigate the effects of nickel in comparison to other T-type channel antagonists on the extracellularly recorded motor root output from the neonatal isolated spinal cord. Similar to previous findings, we demonstrate that nickel reduces pharmacologically evoked locomotor-like rhythmic bursting frequency. Furthermore, the burst amplitude increases and the bursting pattern changes from alternating to synchronous. In contrast, more selective T-type antagonists did not replicate nickel’s effects on locomotor-like bursting. To further probe the differences between T-type antagonists, the more selective antagonists along with nickel were applied to a spontaneously active isolated cord. The results show a difference between the effects of nickel versus other T-type antagonists. Nickel depolarizes and increases bursting amplitude at T-type selective concentrations, which is not seen with the application of selective T-type channel antagonists. The effects of nickel persist in the presence of fast synaptic blockers, suggesting effects on either motoneurons themselves or via slow-synaptic modes of transmission. Although the mechanism of action for nickel has not been elucidated, we demonstrate inconsistencies in the actions of T-type antagonists on the motor rhythm produced by the neonatal spinal cord.

Disclosures: A.P. Lognon: None. S.A. Sharples: None. P.J. Whelan: None.

Poster

319. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 319.01/S10

Topic: F.04. Stress and the Brain

Support: NIH R21MH114184 (JHU; WFC)

Title: Reproductive experience, but not neuropeptide Y (NPY), modulates stress resilience in female rats

Authors: *M. R. DEJOSEPH*¹, M. BOMPOLAKI², M. GILLUM¹, B. L. AVONTS³, J. E. VANTREASE⁴, W. F. COLMERS⁵, J. H. URBAN⁶;
¹Physiol. & Biophysics; Ctr. for Neurobio. of Stress Resilience and Psychiatric Disorders,
Abstract: The prevalence and limited treatments for anxiety disorders demand understanding of innate neural mechanisms promoting resilience. Endogenous NPY is anxiolytic, buffering stress and promoting resilience by decreasing excitatory output of the basolateral amygdala (BLA) through modulating the H current, carried by the hyperpolarization-activated cyclic nucleotide-gated channel subunit 1 (HCN1). We have shown that repeated application of NPY into the BLA induces a lasting behavioral stress resilience associated with the down-regulation of HCN1 expression. While these studies were conducted in male rats, the question remains whether NPY exerts similar actions in females. To test this, female rats received indwelling bilateral cannula in the BLA. After recovery, the animals received injections of NPY (10 pmol/100 nl) daily for 5 consecutive days. Social interaction (SI) was assessed at 1, 7, 14 and 28d post-injection and compared with a pre-injection baseline control. Stress resilience was measured with SI in animals immediately following a single 30 min restraint stress exposure. Brains were collected for determination of HCN1 and cFos expression. Contrary to the robust effects of NPY in males, in female rats NPY did not increase SI at the timepoints measured, nor prevent the effect of restraint stress-induced decreases in SI; no changes in HCN1 protein expression were observed. Using a model of reproductive experience (RE), known to buffer stress responsiveness, we tested whether primiparous (Prim) females experiencing the rearing of one litter would have increased resilience that might engage similar mechanisms as defined in males with NPY treatment. Female rats were mated; after delivery pups were culled or cross-fostered to 10 pups/litter, then weaned on postpartum (PP) day 21. SI was determined in Prim and age-matched nulliparous (Nul) females on PP d14 and 28 along with a single bout of restraint stress to test resilience. SI was not increased in Prim females compared to Nul controls. Restraint stress significantly decreased SI in Nul females, but SI after restraint in Prim females did not differ from baseline control. Determination of brain HCN1 and cFos expression in these animals is ongoing. We have identified a major sex difference in responses to intra-BLA NPY injections, and a model of physiologically inducible stress resilience which can be useful in determining brain circuity important for preventing stress-related disorders.

319. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 319.02/S11

Topic: F.04. Stress and the Brain

Support: NIH Grant R01MH100536

Title: Sex differences in the contribution of projections from the basolateral amygdala to lateral bed nucleus of the stria terminalis in anxiety-like behaviors

Authors: *J. VANTREASE*¹, B. AVONTS¹, J. URBAN², J. ROSENKRANZ¹; ¹Cell. and Mol. Pharmacol., ²Physiol. & Biophysics, Rosalind Franklin Univ., North Chicago, IL

Abstract: Men and women display different symptomology in anxiety disorders and respond differently to treatments, yet our understanding of the sex differences in the neurobiology related to these disorders is limited. The basolateral amygdala (BLA) is a critical component of anxiety neurocircuitry, is hyperactive in patients with anxiety disorders and exhibits sex differences in activation during specific affective tasks. Prior studies have demonstrated that sex differences in BLA neuronal activity exist; however it is not known whether specific subsets of BLA neurons exhibit this disparity. BLA outputs to the lateral bed nucleus stria terminalis (BSTL) play a critical role in the expression of sustained fear and general anxiety-like responses with female rats expressing less general anxiety-like behaviors compared to males. Therefore, the purpose of this study was to determine if BLA neurons projecting to BSTL (BLA-BSTL) in female rats are less active than in males. We utilized in vivo single-unit electrophysiological recordings to record antidromically identified BLA-BSTL neurons in anesthetized male and female Sprague Dawley rats. We observed that spontaneously firing BLA-BSTL neurons in females have lower firing frequencies compared to males, despite females having greater spontaneous BLA neuronal activity overall. To determine if this lower BLA-BSTL neuronal activity in females leads to decreased general anxiety-like behavior, we utilized a combinatorial chemogenic approach to selectively inhibit BLA-BSTL neurons during specific affective tasks using CAV2-Cre injections into the BSTL followed by bilateral Cre-dependent designer receptor exclusively activated by designer drug (DREADD) injections into the BLA; then assessed anxiety-like behavior and social interaction (SI) behavior in an open field 30 min after saline or CNO injections. Preliminary results suggest that inhibition of BLA-BSTL neurons during the open field task increased center exploration in males, but reduced time spent in center in females. Moreover, BLA-BSTL inhibition during SI resulted in a slight increase in SI time in females, but an even greater augmentation in SI was seen in males. Together these data suggest that the sex differences observed in BLA-BSTL neuronal activity may also mediate different anxiety-like behaviors in male and female rats. These results may help explain the apparent disconnect...
between overall sex differences in BLA neuronal activity and its relation to particular anxiety-like behaviors.

**Disclosures:** J. Vantrease: None. B. Avonts: None. J. Urban: None. J. Rosenkranz: None.

**Poster**

319. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 319.03/S12

**Topic:** F.04. Stress and the Brain

**Support:** NIH Grant R01 MH113007

**Title:** Oxytocin modulates the functional neuronal network in the oval nucleus of the bed nucleus of the stria terminalis (BNSTov) in male adult rats

**Authors:** *W. FRANCESCONI, F. BERTON, J. DABROWSKA;
Dept. of Cell. and Mol. Pharmacol., Rosalind Franklin Univ. of Med. and Scien, North Chicago, IL

**Abstract:** Oxytocin (OT) plays a key role in the modulation of social and anxiety-like behaviors. Previous studies demonstrated that OT neurons from the hypothalamus project to the oval nucleus of the bed nucleus of the stria terminals (BNSTov). BNSTov has one of the highest expression of oxytocin receptors (OTR) in a rodent brain. The BNSTov contains exclusively GABA-ergic neurons that were previously categorized based on their intrinsic membrane properties as type I, II and III neurons. The present study was aimed to understand the effects of OT on synaptic transmission as well as excitability of the BNSTov neurons. Therefore, we performed whole cell patch-clamp recordings from functionally identified BNSTov neurons in rat brain slice preparation in adult male Sprague-Dawley rats. We first recorded pharmacologically isolated spontaneous inhibitory postsynaptic currents (sIPSC). The BNSTov neurons were clamped at -70 mV, and the sIPSCs were collected before, during, and after bath application of OT (0.2 μM). Application of OT significantly increased the mean frequency ($P = 0.0098$, one-way ANOVA), but not amplitude of sIPSCs in type II BNSTov neurons. This effect was abolished in the presence of selective OTR antagonist (d(CH$_2$)$_3$,Tyr(Me)$_2$, Thr$_4$, Orn$_8$, des-Gly-NH$_2$$_9$)-vasotocin (OTA, 0.4 μM). The effect of OT was also abolished by application of tetrodotoxin (TTX, 1 μM), therefore the increase of sIPSCs frequency observed in type II BNSTov neurons could be mediated by OT-induced depolarization on other type of BNSTov interneurons. To identify the neuronal type involved in the control of inhibitory input on type II neurons, we investigated the effect of OT on the intrinsic excitability of type I, II and III neurons in a current clamp mode. We found that OT increased the intrinsic excitability on type I neurons as revealed by a left shift of the spike frequency-current relationship ($P = 0.0328$). The increase
in excitability was paired with an increase in the input resistance of the cells \((P = 0.0336)\), suggesting that OT in type I neurons could block hyperpolarizing currents open at the resting membrane potential. Overall, these results present a model of fine-tuned modulation of the BNST\textsubscript{OV} neuronal network by OT, in which OT selectively increases the excitability of regular spiking type I BNST\textsubscript{OV} neurons, which are considered to be intrinsic interneurons. As a result, OT strengthens inhibitory input in type II low-threshold bursting BNST\textsubscript{OV} neurons, which belong to the BNST\textsubscript{OV} projection neurons. As a result, OT would inhibit BNST\textsubscript{OV} output, which might underlie some of the behavioral effects of OT in the BNST\textsubscript{OV}.

**Disclosures:** W. Francesconi: None. F. Berton: None. J. Dabrowska: None.

**Poster**

**319. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 319.04/S13

**Topic:** F.04. Stress and the Brain

**Support:** NIH R01MH113007

**Title:** The expression and distribution of oxytocin receptors in the dorsolateral bed nucleus of the stria terminalis (BNST\textsubscript{DL}) and their interaction with GABA-ergic system

**Authors:** *V. OLIVERA\textsuperscript{1}, S. H. OLSON\textsuperscript{2}, R. CHUDOBA\textsuperscript{3}, J. A. DABROWSKA\textsuperscript{4};
\textsuperscript{1}Dept. of Cell. and Mol. Pharmacol., \textsuperscript{2}Dept. of Cell. and Mol. Pharmacolology, Rosalind Franklin Univ., North Chicago, IL; \textsuperscript{3}Dept. of Cell. and Mol. Pharmacology, Rosalind Franklin Univ. of Med. and Scien, North Chicago, IL; \textsuperscript{4}Chicago Med. Sch. RFUMS, North Chicago, IL

**Abstract:** The bed nucleus of the stria terminalis (BNST) is a heterogeneous brain structure that modulates fear and anxiety-like behaviors. Previously, we have shown that oxytocin receptors (OTRs) in dorsolateral BNST (BNST\textsubscript{DL}) facilitate cued fear acquisition as measured in a fear-potentiated startle (FPS). Notably, inhibition of the BNST with GABA\textsubscript{A} receptor agonist also increased cued fear expression in FPS. First, we investigated the expression and distribution of OTRs in the BNST\textsubscript{DL} of male adult Sprague-Dawley rats. We performed double-immunofluorescence for OTR and marker of GABA-ergic neurons (67 kDa Glutamic Acid Decarboxylase, GAD67), marker of GABA-ergic terminals (vesicular GABA transporter, VGAT), as well as markers of specific populations of neurons of the BNST\textsubscript{DL}, namely Enkephalin, Protein Kinase C delta (PKC\textdelta) or Striatal-Enriched Protein Tyrosine Phosphatase (STEP). While OTRs show little co-localization with VGAT and GAD67-positive processes, OTRs co-localize with ENK-positive processes. As OT was shown to modulate GABA-ergic transmission, we next investigated if activating GABA\textsubscript{A} receptors in the BNST\textsubscript{DL} would affect cued fear in a manner similar to OTR. Here, GABA\textsubscript{A} receptor agonist Muscimol (1 ng, \(n = 9\)) or
vehicle (n = 10) were infused through bilateral cannulas into the BNST<sub>DL</sub> before fear conditioning, during which rats were presented with visual conditioned stimuli (CS) co-terminating with foot-shocks. 24 hours later rats were tested for fear recall, where acoustic startle responses (ASR) to white noise bursts were scored either during or between the CS presentations, which enabled measuring cued and non-cued fear, respectively. All rats showed a significant potentiation of ASR during (P = 0.0002) and between CS presentations (P = 0.0044), but there was no treatment effect, nor interaction between the trial type and treatment (two-way repeated measures ANOVA). In addition, there was no significant difference in the percentage change of cued fear (P = 0.3509), non-cued fear (P = 0.3310), or discrimination index (P = 0.9834, unpaired t-test) between groups. We also performed a trial-by-trial analysis to determine the effects of Muscimol on fear recall as a function of time. Two-way ANOVA showed a significant effect of treatment on ASR measured during fear memory recall (P = 0.0024) but there was no interaction between the treatment and trial type (P = 0.5443), indicating that Muscimol reduced ASR both during and between the CS presentations. We conclude that inhibition of the BNST<sub>DL</sub> reduces ASR during fear memory recall, independent of the trial type, in contrast to OTR, which affects ASR only during CS presentations.


Poster
319. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 319.05/S14

Topic: F.04. Stress and the Brain

Support: NIA Grant K23AG058752

Title: Physical activity as a moderator of the relationship between stress and limbic grey matter in older adults

Memory and Aging Center, Dept. of Neurol., Univ. of California, San Francisco, San Francisco, CA

Abstract: Background: Perceived stress is commonly associated with smaller amygdalar and hippocampal grey matter volumes. Atrophy in the hippocampus and amygdala are established indicators for conversion from “normal aging” to development of cognitive impairment. Thus, it is of great interest to identify strategies to support the function of these limbic structures. We
explored the potential role of physical activity as a moderator of the relationship between perceived stress and grey matter volume in older adults.

**Method:** 34 functionally normal older adults (ages 53-90; CDR =0) wore a FitBit Flex2 device for all waking hours for ≥14 days, blinded to activity levels. Fitbit-derived daily average steps and calories burned were calculated. Participants also completed the Perceived Stress Scale and a structural MRI via a 3-Tesla Siemens scanner. Regions of interest (ROI) included bilateral hippocampal and amygdalar volumes, as well as occipital volume as a control ROI; analyses adjusted for total intracranial volumes.

**Results:** Higher perceived stress was associated with smaller hippocampal and amygdalar volumes (β range -0.28 to 0.36, ps<0.03). There was a significant interaction between perceived stress and average daily calories burned on grey matter volumes in the right hippocampus (stress*calories β=0.29, p=0.04), and right (β=0.33, p=0.03) and left (β=0.27, p=0.04) amygdala, such that the relationship between perceived stress and grey matter volumes attenuated with increasing physical activity. In the left hippocampus, the interaction was not significant (β=16, p=0.22). Parallel moderation models examining the control ROI were not significant (stress*calories β=0.18, p=0.15). Models examining the moderating role of average daily steps did not reach significance for the hippocampi and amygdala (stress*average steps β range 0.15 to 0.23, ps>0.26).

**Conclusion:** Our data provide early support for the protective role of physical activity on the relationship between perceived stress and limbic grey matter volumes in typically aging adults. Specifically, promoting higher intensity physical activity interventions aimed at burning more calories may support grey matter integrity among individuals reporting high stress. Given that limbic grey matter loss is the hallmark of Alzheimer’s disease, these results suggest a possible avenue by which to combat this major public health issue in at-risk adults. Future research should consider a longitudinal study design in order to examine within person effects, which may further corroborate the utilization of high calorie burning routines as protective against the negative effect of stress on cognition.


**Poster**

**319. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 319.06/S15

**Topic:** F.04. Stress and the Brain

**Support:** VA Grant IK2 BX003630
VA Grant IK6 BX003610
Title: Inhibiting the central amygdala-bed nucleus pathway ‘turns-off’ stress-induced visceral pain

Authors: *A. C. JOHNSON*¹,², B. GREENWOOD-VAN MEERVELD¹,³;
¹Res., VA Hlth. Care Syst., Oklahoma City, OK; ²Neurol., ³Oklahoma Ctr. for Neurosci., Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK

Abstract: Introduction: Psychological stress exacerbates abdominal pain experienced by patients with functional gastrointestinal disorders such as irritable bowel syndrome (IBS). Imaging studies of IBS patients have demonstrated abnormal corticolimbic circuitry in response to noxious visceral stimulation. In male rats, we have shown that optogenetic stimulation of central nucleus of the amygdala (CeA) terminals at the bed nucleus of the stria terminalis (BNST), a pathway that is abnormal in IBS patients, increases colonic sensitivity to distension. The hypothesis for this study was that inhibition of the CeA-BNST pathway would reverse stress-induced colonic hypersensitivity. **Methods:** We used stereotaxic procedures to infect neurons in the CeA with viral vectors to express channelrhodopsin (ChR2) or halorhodopsin (HR3.0) in male Fisher 344 rats. Bilateral cannulae were implanted at the BNST for fiber optic stimulation of CeA terminals. After 10 weeks, rats underwent 7 days of water avoidance stress (WAS) or SHAM-stress (1 hr/day). Twenty-four hours after the last WAS/SHAM-stress, laser stimulation at 473 nm for ChR2 (20 Hz, 5 ms) or 532 nm for HR3.0 (continuous) was used to modulate the response to graded, isobaric colorectal distension (20-60 mmHg, 10 min) quantified as the number of abdominal muscle contractions in freely moving rats. Results were analyzed with a repeated measure two-way ANOVA with Bonferroni’s post-hoc analysis (mean ± standard deviation). **Results:** In rats with WAS-induced colonic hypersensitivity, HR3.0-mediated inhibition of CeA terminals at the BNST normalized colonic sensitivity to distension (60 mmHg: WAS+HR3.0: 20.7±6.4, P= 0.01 vs. WAS+ChR2: 39.0±10.0, P=0.99 vs. SHAM+HR3.0: 23.0±0.0). HR3.0 activation did not affect colonic sensitivity in SHAM exposed rats (60 mmHg: SHAM: 22.3 ± 3.1, P= 0.99 vs. SHAM+HR3.0). ChR2 stimulation of CeA terminals at the BNST in SHAM-stressed rats induced colonic hypersensitivity to distension (60 mmHg: SHAM+ChR2: 37.3 ± 8.7, P= 0.01 vs. SHAM). In rats exposed to WAS, ChR2 did not further increase the stress-induced colonic hypersensitivity (60 mmHg: WAS: 34.5 ± 12.5, P= 0.99 vs. WAS+ChR2). **Conclusions:** Neurotransmission from the CeA to the BNST is necessary for the expression of stress-exacerbated colonic sensitivity, without affecting normal responses to colonic stimulation in male rats. These results provide support for developing therapies targeting limbic circuits, which could decrease chronic visceral pain in disorders such as IBS.

Disclosures: A.C. Johnson: None. B. Greenwood-Van Meerveld: None.
The role of the amygdala in the autonomic regulation of stress and anxiety

Authors: *H. H. SUBRAMANIAN*¹, G. HOLSTEGE²;
¹Boston Scientific, Valencia, CA; ²Rijksuniversiteit Groningen, Groningen, Netherlands

Abstract: The amygdala is known to play an important role in the limbic system, which regulates fear and anxiety. Differences in fear and anxiety are associated with changes in breathing, cardiovascular and urinary function. Although the amygdala has been recognized as an important center within the limbic system, it is not known whether it has any direct causal influence on breathing, cardiovascular or urinary functions. In isoflurane anesthetized, vagi-intact, spontaneously breathing cats (n= 4, experiments undertaken at The University of Queensland, Australia) we stereotaxically stimulated the lateral and central areas of the amygdala with metabotropic glutamic acid (L-Glut, 500 mM, pH 7.4), and investigated the changes in diaphragm function, thoracic pressure, blood pressure, heart-rate and urodynamic control. Stimulation in the lateral amygdala generated tachypnea, inspiratory apneusis, hyperpnea, and double diaphragm breathing pattern, while stimulation of the central amygdala induced predominantly dyspnea and bradypnea. Apneas were never generated from either of the two regions of the amygdala. The cardiovascular effects generated from the amygdala were varied and did not show specific topography as respiration. Both the lateral and central amygdala induced hypertension as well as hypotension. Neither lateral amygdala nor central stimulation produced micturition. The results confirm the role of the amygdala in regulating the autonomic system during emotional processing including the levels of fear and anxiety. These effects also constitute a variety of autonomic indexes that can be used for clinical determination of stress and anxiety disorders including Post Traumatic Stress Disorder (PTSD) and panic disorders in humans.

Disclosures: H.H. Subramanian: None. G. Holstege: None.
Voluntary exercise promotes sex-specific exploration and downregulation of neural oxytocin activity in socially isolated prairie voles

*W. WATANASRIYAKUL, M. C. NORMANN, M. COX, S. CIOSEK, S. SUJET, O. I. AKINBO, A. J. GRIPPO;
Psychology, Northern Illinois Univ., DeKalb, IL

Abstract: Loneliness is associated with anxiety, which may be mediated by neuropeptide function in the hypothalamus, including oxytocin (OT). Aerobic exercise improves anxiety symptoms, such as social avoidance and hypervigilance, in humans and animal models. However, the interactions of OT and exercise in the context of social stress and anxiety are unclear. The prairie vole is a valuable translational model to study social stress because this species lives in a similar social structure to humans, including exhibiting social monogamy, biparental care, and negative responses to social stressors. Additionally, exercise may serve a protective role against social stress in this species. Therefore, the current study examined the potential anxiety-reducing effects of voluntary exercise in socially isolated prairie voles. Ninety-one adult male and female prairie voles were randomly selected into 3 experimental groups: paired controls, isolation/sedentary, and isolation/exercise. Animals in the social isolation conditions were housed individually for 8 weeks, and animals in the exercise condition received a running wheel during the last 4 weeks of this isolation period. Control animals were pair-housed with a same-sex sibling for 8 weeks. Running behavior in physically active animals was categorized as low or high by a median split of the mean daily distance traveled. At the end of this 8-week period, all animals were exposed to a 20-min open field test (OFT) to examine anxiety-like behavior. Brains were collected from all animals 2 hours after the OFT was concluded. Overall, males were significantly more active in the OFT compared to females. Additionally, socially isolated males displayed significantly reduced total distance traveled in the OFT (v. paired control males), and low exercise prevented this reduction. No group differences were observed in exploratory behaviors of females. Interestingly, there was a significant positive correlation between running behavior and total distance traveled in the OFT, only in females. Immunohistochemical analyses revealed that socially isolated females had significantly higher activated OT cells (co-labeling of cFos and OT) in the hypothalamic paraventricular nucleus (v. paired females), and high exercise attenuated this elevation. No group differences were observed
in the OT activity of males. In summary, exercise protected against behavioral changes in males but neural changes in females. The current findings inform our understanding of the potential benefits of exercise for socially isolated or lonely individuals.


**Poster**

319. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 319.09/S18

**Topic:** F.04. Stress and the Brain

**Support:** National Academy of Sciences of Armenia

**Title:** How the hypothalamus regulate activity of solitary tract neurons and heart rate variability during psycho-emotional stress in rats?

**Authors:** *N. BEHNAM DEHKORDI*\(^1\), V. SARKISIAN\(^1\), E. AVETISYAN\(^1\), A. PETROSYAN\(^1\), N. SAHAKYAN\(^2\), S. SHOGERYAN\(^2\);
\(^1\)Natl. Acad. of Sci. Republic of Armenia, Yerevan, Armenia; \(^2\)Armenian State Pedagogical Univ., Yerevan, Armenia

**Abstract:** **Objective:** Central organization of emotional and visceral responses of hypothalamic structures and particularly, the paraventricular nucleus (PVN) determines the relationship of psycho-emotional stress with changes in the regulation of autonomic reactions. However, to date there is no comprehensive studies of the mechanisms of central PVN regulation of impulse activity of solitary tract (ST) visceral sensory neurons, as well as their effects on the cardiovascular system in health and psycho-emotional stress

**Methods:** In rats under urethane anesthesia bipolar stimulating electrodes introduced in the anteromedial region of the hypothalamus. Extracellular recording of neuronal activity was carried out from the medial region of ST. Record of background impulses lasted for 10 seconds, tetanic stimulus duration was 1 s and post-stimulus changes the behavior of neurons in the PVN was recorded for 10 to 200 or 400 ms / bin. Analysis of cardiac activity was carried out by method of mathematical analysis of heart rate variability (MA HRV). We studied the most important indices: heart rate (HR), vegetative balance index (PSI) and the tension index of regulatory systems (TIRS). The data is processed using a Microsoft Excel spreadsheet, the level of statistical significance was determined by Student's t-test.

**Results:** Significant reactivity of solitary neurons (62%) to descending paraventricular inputs was revealed. The tetanic stimulation of PVN (100-Hz during 1-sec) resulted in pronounced tetanic depression with post-tetanic potentiation. In the mathematical analysis of some
functionally significant HRV parameters: the heart rate (HR), the vegetative equilibrium index (IWR), the intensity index of the regulatory systems before and immediately after immobilization a beneficial effect of the frequency stimulation of PVN was found.

**Conclusion:** Our results suggest that some decrease in the stress reaction is evidently due to the initiation of the neuro-regulative inhibitory control of PVN supporting the realization of vago-vagal reflexes via STN neurons.

**KEYWORDS:** Paraventricular nucleus of the hypothalamus, solitary tract nucleus, psycho-emotional stress, tetanic potentiation and depression, extracellular recording.

**Disclosures:** N. Behnam Dehkordi: None. V. Sarkisian: None. E. Avetisyan: None. A. Petrosyan: None. N. Sahakyan: None. S. Shogeryan: None.

**Poster**

319. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 319.10/T1

**Topic:** F.04. Stress and the Brain

**Support:** CIHR

**Title:** Transcriptional responses to repeated restraint stress exposure in male and female rats

**Authors:** *T. J. PHILIPPE*¹, M. DORDEVIC¹, P. PAVLIDIS², T. UNDERHILL³, V. G. VIAU¹; ¹Cell Physiological Sci., ²Psychiatry, ³Univ. British Columbia, Vancouver, BC, Canada

**Abstract:** Experience with a controllable challenge can protect an organism from the negative consequences of stress. Thus, determining the molecular mechanisms of adaptive responses has important implications for understanding stress resistance and vulnerability. Here we used a model of stress hypothalamic-pituitary-adrenal (HPA) axis habituation and compared transcriptional responses in male and female Sprague Dawley rats exposed to a single bout of 2h restraint or to multiple bouts repeated daily for 5 consecutive days. Serial blood sampling indicated comparable declines in corticosterone responsiveness to repeated restraint in males and females. However, this was met by very limited overlap, if not completely different transcriptional profiles between the sexes depending on the brain region surveyed. RNA sequencing revealed 3 types of responses in the raphe nucleus, reflected by 1) transient changes in differentially expressed genes (DEGs) that returned to baseline after repeated restraint; 2) sustained increments/decrements in DEGs unique to the repeat restraint condition; and 3) sustained changes in DEGs regardless of restraint experience. Distinct transcriptional patterns also occurred within forebrain regions known to be targeted by the raphe-serotonin system and/or mediating adaptive neuroendocrine and behavioral responses. DEGs uniquely associated
with the repeat restraint condition was the predominant response type in the septum and zona incerta. However, changes in gene expression in the septum occurred only in males, whereas changes in the zona incerta occurred only in females. In the paraventricular nucleus of the hypothalamus, the final common pathway regulating the HPA axis, only females responded to show DEGs induced by both single and repeated restraint. Many of these DEGs in the paraventricular nucleus, zona incerta and septum belong to key neurotransmitter (serotonergic, GABAergic) and neuroendocrine systems, in addition to those mediating synaptic remodeling and signaling pathways. The extent to which these transcriptional alterations contribute to the state of habituation, or otherwise respond to stress, remains to be seen. Nonetheless, the current findings provide several testable frameworks for determining factors controlling sex differences in adaptive responses.


Poster

319. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 319.11/T2

Topic: F.04. Stress and the Brain

Support: CONACYT-CB_238744
UNAM-PAPIIT-IN216918
NIMH-IRP-MH002386

Title: Predator odor evokes stress response and fos activation in sensorial and limbic regions: Role of PACAP-PAC1 signaling for circuit interactions

Authors: *L. Zhang¹, V. S. Hernández¹, S. Sweat², L. E. Eiden²;
¹Av. Univ. 3000, Mexico City, Mexico; ²Sec Molec Neurosci, NIH, NIMH-IRP, Bethesda, MD

Abstract: Pituitary adenylate cyclase-activating polypeptide (PACAP) signaling has been suggested as a pleiotropic transmitter for stress responding. We have recently observed, via RNAscope methodology, that PACAP and PAC1 expression is distributed with either excitatory or inhibitor co-transmitters in the mouse central nervous system in a pattern showing a marked dominance in sensorial pathways and limbic circuits. This pattern suggests that PACAP/PAC1 signaling may be importantly involved in emotion-based decision-making and motor coordination for stress coping. Here, we have assessed the effect of predator odor (cat urine) exposure on the behavior of wild-type compared to PACAP knock-out C57Bl6/N mice. Cat urine material was collected from domestic cat litter boxes and was inserted into a flask (A) (2cm of opening bore) within a custom-made wooden box (B) (28cm x 28cm x 28 cm, with a sliding
After introducing the subject, the lid of A was opened and the lid of B was closed. Video recording was made during a 10 min period, after which animals were euthanized by cervical dislocation and brains rapidly removed and processed for double in situ hybridization histochemistry using the RNAscope 2.5 HD Duplex Assay. The neuronal activation in the olfactory bulb (OB), piriform cortex (Pir), medial and central amygdala (MeA and CeA), bed nucleus of *stria terminalis* (BNST), ventromedial hypothalamic nucleus (VMH), lateral septum (LS), parabrachial nucleus (PBN) and hippocampus were assessed for *fos* expression within PACAP, PAC1, VGluT2 and VGAT-positive cells. Cat urine exposure triggered purposeful movement such as object exploration/complete retreat and freezing behaviors in both wild-type and knock-out mice, with the PACAP-deficient (knock-out, ko) group showing significantly less of both behaviors than the wild-type group (p < 0.001 and p < 0.05 respectively). Spatial preference *heat map* showed that the wt mice had clear border preference in the 3 quadrants other than the quadrant where A was located, while the ko mice showed no difference in this parameter. *Fos* mRNA expression was significantly less in the PACAP-deficient compared to the wild-type group in OB (mitral and glomerular layers), MeA and CeA, BNSToval and BNSTam, VMH, PBN (p < 0.001 in all cases), with similar expression in both groups in the dorsal and ventral DG and in LS. These data provide some of the first evidence implicating the sensorial hypo-functioning and stress hypo-responding in PACAP-KO subjects and offer an initial step towards identifying the PACAP-PAC1 signaling in sensory-emotional circuits in stress susceptibility and responding.


Poster

319. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 319.12/T3

Topic: F.04. Stress and the Brain

Support: NIH grant MH28380 to RTR
PA Dept. of Health Funds to MER

Title: Sexually diergic effects of environmental enrichment and removal on baseline and restraint stress HPA axis responses in rats

Authors: *M. E. RHODES*¹, G. C. NOEL¹, E. P. ROHM¹, B. R. SCHEIDER¹, R. T. RUBIN²; ¹Biol., St. Vincent Col., LATROBE, PA; ²Psychiatry & Biobehavioral Sci., David Geffen Sch. of Medicine, UCLA, Los Angeles, CA

Abstract: Structural and social aspects of housing and husbandry conditions can influence the physiology and behavior of laboratory animals. Because many of our experiments incorporate
singly-housed, jugular vein-cannulated (JVC), male and female rats into pharmacological dosing studies, alleviating potential stress from single housing is imperative. Our laboratory has previously demonstrated that housing animals during the acclimation period with commonly used environmental enrichment (EE) toys resulted in lower hypothalamic-pituitary-adrenal (HPA) axis activity before and after mild stress. The specific EE toys, Nestlets® and Kong® Toys, were chosen because of their potential to accentuate nesting and gnawing/playing behaviors, respectively. The present study used these same toys to examine the effects of EE, and the removal of EE, on stress-responsive hormones of the HPA axis under basal conditions and after acute restraint stress in singly-housed, JVC, male and female rats. All animals were presented with EE upon arrival in our animal facility (Day 0). On Days 1-7, a daily blood sample was collected from each animal. After the blood sample collection on Day 4, EE was removed from randomly selected cages, such that two groups were formed: 1) male and female animals that remained housed with EE, and 2) male and female animals that had lost EE. On Day 8, blood samples were collected from each animal before and after 15 min restraint stress. Plasma adrenocorticotropic hormone (ACTH) and corticosterone (CORT) concentrations were determined by highly specific immunoassays. Consistent with our previous findings, baseline ACTH and CORT decreased over time in male and female groups housed with EE. Removal of EE resulted in sexually diergic baseline hormone effects: male ACTH and CORT increased, and female ACTH and CORT decreased, after EE removal. Moreover, removal of EE resulted in sexually diergic hormone responses to restraint stress: Overall, male ACTH and CORT responses were higher and returned to baseline more slowly, and female ACTH and CORT responses were lower and returned to baseline more rapidly, compared to males and females that retained EE. These data indicate that sudden changes to the housing environment of laboratory animals may have both immediate and lasting effects on their physiology and likely their behavior. EE provides a distraction from standard housing conditions and decreases the HPA stress response in laboratory animals. It also appears that the loss of EE influences baseline and stress-induced HPA axis activity in a sexually diergic manner, increasing hormone secretion in males and decreasing it in females.


Poster

319. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 319.13/T4

Topic: F.04. Stress and the Brain

Support: JSPS KAKENHI 16K19006 (N.K.)
JSPS KAKENHI 19K06954 (N.K.)
Title: A cortico-hypothalamic pathway that mediates sympathetic and behavioral responses to psychosocial stress

Authors: *N. KATAOKA, Y. SHIMA, K. NAKAMURA;
Dept. of Integrative Physiol., Nagoya Univ. Grad. Sch. of Med., Nagoya, Japan

Abstract: Psychological stress causes a variety of sympathetic and behavioral responses in mammals, which help animals cope with stressors. We have reported that psychological stress induces thermogenesis in brown adipose tissue (BAT), hyperthermia and tachycardia by activating a monosynaptic neural pathway from the dorsomedial hypothalamus (DMH) to the rostral medullary raphe. Here we found that the DMH receives glutamatergic sympathoexcitatory stress inputs from the dorsal peduncular cortex and dorsal tenia tecta (DP/DTT), located at the ventral limit of the medial prefrontal cortex. To determine how important the DP/DTT-DMH neural pathway is for the drive of sympathetic stress responses, we injected anterograde and retrograde adeno-associated viruses (AAVs) into the rat DP/DTT and DMH, respectively, to selectively ablate DP/DTT-DMH projection neurons with Cre-dependent expression of a genetically engineered caspase-3. Ablation of DP/DTT-DMH neurons did not affect the basal control of body temperature, but did eliminate BAT thermogenesis and hyperthermia induced by social defeat stress, a psychosocial stress model. We further tested the effect of optogenetic inhibition of the DP/DTT-DMH pathway on stress behavior. Social interaction tests were conducted by using rats given social defeat stress. The defeated rats exhibited reduced social interaction with the dominant rats by which they were defeated during the first confrontation. However, optogenetic inhibition of DP/DTT-DMH projection neurons, which expressed iChloC-mCherry, a chloride-conducting channelrhodopsin, restored social interaction, and also eliminated sympathetic stress responses including hyperthermia and tachycardia. These results indicate that the DP/DTT-DMH monosynaptic excitatory pathway transmits stress signals essential for the hypothalamus to drive sympathetic and behavioral responses for stress coping.

Disclosures: N. Kataoka: None. Y. Shima: None. K. Nakamura: None.

Poster

319. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 319.14/T5
**Topic:** F.04. Stress and the Brain

**Support:**
- Mathison Centre for Mental Health Research and Education
- Brain Canada
- Canadian Institutes of Health Research

**Title:** Topographical and projection specific activation of basolateral amygdala neurons in response to stress

**Authors:**
*R. J. AUKEMA*¹, S. L. BAGLOT¹, B. K. LAU², G. N. PETRIE¹, A. S. NASTASE¹, M. MORENA³, S. KADHIM¹, S. L. BORGLAND⁴, M. N. HILL²;
²Hotchkiss Brain Inst., ³Cell Biol. & Anat. and Psychiatry, ⁴Physiol. Pharmacol., ¹Univ. of Calgary, Calgary, AB, Canada

**Abstract:** The basolateral amygdala (BLA) is an important brain region activated by psychological stress, functioning to integrate sensory information with higher-order cognitive information such as learned behavior and motivational drive. In turn, it projects to several brain regions capable of modulating various behavioral and physiological processes. However, it is unclear which projection populations are recruited during stress exposure and how each population individually contributes to the overall stress response. Using adult male rats, we first mapped the topographical distribution of c-fos (a marker of neural activity) within the BLA in response to both appetitive (female odor, palatable food) and aversive (restraint, predator odor, foot shock, swim stress) stimuli. Noticeably, c-fos expression in response to these stimuli was not homogenously distributed, but rather expression was biased towards certain aspects of the BLA. We then investigated which BLA projection populations are recruited by exposure to acute restraint stress. We first mapped the topographical distribution of five projection populations within the BLA (targeting the ventral hippocampus, nucleus accumbens, prelimbic cortex, central amygdala, and lateral hypothalamus) using the retrograde tracer choleratoxin subunit B (CTb). Consistent with prior literature, each projection population exhibited a unique topographical distribution within the BLA. Next, we identified which of these populations were responsive to acute restraint stress. Discrete BLA projection populations were identified by choleratoxin subunit B (Ctb) and examined for colocalization with c-fos (indicating neural activation) following 30-minute restraint stress exposure. Populations targeting the nucleus accumbens, prelimbic cortex, and ventral hippocampus were robustly activated by stress. To a lesser extent, populations targeting the central amygdala and lateral hypothalamus were also activated. This suggests that these projections are important in the collective stress response and provides a framework to now begin investigating the individual role of each projection. Given that distinct amygdala projection neuron populations can influence discrete - and sometimes even opposite - behaviors, it is possible that each population uniquely contributes to discrete aspects of the collective stress response.

Sexually dimorphic effects of stress and age on orexin receptor mRNA expression in the paraventricular thalamic nucleus

Orexins are neuropeptides involved in states of motivational relevance such as addiction, reward, and anxiety, and are important for responses to both acute and repeated stress. Orexins have an anxiogenic effect when administered in the posterior paraventricular thalamic nucleus (pPVT), an area that coordinates input from stress-activated neurotransmitter systems to structures that regulate motivation and mood. Dysregulation of orexins is seen in stress-related psychiatric disorders, such as Major Depressive Disorder. Incidence of these disorders is twice as high in women compared to men, and sex differences in the prevalence and symptoms of these disorders emerge during adolescence. In previous work, we have shown that orexins are important in mediating sex-differences in the response to repeated stress and in cognitive function (Grafe et al., Biological Psychiatry 2017). Less is known about sex differences or the impact of stress on expression of orexin receptors 1 and 2 (HCRTR1/2). This study examined the quantity and distribution of both orexin receptor mRNA in the pPVT across age, sex, and stress status. We ran cohorts of adult (PND 74) and early adolescent (PND 30) male and female rats through five days of social defeat, a repeated stress paradigm in which an experimental “intruder” rat is placed in the home cage of a dominant “resident” rat. This paradigm allows us to further separate the rats into vulnerable and resilient groups. Using an in-situ hybridization protocol from RNAscope, we co-stained HCRTR1 and 2 mRNA in the pPVT to quantify the overall number of transcripts as well as cell type (the number of cells containing both orexin transcripts versus a single orexin transcript type). In general, the results indicated similar findings with both HCRTR1 and HCRTR2. Male adolescent and adult defeated rats exhibit decreased orexin receptor mRNA expression compared to non-stressed controls, and in adults this effect of defeat is primarily seen in the vulnerable group. Female adults have decreased orexin receptor mRNA expression compared to adolescents regardless of defeat status, suggesting a stress-independent developmental shift in orexin receptor mRNA expression in the
pPVT of females. Thus, the expression of mRNA both orexin receptors was influenced primarily by stress in male rats but by age in female rats. These findings are a starting point to further investigate the neural differences that underlie sex-specific responses to repeated stress across age.

**Disclosures:** A. Vigderman: None. S. Luz: None. A. Shingala: None. S. Bhatnagar: None.

**Poster**

319. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 319.16/T7

**Topic:** F.04. Stress and the Brain

**Support:** FI2GM119962

**Title:** Traumatic stress induces long lasting violent aggression through potentiation of a medial amygdala circuit

**Authors:** *J. NORDMAN*¹, Z. LI²;

¹NIH, Bethesda, MD; ²NIMH, Bethesda, MD

**Abstract:** Traumatic stress has long been shown in humans and rodents to promote excessive and recurring violent aggression, co-morbid with psychiatric diseases such as PTSD, intermittent explosive disorder, and schizophrenia. In individuals suffering from PTSD, hyperactivity of the amygdala is strongly correlated with stress responses that trigger anger and violence. The medial amygdala (MeA) is an evolutionarily conserved subnucleus of the amygdala that regulates attack behavior and has been implicated in stress induced aggression. The precise contribution of the MeA in traumatic stress induced aggression, however, requires further elucidation. In this study we used a modified foot shock protocol, a common method for inducing aggression in rodents, to evaluate the circuit level mechanisms of the MeA in driving long lasting attack behavior associated with traumatic stress. We observed that foot shock increased aggression for weeks after training. Previous studies have shown that the MeA regulates aggression through two canonical members of the aggression circuit, the ventromedial hypothalamus (VmH) and bed nucleus of the stria terminalis (BNST). Based on previous studies and our preliminary findings, we hypothesized that traumatic stress induces aggression through potentiation of these same pathways. In vivo electrophysiological recordings, a method for detecting neural activity in an awake and freely moving animal, revealed significant and sustained increases in optically evoked excitatory postsynaptic potentials, indicative of long-term potentiation. Intriguingly, low frequency photostimulation (LFPS), an optogenetic strategy for inducing synaptic depression in the brain, suppressed long-lasting aggression associated with our foot shock protocol when applied directly to the MeA, confirming that potentiation of these circuits is integral for the
observed attack behavior. Notably, these effects were irrespective of non-violent social behavior or fear memory. These results reveal an important function for the MeA in traumatic stress induced aggression and may be useful in developing therapeutic strategies to treat excessive aggression associated with traumatic stress and psychiatric disease.

**Disclosures:** J. Nordman: None. Z. Li: None.

**Poster**

**319. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 319.17/T8

**Topic:** F.04. Stress and the Brain

**Support:** NIH Grant R15 MH104485
NRT Grant USD-N3 DGE-1633213

**Title:** Stress responses are bidirectionally regulated through amygdalar orexin 1 and 2 receptors

**Authors:** *J. D. W. YAEGER¹, K. T. KRUPP¹, N. T. JONES¹, B. MEYERINK³, T. R. SUMMERS², J. T. CAIN³, C. H. SUMMERS²;
²Dept. of Biol., ¹Univ. of South Dakota, Vermillion, SD; ³Sanford Res., Sioux Falls, SD

**Abstract:** The orexin (Orx) system regulates adaptive stress responses; therefore, maladaptive reactions to stress (susceptibility) may arise as a result of irregular orexin function. Orexin A (OrxA) and B (OrxB) are produced in the lateral/dorsomedial-perifornical (LH/DMH-PeF) regions of the hypothalamus. These neuropeptides activate neuronal targets through two receptors: orexin 1 (Orx1) and 2 (Orx2). Although OrxA stimulates both receptors, OrxB exhibits a binding preference for Orx2. These receptors are dispersed broadly throughout the central nervous system, including the amygdala, a region associated with emotional interpretation/learning. Within the basolateral amygdala (BLA), susceptible mice express higher mRNA levels of Orx1 and lower levels of Orx2 compared to controls. Here, pharmacological manipulations of the Orx receptors in the BLA (intra-BLA) were performed after exposure to a social stress paradigm called the Stress Alternatives Model (SAM). The SAM is composed of an oval shaped, open field arena in which a small C57BL/6 mouse is paired, after a tone (conditioned stimulus), with a large CD-1 that aggressively pursues the smaller animal (unconditioned stimulus) over the course of four, 5-minute sessions. The SAM provides an opportunity for the smaller mouse to avoid defeat by escaping through tunnels too small for the aggressor. By the end of the second trial, mice select a stress-related phenotype from which they do not deviate in subsequent trials: Escape/Resilient or Stay/Susceptible. Therefore, treatments can be administered before the third session, after phenotype commitment, in an attempt to pharmacologically shift the stress state of the animal. In a series of experiments, the SAM was
paired with the open field (OF) and social interaction/preference (SIP) tests. With intra-BLA Orx1 antagonism (SB-674042; 0.3 nmol), 63% of Stay mice are rescued to the Escape phenotype. Also, Orx1 blockade reduced fear responses to both contextual and cued stimuli, decreased behavioral inhibition in the SAM and OF, and enhanced social learning in a non-social environment (OF). Intra-BLA infusion of Orx1 increased cued fear responses, enhanced freezing, and diminished social learning. Inhibition of BLA Orx2 (MK-1064; 0.1 nmol) exaggerated freezing responses in the SAM and OF, and decreased social preference in the SIP test. Activating intra-BLA Orx2 ([Ala11, d-Leu15]-Orx2; 0.1 nmol) produced the opposite effect in the SIP test. Together, the results illustrate a bidirectional role of Orx receptors in the BLA, with Orx1 activity contributing to negative stress responses and Orx2 stimulation promoting adaptive behaviors.


Poster

319. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 319.18/T9

Topic: F.04. Stress and the Brain

Support: Strategic Priority Research Program of the Chinese Academy of Sciences (XDB02050000 to G.-Q.B.) NSFC Grant 91732304 NSFC Grant 91632303

Title: Brainwide mapping of stress induced neuronal activation for large cohort

Authors: *H. WANG, C. YANG, Y. SHEN, S. HUANG, L. DING, Y. CHENG, Q. ZHU, P.-M. LAU, G.-Q. BI; Univ. of Sci. and Technol. of China, Hefei, China

Abstract: Severe or chronic stress is thought to be related to mental disorders such as post-traumatic stress disorder and depression. In rodent models, multiple brain areas have been found to be activated by various stress stimuli. Meanwhile, it is also known that, similar to human beings, model animals also exhibit individual differences in their behavior coping with stress conditions. In an attempt to unbiasedly evaluate the activation of neural circuits in response to behavioral stress, we performed a “large” cohort brain-wide mapping of neuron activity trace at subcellular resolution, using our recently developed high-throughput volumetric imaging system (VISoR). Analysis of neuronal activation based on c-Fos expression in over 20 mice under resting or stressed conditions revealed marked variability in the activation of multiple subcortical
areas by stress across different individual animals. Intriguingly, stress-induced activation of several brain areas exhibited strong lateralization. These results corroborate the idea that stress response of an individual animal can be very sensitive to its genetic and environmental factors, and suggest the necessity of sufficient cohort size for studying the circuit basis of specific behaviors.

**Disclosures:**  H. Wang: None.  C. Yang: None.  Y. Shen: None.  S. Huang: None.  L. Ding: None.  Y. Cheng: None.  Q. Zhu: None.  P. Lau: None.  G. Bi: None.

**Poster**

**319. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 319.19/T10

**Topic:** F.04. Stress and the Brain

**Support:** 1R56MH119456-01

**Title:** Agrp and POMC neurons are involved in mediating chronic stress-induced depressive behaviors

**Authors:** *X. FANG, J. WANG, S. JIANG, Y. LEI, X.-Y. LU; Augusta Univ., Augusta, GA

**Abstract:** The arcuate nucleus (ARC) of the hypothalamus contains two distinct subpopulations of neurons, expressing orexigenic agouti-related peptide (AgRP) and anorexigenic pro-opiomelanocortin (POMC). It is unknown that how AgRP and POMC neurons's response to chronic stress and the roles they play in the development of depressive-like behaviors. We demonstrated that chronic unpredictable stress (CUS) induces depressive-like behavioral phenotypes in mice, including anhedonia and behavioral despair. By using this model and electrophysiological recordings, we found that CUS decreased the firing rate of AgRP neurons but increased the firing rate of POMC neurons. Furthermore, synaptic input onto AgRP and POMC neurons were also differentially altered by CUS. Chemogenetic stimulation of these neurons cause opposite effects on CUS-induced behavioral changes. Our results suggest that AgRP and POMC neurons play an important role in chronic stress adaptation and associated behavioral deficits.

**Disclosures:** X. Fang: None. S. Jiang: None. J. Wang: None. Y. Lei: None. X. Lu: None.
**Title:** Resilience gene expression profiles and metabolic pathways in the amygdala associated with corticosterone induced posttraumatic growth

**Authors:** J. LI¹, J. CHEN², G. WOODWARD³, C. LI¹, E. XING³, L. ZHANG³, *H. LI³; ¹2Department of Bioinformatics Biostatistics and Bioinformatics, Georgetown Univ., Washington DC 20057, WA; ²Neurol., ³Psychiatry, Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD

**Abstract:** Resilience in the face of chronic stress or traumatic stress exposure is one of the critical neurobiological processes preventing the development of posttraumatic stress disorder (PTSD). Activating of glucocorticoid receptor signaling and subsequently mitigating the stress-induced symptoms after the traumatic events have been implicated in the posttraumatic growth (PTG). To discover molecule targets for pharmacological intervention in the PTG, current study examines the mitochondrial gene expression profile associated with glucocorticoid receptor signaling and the occurrence of stress-induced psychiatric symptoms utilizing mitochondria gene array and a rat model of PTSD. Our unsupervised clustering analysis revealed differential expression of 129 genes in the amygdala among 1500 mitochondria focused informative genes in the amygdala were dysregulated from three groups of experiments, including controls (n=10), stressed (n=10) and corticosterone treated (n=10). These results suggested that stress dysregulated two main category genes including corticosterone induced DRG and another is neuronal and/or other hormonal other than glucocorticoid involved DRG systems. These other than corticosterone activated systems appear to be involved in multiple neuronal and hormonal systems which contribute to the key symptom of PTSD, an exaggerated fear response. Thus, the corticosterone activated system appear to be involved in resilience to stress and attenuated the development of delayed and exaggerated fear response.

**Disclosures:** J. Li: None. J. Chen: None. G. Woodward: None. C. Li: None. E. Xing: None. L. Zhang: None. H. Li: None.
Title: Genetic and endocrinological analysis of stress response and anxiety-like behavior by cross-fostered hatano rats

Authors: *T. AKIMOTO;
Meiji Univ., Kawasaki-shi, Japan

Abstract: The fosterer is important factor in determining the traits of offspring because fostering behavior and the fosterer’s hormones affect anxiety-like behavior and stress response of offspring. Cross-fostering is one method to determine whether a certain trait of a child is characterized by the fosterer. Hatano high- (HAA) and low- (LAA) active avoidance rats are inbred strains selected from SD rats. The strains have different breeding behavior and blood hormone concentrations. Previously, we did cross-fostering using Hatano rats and clarified that the anxiety-like behavior of their offspring differs depending on the strain of the fosterer. However, the mechanisms of this behavioral change, as well as stress response, are still unclear. Therefore, in this study, we measured the stress response reactivity and its related mRNA expression in the brains of cross-fostered Hatano rat offspring to clarify the genetic and endocrinological mechanisms of the fosterer effects on offspring traits. On postnatal day 1, HAA and LAA offspring were either fostered by dams of the opposite strain or an unrelated foster dam of the same strain. Then, at 9 weeks of age, restraint stress was applied to the offspring and we measured adrenocorticotropic hormone (ACTH) to observe stress responsiveness. HAA offspring had significantly higher concentrations of ACTH than LAA offspring. In addition, HAA offspring reared by HAA dam also had significantly higher ACTH concentrations than HAA offspring reared by LAA dam after restraint stress. These results indicate that the characteristics of the foster dam affected the stress responsiveness of the offspring. Next, we assessed the anxiety-like behavior and stress reactivity of 5-week-old and 9-week-old cross-fostered Hatano rats by measuring the expression of related brain mRNA using RT-qPCR. Expression of Kcnj5 mRNA was significantly higher in the hippocampi of all 5-week old offspring reared by LAA dam than those of any offspring. In addition, expression of GR mRNA was initially higher in the hippocampus of 9-week-old HAA offspring reared by HAA dam than in HAA offspring reared by LAA dam, but after 60 minutes of restraint stress this significant difference disappeared. Furthermore, the expression of corticotropin releasing factor (Crf) hnRNA in 9-week old amygdala was significantly higher in the offspring reared by HAA dam than LAA dam before restraint stress, as was expression of CRH mRNA after restraint stress.
In summary, this study clarified that fosterer characteristics affect the genes involved in anxiety-like behavior and stress responsiveness in the brain of offspring.

Disclosures: T. Akimoto: None.

Poster

319. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 319.22/T13

Topic: F.04. Stress and the Brain

Support: R01MH112739

Title: Identification of a tachykininergic projection onto melanin-concentrating hormone (MCH) neurons in the lateral hypothalamic area (LHA)

Authors: *A. FUJITA\textsuperscript{1,2,3}, L. ZHONG\textsuperscript{1}, A. C. JACKSON\textsuperscript{1,2,3}; \textsuperscript{1}Physiol. and Neurobio., \textsuperscript{2}Connecticut Inst. for the Brain and Cognitive Sci., \textsuperscript{3}Biomed. Engin., Univ. of Connecticut, Storrs Mansfield, CT

Abstract: The lateral hypothalamic area (LHA) is a highly conserved, though heterogeneous, brain region critical for maintaining physiological and behavioral homeostasis. A unique neuronal population within the LHA is defined by the expression of melanin-concentrating hormone (MCH), and regulates multiple homeostatic functions including sleep-wake states, energy balance and stress. However, cellular and functional diversity among MCH neurons is not well understood. Previous anatomical and molecular data suggests that MCH neurons may be parsed into at least two transcriptionally-distinct subpopulations, with one enriched in transcript for the neurokinin 3 receptor (NK3R), a G-protein coupled receptor for the tachykinin neuropeptide family member neurokinin B (NKB), encoded by Tac2. This tachykininergic ligand-receptor system has been implicated in reproduction, fear memory and anxiety in other brain regions, but the function of NKB interactions with a subpopulation of MCH neurons is unknown. To begin to understand possible functional connectivity between NKB-expressing neurons and LHA MCH neurons, we first asked which NKB+ neuronal populations in the brain may be innervating the LHA. To accomplish this, we used Tac2-Cre mutant mice crossed to an enhanced yellow fluorescent protein (EYFP) reporter line (Tac2-Cre;EYFP), bilaterally injected in the LHA with a cre-dependent retrograde virus (rAAV-Flex-tdT). In the course of mapping retrogradely-labeled Tac2/EYFP+ neurons in the brain, we identified the bed nucleus of the stria terminalis (BNST) as one of several candidate brain regions that may be innervating the LHA. We subsequently performed anterograde tracing through bilateral injections of a cre-dependent anterograde virus (AAV-DIO-ChR2-EYFP) in the BNST and mapped fibers in the LHA, apposed to MCH neurons. Finally, using optogenetics in slices, we interrogated fast
neurotransmitter release from BNST Tac2+ fibers onto identified MCH neurons using a dual-viral approach. These data provide a foundation for characterizing a novel NKB/MCH neural circuit, which will lead to further, circuit-level insight into the function of anatomically and molecularly distinct subpopulations of MCH neurons.

**Disclosures:** A. Fujita: None. L. Zhong: None. A.C. Jackson: None.

**Poster**

**319. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 319.23/T14

**Topic:** F.04. Stress and the Brain

**Support:** NIH Grant MH 107435

NIH T32 Training Grant

**Title:** Endocannabinoid regulation of basolateral amygdala input to posterior tail of dorsal striatum and effects of stress

**Authors:** *V. KONDEV, D. MARCUS, G. BEDSE, S. PATEL;* 
Vanderbilt Univ., Nashville, TN

**Abstract:** The role of the basolateral amygdala (BLA) is complex, positioned for emotional responding, valence processing, and the modulation of fear and stress-related behaviors. It has been suggested that the many roles of the BLA may be due to the anatomical projection targets; specifically, that the BLA may encode information about stimuli in a projection-specific manner. While significant research has been done on BLA projections to the ventral striatum, BLA input to the dorsal striatum has received relatively less attention. Recent evidence suggests that the posterior tail of the dorsal striatum (TS) may represent a functionally distinct striatal territory, related to external threat and avoidance reinforcement. However, the question remains what the significance of the BLA-TS projection is and how it is regulated. Here, we show that BLA-TS synapses are stronger than to anterior dorsal striatum, glutamatergic, and express functional endocannabinoid (eCB) CB1 receptors. Furthermore, using *in vivo* optogenetics, we assess the functional role of this circuit in the regulation of anxiety and fear-related behaviors. These data will begin to identify a novel functional role of BLA-TS glutamatergic projection and the capacity for experience-dependent synaptic plasticity in this circuit. Elucidating diverse BLA efferent projections could provide insight into the diverse functional properties of the BLA and elucidate fundamental mechanisms by which emotional information gains access to basal ganglia circuits.

**Disclosures:** V. Kondev: None. D. Marcus: None. G. Bedse: None. S. Patel: None.
Title: Pubertal- and sex-dependent changes in the activity of brain nuclei controlling hormonal stress reactivity

Authors: M. R. BAKER, *R. D. ROMEO;
Barnard Col. of Columbia Univ., New York, NY

Abstract: Adolescence is associated with the maturation of the hypothalamic-pituitary-adrenal (HPA) axis, the major neuroendocrine axis mediating the hormonal stress response. Specifically, prepubertal males and females show greater hormonal stress reactivity than adults. Adolescence is also a developmental stage marked by a variety of stress-related vulnerabilities, including mood disorders, such as depression and anxiety. Thus, it is possible that this heightened stress reactivity plays a role in these vulnerabilities. Along with increased hormonal responsiveness prior to puberty, stressors also induce greater activity, as measured by FOS immunohistochemistry, in the paraventricular nucleus of the hypothalamus (PVN). As the PVN initiates the hormonal stress response, the heightened reactivity observed in prepubertal animals may in part be mediated by greater central drive to the PVN at this development stage. The current study examined this possibility by measuring stress-induced FOS responses in forebrain nuclei known to contribute direct excitatory and inhibitory inputs to the PVN in prepubertal and adult male and female rats. Replicating previous work, we found that both prepubertal males and females showed a greater number of stress-induced Fos-positive cells in the PVN immediately and 40min after termination of a 30min session of restraint compared to adult males and females. We also found that the anterior bed nucleus of the stria terminalis (aBST), a nucleus with direct excitatory projections to the PVN, showed a significantly higher number of FOS-positive cells following restraint, with the increase greatest in adolescent males. Though additional nuclei projecting to the PVN, such as the medial preoptic nucleus, the dorsomedial hypothalamus, and posterior bed nucleus of the stria terminalis, are currently being analyzed, these data suggest that greater activation of the aBST contributes to these pubertal-related increases in stress-induced HPA reactivity, at least in males. As adolescence is a period often marked by the onset of stress-related mood disorders, some of which show sex differences in their prevalence, it will be crucial to continue research aimed at understanding the neural mechanisms that mediate changes in stress reactivity during adolescence in both males and females.

Title: Interneuron-specific expression of α1A adrenergic receptors drives patterned inhibitory synaptic transmission in the basolateral amygdala

Authors: X. FU, J. G. TASKER; Cell and Mol. Biol., Tulane Univ., New Orleans, LA

Abstract: The central noradrenergic system plays a critical role in regulating the stress response, memory formation and anxiety behaviors. Recent clinical trials have shown the promise of the α1 adrenergic receptor blocker prazosin in treating posttraumatic stress disorder (PTSD). However, the distribution of the α1 adrenergic receptors in brain areas involved in emotional processing, like the amygdala, is controversial due to the poor specificity of adrenergic receptor antibodies. Here, we used mice with a genetic substitution of the α1A-subtype adrenergic receptor with β-galactosidase to investigate the location as well as the function of the α1A receptors in the basolateral amygdala (BLA). We found that the expression of α1A adrenergic receptors is restricted to GABAergic interneurons in the BLA, although not all the BLA GABA neurons show α1A receptor expression. Using whole-cell recordings of principal neurons in slices of BLA, we found that α1 adrenoreceptor activation induced specific patterns of synchronized inhibitory synaptic inputs from parvalbumin and CCK interneurons, both of which were eliminated in the α1A knockout animals. Our data suggest that α1A adrenergic receptors regulate local inhibitory neural circuits to drive patterned neural output from the BLA.

Disclosures: X. Fu: None. J.G. Tasker: None.
Support: NIH grant R01 MH104373

Title: Long lasting feedback inhibition of the HPA response to physical stress via glucocorticoid-induced changes in alpha-1 adrenergic receptor trafficking

Authors: *G. L. WEISS, A. NEILSON, S. NGUYEN, L. HARRISON, J. G. TASKER; Cell and Mol. Biol., Tulane Univ., New Orleans, LA

Abstract: Systemic release of glucocorticoids is regulated by negative feedback inhibition of corticotropin releasing hormone (CRH) neurons in the hypothalamus. Noradrenergic afferents provide a major excitatory input to the CRH neurons to drive sensory activation of the hypothalamic-pituitary-adrenal (HPA) axis by physiological stressors. Norepinephrine (NE) causes a robust increase in glutamatergic excitatory synaptic inputs to CRH neurons recorded by patch-clamp electrophysiology, which is completely abolished by prior restraint stress or glucocorticoid preincubation of brain slices. The NE desensitization lasts for at least 4 hours, extending beyond the expected two-hour time course of glucocorticoid release and recovery from a single stressor. Here, we found that mice subjected to prior restraint stress had a significantly attenuated glucocorticoid response to a physiological immune challenge (I.P. lipopolysaccharide, LPS) within a 4-hour window, but their response to a psychological stressor, predator odor exposure, was unaltered. The hypothalamic cell line N42 was used as an expression system to track intracellular alpha 1B receptor trafficking. Live-cell and FRET imaging revealed that dexamethasone suppresses alpha 1B receptor recycling to the membrane by increasing trafficking to late endosomes and decreasing trafficking to rapidly recycling endosomes. Analysis of post-translational modifications to b-arrestin showed that glucocorticoids alter b-arrestin ubiquitination and nitrosylation, which provides a mechanism for alpha 1 receptor desensitization. Modality-dependent HPA desensitization to stress may be adaptive by suppressing the neuroendocrine response to sensory stressors and allowing the animal to attend to life-threatening psychological stressors. However, this may be maladaptive to animals experiencing severe immune challenge, such as sepsis, as glucocorticoids are required to prevent an overactive immune response.

Disclosures: G.L. Weiss: None. A. Neilson: None. S. Nguyen: None. L. Harrison: None. J.G. Tasker: None.

Poster

319. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: 319.27/T18

Topic: F.04. Stress and the Brain

Support: NIH grant MH059911
Glucagon-like peptide 1 receptor signaling activates identified projection neurons within the central nucleus of the amygdala

Authors: *N. V. Povysheva1, H. Zheng2, L. M. Rinaman3;
1Univ. of Pittsburgh Dept. of Neurosci., Pittsburgh, PA; 2Psychology, 3Dept. of Psychology, Florida State Univ., Tallahassee, FL

Abstract: GLP1 receptor (GLP1R) signaling within the central nucleus of the amygdala (CEA) and the interconnected anterior lateral bed nucleus of stria terminalis (aBST) increases anxiety and other stress-related behaviors in rats. Here we examined whether GLP1 signaling activates CEA neurons that project to the aBST. Red fluorescent retrobeads were stereotaxically injected into the dorsal aBST in young adult male rats. One week later, whole-cell recordings were made in labeled neurons in brain slices containing the CEA. Ex-4 (400 nM), the synthetic GLP1 analogue, was bath applied to activate GLP1Rs. In current-clamp mode, Ex-4 produced an upward shift in the membrane potential of labeled neurons (p<0.01; n=11), which led to spontaneous firing in 4 out of 11 neurons. In other neurons (n=6), Ex-4 effects were blocked by prior application of the GLP1R antagonist Ex-9. Blockade of synaptic transmission eliminated the depolarizing effects of Ex-4 (n=6), suggesting an indirect effect of GLP1R signaling within the slice. In voltage-clamp mode, Ex-4 did not affect the amplitude or frequency of spontaneous IPSCs (n=7), but reduced the amplitude of miniature IPSCs (p<0.05, n=7). Ex-4 increased the amplitude of spontaneous EPSCs (p<0.05; n=5), but did not affect their frequency, and did not affect the amplitude or frequency of miniature EPSCs. Thus, GLP1R signaling promotes activation of dorsal aBST-projecting CEA neurons through membrane depolarization, enhanced excitatory drive, and reduced inhibitory drive, consistent with GLP1R-mediated recruitment of CEA-aBST circuits that contribute to anxiety and stress responsiveness.


Poster

319. Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 319.28/T19

Topic: F.07. Autonomic Regulation

Support: Natural Science Foundation of China (81471164)
Natural Science Foundation of China (31800881)
Natural Science Foundation of China (31671116)
Natural Science Foundation of China (9132306)
Natural Science Foundation of Guangdong Province (2015A030313877)
Key Research Program of Frontier Sciences of Chinese Academy of Sciences (QYZDB-SSW-SC056)
Identification of a central neural circuit that regulate bone mass

Authors: *F. YANG¹, Y. LIU¹,², S. CHEN¹,², D. GAO¹,², J. SHAO¹,², L. WANG¹;
¹The Brain Cognition and Brain Dis. Inst., Shenzhen Inst. of Advanced Technology, CAS, Shenzhen, China; ²Univ. of Chinese Acad. of Sci., Beijing, China

Abstract: The central nervous system is crucial in controlling energy expenditure and metabolism homeostasis; the extreme and isolated microenvironment could not only induce the psychological changes through central neural networks but also send signals to regulate the peripheral metabolism in a top-down manner. The homeostasis of bone metabolism is finely regulated by the central nervous system and recent studies have suggested that mood disorders such as anxiety are closely related with bone metabolic abnormalities. However up to now the central neural circuits regulating bone metabolism is not clear. In this study we found that the confined isolation of crewmembers could result in both elevated anxiety and decreased bone density; then we established a mouse model of anxiety-induced bone loss to understand the neural circuitry underlying the anxiety-induced bone loss. We firstly demonstrated that the GABAergic neural circuitry in ventromedial hypothalamus (VMH) mediated the anxiety-induced bone loss; a specific group of GABAergic neurons in bed nucleus of the stria terminalis (BNST) sent neural projections to VMH, and this BNST-VMH neural circuitry was both sufficient and necessary to drive the anxiety-induced bone loss through nucleus of solitary tract (NTS). Our findings thus not only provide important clues to understand the neural mechanism of anxiety induced bone loss, but also help to further understand the mechanism of brain top-down control of bone metabolism.


Poster

320. CRF in Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 320.01/T20

Topic: F.04. Stress and the Brain

Support: USDA National Institute of Food and Agriculture Grant 2014-67015-21655

Title: Early post hatch exposure to stressors altered growth and induced DNA hypomethylation of corticotropin releasing factor in the hypothalamic paraventricular nucleus of chicks predisposed to anorexia
**Authors:** *Y. XIAO, J. WANG, P. B. SIEGEL, M. A. CLINE, E. R. GILBERT; Dept. of Animal and Poultry Sci., Virginia Polytechnic Inst. and State Univ., Blacksburg, VA

**Abstract:** When subjected to a combination of nutritional and thermal stressors at hatch, chicks from a line selected for low body weight (LWS), which are predisposed to anorexia, had increased expression of neuropeptide Y (NPY) in the arcuate nucleus (ARC) and corticotropin-releasing factor (CRF) in the paraventricular nucleus (PVN) of the hypothalamus at 5 days post-hatch. Also, they were refractory to the orexigenic effect of exogenous NPY on day 5 post-stress. We hypothesized that stressor-induced changes in gene expression involved epigenetic regulation, and determined global DNA methylation and DNA methyltransferase (DNMT) activity, as well as NPY and CRF gene-specific methylation. Stressor-exposure was associated with reduced yolk sac resorption, body weight, lean mass, and fat mass during the first 5 days post-hatch. Stressor-exposure induced global hypermethylation and increased DNMT activity in the ARC but not PVN. There was no effect on methylation status of CpG sites near the NPY gene in the ARC. In the PVN of stressor-exposed chicks, there was hypomethylation of a CpG site that is in the core binding domain of methyl cytosine binding domain protein 2 (MBD2) in the CRF gene promoter. These results demonstrate that early-life exposure to stressors intensifies the anorexic condition in LWS chicks. There was reduced utilization of yolk reserves, impaired growth, and hypomethylation of a CpG site on the CRF promoter that is a target for a transcriptional repressor, MBD2. These findings provide novel insights on molecular mechanisms through which stressful events induce or intensify anorexia in individuals predisposed to the condition and provide a novel molecular target for subsequent studies.

**Disclosures:** Y. Xiao: None. J. Wang: None. P.B. Siegel: None. M.A. Cline: None. E.R. Gilbert: None.

**Poster**

**320. CRF in Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 320.02/U1

**Topic:** F.04. Stress and the Brain

**Support:** Bowles Center for Alcohol Studies

**Title:** Neuroactive steroid (3α,5α)3-hydroxypregnan-20-one alters the extrahypothalamic CRF signal and stress response with sex and regional specificity

**Authors:** *G. BOERO, I. BALAN, C. A. TODD, T. K. O'BUCKLEY, A. L. MORROW; Dept. of Psychiatry and Pharmacology, Bowles Ctr. for Alcohol Studies, Univ. of North Carolina Sch. of Med., Chapel Hill, NC
Abstract: Alterations in steroidogenesis and dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis activity have been observed in several neuropsychiatric disorders, including drug addiction. Corticotropin-releasing factor (CRF) regulates the stress response in the hypothalamus, but also modulates neurotransmission across the brain through CRF receptors. Acute stress increases CRF in hypothalamus (HYP) as well as the endogenous neurosteroid (3α,5α)3-hydroxypregnan-20-one (3α,5α-THP, allopregnanolone) which subsequently attenuates this CRF response, and enhances GABAergic transmission throughout brain. To test the possibility that 3α,5α-THP inhibits CRF signaling in extrahypothalamic regions, we examined the effects of 3α,5α-THP on the CRF signal and stress response in the central nucleus of the amygdala (CeA), hippocampus (HP), nucleus accumbens (NAc) and ventral tegmental area (VTA), both in male and female rats. Sprague Dawley rats were injected with 3α,5α-THP (15mg/kg, ip) in hydroxypropyl-beta-cyclodextrin (45%) or an equivalent volume of vehicle and after 15 min subjected to 30 min of restraint stress, sacrificed instantly or after 3hrs, and brains were harvested for western blot measurements of CRF. We observed a sex difference in basal CRF expression in HYP (males +72% vs females, p&lt0.05; 2-way ANOVA, Tukey’s post-hoc test), but not in CeA, HP, NAc or VTA. In males, 3α,5α-THP reduced basal levels of CRF in CeA (-64%, p&lt0.01), HP (-30%, p&lt0.05) and VTA (-54%, p&lt0.001), but not in the HYP or NAc. In contrast, 3α,5α-THP reduced basal levels of CRF only in the VTA (-38%, p&lt0.05) of female rats. Restraint stress for 30 minutes exhibited sex dependent effects in the HP and VTA. In male rats, stress reduced CRF (-72%, p&lt0.01) in the HP, but increased CRF (+36%, p&lt0.05) of female rats in the same area. In addition, stress increased CRF levels (109%, p&lt0.01) 3 hrs. after the start of restraint stress in VTA of male rats, but decreased CRF (-49%, p&lt0.05) in VTA of female animals. Furthermore, stress increased CRF in the HYP (178%, p&lt0.001) of female rats. 3α,5α-THP did not alter the stress-induced increase in CRF in VTA of male rats, but prevented the stress-induced change in CRF in female rat HP, HYP and VTA. The data indicate that 3α,5α-THP inhibits basal CRF protein expression in the VTA of both male and female rats but only inhibits stress-induced CRF in the VTA of female rats, suggesting that female rat VTA is particularly responsive to the effects of 3α,5α-THP. Further studies will focus on the mechanisms of 3α,5α-THP regulation of CRF across brain.


Poster

320. CRF in Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 320.03/U2

Topic: F.04. Stress and the Brain

Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes-Brazil)
Title: Anxiolytic effects of the antagonism of the CRF binding protein and the CRF receptor type 1 in the bed nucleus of stria terminalis in socially stressed male rats


1Psychology Inst., Federal Univ. of Rio Grande do Sul - UFRGS, Porto Alegre, Brazil; 2Dept. of Pharmacol., Federal Univ. of Rio Grande do Sul -UFRGS, Porto Alegre, Brazil; 3Psychology, Tufts Univ., Medford, MA

Abstract: Corticotropin-releasing factor (CRF) integrates endocrine, immune and behavioral stress responses in mammals. Clinical and preclinical evidence demonstrates that inappropriate CRFergic activity is a common factor shared by neuropsychiatric conditions associated with stress, for instance, anxiety-, depressive-, and substance abuse disorders. In this study, we submitted rodents to social defeat using an intermittent resident-intruder paradigm to mimic the human patterns of exposure to social stress. Our study aimed to provide insights for a better understanding of the role of the CRFergic system on anxiety and depressive-like symptoms induced by social stress. Social defeat was applied on three cohorts of male Wistar rats (n = 15-16 per cohort) by short confrontations with a reliably aggressive resident every three days for 10 days. Control animals (n = 14-17 per cohort) were left undisturbed in their home cages. Animals were evaluated for anhedonia before and after stereotaxic surgery for guide-cannula implantation in the bed nucleus of stria terminalis. Each cohort received different intra-BNST microinjections of selective CRFergic antagonists. Drugs used were the CRF binding protein antagonist CRF$_{6-33}$, the CRF receptor type 1 antagonist CP316311, and the CRF receptor type 2 antagonist Astressin 2B. The experience of intermittent episodes of social defeat stress disrupted social interaction behaviors in two out of the three cohorts. Microinjections of CRF$_{6-33}$ and CP316311 in the bed nucleus of the stria terminalis, separately, restored social approach behavior in stressed animals. Effects of Astressin 2B could not be evaluated since animals did not present stress-induced symptoms during the social preference tests. These findings suggest a distinct interpretation for the CRF sequestrating role of the CRF binding protein, as proposed in the literature. It seems that the CRF binding protein enhances CRF receptor type 1 signaling, and that the antagonism of this protein also prevents the onset of anxiety responses.

**Poster**

**320. CRF in Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus**

**Location:** Hall A  
**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM  
**Program #/Poster #:** 320.04/U3  
**Topic:** F.04. Stress and the Brain  
**Support:** FAPESP: nº 2018/14284-4  

**Title:** Role of CRF$_1$ receptors in the lateral hypothalamus in cardiovascular responses during acute and repeated restraint stress

**Authors:** *L. BARRETTO-DE-SOUZA, R. BENINI, C. C. CRESTANI;* Princípios Ativos Naturais e Toxicologia, Faculdade De Ciências Farmacêuticas De Araraquara, Araraquara, Brazil

**Abstract:** **Introduction:** The lateral hypothalamus (LH) is an important hypothalamic area that has been implicated in the integration of physiological and behavioral responses to stress. In this sense, studies have shown the involvement of this hypothalamic area in the cardiovascular responses to aversive stimulus. The CRFergic system has been shown to be an important mechanism in the central nervous system involved in the etiology of the physiological adjustments evoked by exposure to aversive situations. Data also showed that exposure to chronic stress affects the expression of CRF receptors and the levels of CRF and urocortins in brain regions involved in the control of stress responses. However, the role of CRFergic neurotransmission within the HL in the cardiovascular responses during acute and repeated restraint stress has not been investigated. **Objectives:** Here, was investigated the effect of bilateral microinjection into the LH of the selective CRF$_1$ receptor antagonist CP376395 in the cardiovascular responses induced during the first and the 10th session of restraint stress in rats. **Materials and Methods:** Male Wistar rats (250g) had cannula-guide bilaterally implanted within the LH. A catheter was implanted into the femoral artery for mean arterial pressure (MAP) and heart rate (HR) recording. The restraint stress was realized by placing the animals in a plastic cylindrical tube for 60 minutes. Independent sets of animals received CP376395 (5 mol/100nL) or vehicle into the LH 10 minutes before the onset of the first or 10th session of restraint stress. **Results:** Bilateral microinjection of CP376395 into the LH did not alter the increase in MAP during either the first (F (1, 16) = 3,043 P>0.05) or the 10th (F (1, 12) = 0,1447 P>0.05) session of restraint stress. However, the blockade of the CRF$_1$ receptor within the LH decreased the restraint-evoked tachycardiac response in both the first (F (1, 16) = 4,659 P<0.05) and 10th (F (1, 12) = 5,903 P<0.05) restraint session. The effects of the LH pharmacological treatment in tachycardic response to restraint was not statistically different between the restraint sessions (F (1, 12) = 1,719 P>0.05). **Conclusion:** The CRFergic neurotransmission in the LH,
acting through activation of the CRF₁ receptor, is involved in control of the tachycardiac response during aversive threats

**Disclosures:** L. Barretto-de-Souza: None. R. Benini: None. C.C. Crestani: None.

**Poster**

320. CRF in Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 320.05/U4

**Topic:** F.04. Stress and the Brain

**Support:** VA Merit Award 1 I01 BX004712-01
VA Advanced Research Fellowship
Great Plains Veterans Research Foundation

**Title:** Inhibition of calcineurin attenuates stress-induced expression of CRF and IL-2 in the central nucleus of the amygdala

**Authors:** *P. J. RONAN¹, T. I. MEHTA¹, T. P. BERESFORD²;
¹Psychiatry and Basic Biomed. Sci., VA Research/USD Sanford Sch. of Med., Sioux Falls, SD;
²Psychiatry, Denver VA/UC Denver, Denver, CO

**Abstract:** We have shown that immunosuppressants acting through inhibition of calcineurin (CLN) in brain reduce alcohol intake in rodents. Calcineurin is an abundant phosphatase in brain and plays a key role in the transcription of both cytokines and signaling molecules related to addiction. Ethanol withdrawal is known to activate corticotropin releasing factor (CRF) signaling in the central nucleus of the amygdala (CeA). We sought to determine whether inhibition of calcineurin could prevent stress-induced transcription of CRF and cytokines in the CeA. Rats (n=8) were given either CsA (30 mg/kg, I.P.) or vehicle, exposed to 30 minutes of restraint stress and returned to their home cage for 90 minutes. Brains were rapidly dissected and frozen in isopentane on dry ice. The CeA was microdissected from 300 um frozen sections and qRT-PCR was performed. Stress-induced expression of both CRF and interleukin 2 (IL-2) mRNA was significantly attenuated in CsA treated animals relative to controls but c-fos mRNA, an immediate early gene product indicative of neuronal activation, was increased by CsA treatment. Conclusion: These data suggest a complex interaction resulting in decreased stress-induced expression of both CRF and IL-2 in the central nucleus of the amygdala while at the same time effecting an increase in CeA neuronal activation in response to stress. The significant anti-drinking effect of cyclosporine-A may be due to calcineurin's role in multiple pathways involved in brain stress, reward, and neuroimmune responses. Deciphering proximal mechanisms underlying the anti-drinking effects of calcineurin inhibition holds promise for the development of effective treatments where few exist.

Poster

320. CRF in Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 320.06/U5

Topic: F.04. Stress and the Brain

Support: NIH grant R01110350126A

Title: The role of central amygdala CRF+ neurons in conditioned place avoidance in adult Wistar rats

Authors: *T. J. TEMPLETON¹, M. M. WEERA², N. W. GILPIN³;

Abstract: Post-traumatic stress disorder (PTSD) is a costly debilitating psychiatric condition that affects a subset of those exposed to a traumatic event. The neurocircuitry underlying this disorder is still unclear and treatments are often ineffective. Diagnostic criteria include symptoms from four clusters including avoidance of trauma-associated stimuli. Avoidance behavior is highly predictive of PTSD severity, duration, and treatment response. The central amygdala (CeA) contains a dense population of corticotropin-releasing factor (CRF) neurons that mediate behavioral responses to stressful stimuli. CRF in the CeA can be synthesized locally or imported via afferent projections from other brain regions. The purpose of this work is to understand the role of CeA CRF in the long-term effects of traumatic stress on brain and behavior. We hypothesized that selective activation of CRF+ cells in the CeA would produce avoidance of conditioned stimuli in otherwise experimentally naïve animals. In this study, male and female CRF:cre transgenic rats received intra-CeA infusions of the Cre-dependent adeno-associated viral vector (AAV5-hSyn-DIO-hM3Dq-mCherry) or control virus (AAV5-hSyn-DIO-mCherry). After four weeks, rats were submitted to the following place conditioning (CPA) protocol: on the first day, rats freely explored both chambers, each with specific visual and tactile cues, of a two-chamber apparatus. During ensuing conditioning sessions, in an alternating order across days, rats were confined to one of the two chambers and systemically injected (i.p.) with the DREADD agonist clozapine-n-oxide (CNO). Each individual rat was repeatedly injected with the same single dose of CNO (0, 2, 4 mg/ml/kg BW) throughout the experiment. On alternating days, all rats were administered a vehicle (20% DMSO in saline) injection (1 ml/kg BW) in the other chamber. After 6 conditioning days (3 CNO and 3 vehicle), rats were tested for time spent in the two chambers and a post-test minus pre-test score was calculated for each rat. The conditioning (3 CNO injections/3 saline injections; one per day) and post-test (one day) procedures were repeated twice more (3 weeks for the entire procedure). Preliminary results
suggest that chemogenetic activation of CRF+ cells supports conditioned avoidance behavior in males, but not female rats. These findings suggest that activation of CeA CRF cells is aversive for male but not female rats. Results from this study suggest there are sex differences in the role of amygdalar CRF in mediating aversion and perhaps in mediating traumatic stress effects on behavior.

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**Disclosures:** T.J. Templeton: None. M.M. Weera: None. N.W. Gilpin: F. Consulting Fees (e.g., advisory boards); Glauser Life Sciences.

**Poster**

320. CRF in Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 320.07/U6

**Topic:** F.04. Stress and the Brain

**Support:** CIHR 422007

**Title:** Vasopressin has sex-specific effects on short-term potentiation in CRH neurons after stress

**Authors:** *S. P. LOEWEN, J. S. BAINS;* Hotchkiss Brain Inst., Univ. of Calgary, Calgary, AB, Canada

**Abstract:** A single stress primes glutamate synapses onto corticotropin-releasing hormone (CRH) neurons in the hypothalamic paraventricular nucleus (PVN). This priming allows these synapses to undergo robust short-term potentiation (STP) in response to tetanic stimuli. We have recently shown that the presence of a partner decreases STP following a single stress in female, but not male mice. These findings indicate that social interactions have sexually dimorphic effects on the long-term synaptic consequences of stress. How this occurs, at the cellular level, is not known. Increasing evidence suggests that neurons in PVN are sensitive to neuromodulation by locally released substances. Here, we examined the effects of vasopressin (AVP) on stress-induced STP in CRH neurons. Hypothalamic slices were obtained from male and female Crh-IRES-Cre; Ai14 mice subjected to a footshock protocol (0.5 mA for 2 s delivered every 30 s over 5 min). A subset of slices were incubated in 100 nM AVP dissolved in artificial cerebrospinal fluid for 30 min. Following AVP incubation, whole-cell recordings were obtained from CRH neurons. AVP significantly reduced STP in CRH neurons from females (non-incubated, 152.2 ± 8.2%, n=17; AVP-incubated, 120.2 ± 5.7%, n=16; p=0.003), but not males (non-incubated, 137.1 ± 7.6%, n=12; AVP-incubated, 126.1 ± 8.0%, n=9; p=0.3). The effects of AVP on STP in female mice were prevented by pre-incubation in a V1a receptor antagonist (SR 49059, 147.5 ± 9.0%, n=10). Furthermore, the AMPA:NMDA ratio was significantly reduced in cells following AVP incubation compared to cells from non-incubated control slices (non-incubated, 3.8 ± 0.5, n=9;
AVP-incubated, 2.2 ± 0.4, n=5; p=0.04). These findings indicate that AVP may reduce STP in CRH neurons by enhancing NMDAR-mediated currents and/or decreasing AMPAR-mediated currents in these cells, and implicate this neuropeptide as a potential mediator of social stress buffering in female mice.

**Disclosures:** S.P. Loewen: None. J.S. Bains: None.

**Poster**

**320. CRF in Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 320.08/U7

**Topic:** F.04. Stress and the Brain

**Support:** CIHR

**Title:** Fear and anxiety in the hypothalamus

**Authors:** *T. FUZESI, D. G. ROSENEGGER, N. DAVIU, T. CHOMIAK, L. A. MOLINA, N. P. RASIAH, J. S. BAINS;
Hotchkiss Brain Inst., Calgary, AB, Canada

**Abstract:** In addition to controlling the endocrine response to stress, corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus (PVN) also regulate specific stress-related behaviors. Evidence suggests these two processes may be controlled independently, but precisely how these neurons encode both rapid behavior and slow hormone release is not known. Here, we used in vivo single fibre photometry and the genetically encoded Ca reporter, GCaMP6s to assess real-time calcium changes in PVN CRH neurons in freely behaving mice. We examined PVN CRH activity in conditions with increasing degrees of negative valence (homecage, novel environment and footshock). Consistent with recent reports, CRH neurons show a rapid, transient increase in Ca2+ in response to an acute footshock stress. By contrast, exposure of the animal to a mild stress (placement in a novel environment) elicits an increase in the GCaMP signal (compared to homecage). This increase persists for the duration of the exposure to the novel environment. Repeated exposure (4 days) to the same novel environment had no effect the amplitude of this persistent increase. Exposure to an environment associated with footshock also induced a sustained elevation, however with a higher amplitude. These observations indicate that PVN CRH neurons show distinct activity profiles that reflect the duration and intensity of the stressor.

Poster

320. CRF in Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 320.09/U8

Topic: F.04. Stress and the Brain

Title: PVN-CRH neurons control innate escape behaviors

Univ. of Calgary, Calgary, AB, Canada

Abstract: Flight or freeze are instinctive responses to threat. These behaviors are one component of a broader stress response that includes the recruitment of hypothalamic PVN CRH neurons. Here we reveal an unexpected role for these neurons as modifiers of the flight response to threat. We used single fiber photometry to record in vivo calcium responses in PVN CRH neurons during different situations that require mice to engage in an innate escape behavior. Our results demonstrate that flight/escape responses to an advancing shadow are preceded by an increase in calcium in CRH neurons. In addition, inhibition of this cell population decreases the probability of escape, suggesting a permissive role for CRH neurons. Further, we show that these behaviors, although instinctive, are also trainable. We show a differential entrainment of CRH neurons to an uncontrollable (learned helplessness) vs controllable (learned avoidance) stress training protocol. Following controllable, but not uncontrollable stress, CRH neurons show associative learning. When challenged with a looming shadow, controllable stress subjects show an increase in CRH calcium and a flight response, but uncontrollable stress subjects show a bias toward freezing and no increase in CRH calcium. These findings provide evidence that CRH neurons encode a preparatory signal for an innate survival behaviors to a threat and demonstrate that behavioral training can alter this signal and modify instinctive behavior.

Title: An *in vivo* electrophysiology study of neurons in the paraventricular nucleus of the hypothalamus responding to stress

Authors: *A. Ichiyama*¹, K. Scott², B. L. Allman², W. Inoue¹; ²Anat. and Cell Biol., ¹Univ. of Western Ontario, London, ON, Canada

Abstract: A hallmark of the stress response, the activation of the hypothalamic-pituitary-adrenal (HPA) axis, starts with the release of corticotropin releasing hormone (CRH) from neuroendocrine neurons in the paraventricular nucleus of the hypothalamus (PVN). Although it is generally believed that CRH release is encoded by the firing activities of PVN-CRH neurons, the *in vivo* firing activity of these neurons remain unknown as traditional electrophysiology methods are unable to distinguish neurons intermingled in the PVN by their neurochemical identities. To investigate this, we used a combination of optogenetics and electrophysiology to “tag” the *in vivo* firing activity of CRH neurons by expressing light-activated channelrhodopsin (ChR2).

In anesthetized, head-fixed mice, we first recorded the spontaneous single unit firing activities of unidentified PVN neurons during a no stress baseline and following stressful sensory stimuli (sciatic nerve stimulation). Using off-line wave form analysis, we found that sciatic nerve stimulation elicits heterogeneous responses from PVN neurons. While most units increased firing frequency, we found units that were non-responsive or even decreased firing.

We then used optogenetics to identify the spontaneous activity of PVN-CRH neurons. Light stimulation of ChR2-expressing CRH neurons elicited time-locked increases in firing activity, which provided fingerprint waveforms of light-responding PVN-CRH neurons. This allowed us to identify the activity of PVN-CRH neurons from spontaneous recordings. Finally, we observed both light and sciatic nerve stimulation induced increased activity in the same recording. This is the first demonstration of firing activities of identified PVN-CRH neurons *in vivo*.

Title: Neuronal hypertrophy induced by repeated stress decreases the intrinsic excitability in stress habituation

Authors: S. MATOVIC¹, A. ICHIYAMA³, H. I GARASHI², E. W. SALTER⁴, M. S. HENRY⁵, J. K. SUNSTRUM⁶, X. WANG³, N. VERNOUX⁷, E. KUEBLER⁸, J. MARTINEZ-TRUJILLO⁸, M.-E. TREMBLAY⁹, *W. INOUE³;
¹Neurosciences, ²Dept. of Physiol. and Pharmacol., Robarts Res. Inst., London, ON, Canada; ³Univ. of Western Ontario, London, ON, Canada; ⁴Physiol., Univ. of Toronto, Etobicoke, ON, Canada; ⁵Ctr. De Recherche Du CHU De Québec, Quebec, QC, Canada; ⁶Neurosci., The Univ. of Western Ontario, London, ON, Canada; ⁷Laval Univ., Quebec City, QC, Canada; ⁸University of Western Ontario, London, ON, Canada; ⁹Univ. Laval, Quebec, QC, Canada

Abstract: Activation of the hypothalamic-pituitary-adrenal (HPA) axis is a hallmark of the stress response conserved across vertebrates. Although adaptive in the short-term, protracted recruitment of this energetically costly response can be maladaptive. Indeed, the HPA axis is flexible and can habituate after repeated stress exposures. Despite the biological and clinical importance of HPA axis habituation, surprisingly little is known about the neural plasticity mechanisms through which repetition of stressful experiences refines the sensitivity of the stress axis to the stressor. Here we report a neural correlate for HPA axis habituation. Using a mouse model of repeated restraint and slice patch-clamp electrophysiology, we studied hypothalamic corticotropin-releasing hormone neurons that form the apex of the HPA axis. We found that the intrinsic excitability of these neurons substantially decreased after daily repeated stress in a time course that coincided with their loss of stress responsiveness in vivo. This intrinsic excitability plasticity co-developed with an expansion of surface membrane area, resulting in an increase in input conductance with minimal changes in conductance density. Moreover, repeated stress augmented ruffling of the plasma membrane, suggesting an ultrastructural plasticity that may efficiently accommodate membrane area expansion. We report a novel structure-function relationship for intrinsic excitability plasticity that correlates with habituation of the neuroendocrine stress response.


Poster

320. CRF in Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 320.12/U11

Topic: F.04. Stress and the Brain
Support: Ontario Graduate Scholarship
CIHR Grant 148707
NSERC Grant RGPIN/06106-2015

Title: Synaptic sensitization to stress mediated by hyperpolarization-activated cyclic nucleotide-gated (HCN) channels

Authors: *J. K. SUNSTRUM*1,2, E. W. SALTER3, W. INOUE4,2;  
1Neurosci., The Univ. of Western Ontario, London, ON, Canada; 2Robarts Res. Inst., London, ON, Canada; 3Physiol., Univ. of Toronto, Toronto, ON, Canada; 4Physiol. and Pharmacol., Univ. of Western Ontario, London, ON, Canada

Abstract: The hormonal and behavioural response to stress can habituate (diminish its responsiveness) to repeated familiar stressors while maintaining its responsiveness to unfamiliar (novel) stressors. A prevailing theory of stress habituation predicts two opposing forms of plasticity, one that diminishes and another that sensitizes the stress response. While this may involve plasticity in segregated populations of neurons, whether a single population of neurons can accommodate the opposing forms of stress-induced changes remains unknown. The hormonal and behavioural response to stress is controlled by corticotropin releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus (PVN). Our recent study found that, in a mouse model of repeated stress, the intrinsic excitability of CRH neurons decreases, a mechanism in line with habituation. Here, we investigated a mechanism that enables a sensitized response to an unfamiliar stressor in the same stress model. Considering that afferent glutamatergic synapses are the major excitatory inputs onto CRH neurons, we hypothesized that potentiation of these excitatory synapses represents a neural correlate for sensitized responses to an unfamiliar stressor. Crh-IRES-Cre;Ai14 mice underwent the well-established repeated restraint stress paradigm (21 days of 1 hour per day restraint). We then used ex vivo whole-cell patch clamp electrophysiology to study CRH neuron synaptic activity. We found that pharmacologically stimulating cAMP (either by activating adenylyl cyclase or blocking cAMP breakdown by phosphodiesterase) potently increased the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) in CRH neurons (p < 0.0001 and p = 0.0006, respectively). In line with our hypothesis, this cAMP-induced increase in sEPSC frequency was even greater following our repeated stress paradigm (p <0.0001), suggesting CRH neurons are sensitized to cAMP facilitation of glutamate release following stress. Furthermore, this cAMP-induced facilitation was dependent on presynaptic hyperpolarization-activated cyclic-nucleotide-gated (HCN) channels. This work identifies a novel homeostatic mechanism that supports sensitization of the stress response in the same model known to show habituation to repeated stressors.

**Poster**

320. CRF in Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 320.13/U12

**Topic:** F.04. Stress and the Brain

**Support:** NIMH Grant R01 MH113007

**Title:** Inhibition of corticotropin-releasing factor (CRF) neurons in the oval nucleus of the bed nucleus of the stria terminalis does not affect anxiety-like behavior in adult male rats

**Authors:** *R. CHUDOBA*¹²³, V. OLIVERA¹²³, P. LIS¹, C. EDMONSON¹, S. OLSON¹², J. A. DABROWSKA¹²³⁴;

¹Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL; ²Ctr. for the Neurobio. of Stress Resilience and Psychiatric Disorders, North Chicago, IL; ³Sch. of Grad. and Postdoctoral Studies, Discipline of Cell. and Mol. Pharmacol., North Chicago, IL; ⁴Chicago Med. Sch. RFUMS, North Chicago, IL

**Abstract:** Corticotropin-releasing factor (CRF) is a neuropeptide responsible for regulating autonomic, endocrine, and behavioral responses to stress. A significant population of CRF-producing neurons is found in the oval nucleus of the bed nucleus of the stria terminalis (BNSTOV), a brain region that mediates behavioral responses to stressors such as fear and anxiety. Here, we used a Cre-CRF transgenic rat model that expresses Cre-recombinase driven by the CRF promoter to investigate the role of CRF neurons in the BNSTOV in modulating anxiety-like behavior and contextual fear. We infused male adult rats bilaterally with Cre-dependent adeno-associated viral vector driving expression of inhibitory designer receptors exclusively activated by designer drugs (DREADD) to selectively silence CRF neurons in the BNSTOV and investigate the corresponding behavioral effects. Four weeks after stereotaxic AAV injections in Cre-/- and Cre+/- rats, we investigated if silencing CRF neurons with DREADDs selective ligand, clozapine-N-oxide (CNO), affected anxiety-like behavior using the elevated plus maze (EPM) and open field test (OFT), or contextual fear conditioning (CFC) and acoustic startle response (ASR). In the EPM, percentage time spent in open arms of the maze did not differ between Cre-/- (n = 18) and Cre+/- (n = 13) rats after CNO injection (P = 0.3678). Similarly, in the OFT, percentage time spent in the area center did not differ between Cre-/- (n = 9) and Cre+/- (n = 11) rats (P = 0.8319) nor did silencing CRF neurons affect locomotion (P = 0.4807), suggesting that inhibiting CRF neurons does not affect baseline anxiety-like behaviors. In another experiment, CNO was injected before CFC to determine if silencing CRF neurons affects acquisition of contextual fear in a fear-potentiated startle experiment. Although all rats displayed significant potentiation of the ASR during exposure to context previously paired with foot-shocks (P = 0.0004), there was no genotype effect (P = 0.9218) nor interaction between trial...
type and genotype ($P = 0.7637, n = 21$). Next, we tested ASR where all rats were first tested for their baseline ASR (pre-test) and ASR after CNO injection (post-test). Although time (pre-test vs. post-test, $P = 0.6093$) and genotype ($P = 0.7425$) did not affect ASR, we observed a significant interaction between time and genotype ($P = 0.0397$), suggesting that IP injection before post-test affected startle amplitudes differently in Cre-/- (n = 19) and Cre +/- (n = 14) rats. Overall, our results suggest that although silencing CRF neurons in the BNST does not affect baseline anxiety-like behavior in male rats, it might attenuate anxiety triggered by exposure to stress.

**Disclosures:** R. Chudoba: None. V. Olivera: None. P. Lis: None. C. Edmonson: None. S. Olson: None. J.A. Dabrowska: None.

**Poster**

320. CRF in Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 320.14/U13

**Topic:** F.04. Stress and the Brain

**Title:** Stress experience and hormone feedback tune distinct components of CRH neuron activity

**Authors:** *J. S. KIM, K. J. IREMONGER;* Univ. of Otago, Dunedin, New Zealand

**Abstract:** Neural circuits in the hypothalamus are essential for coordinating organism’s responses to real or perceived threats. These experiences can evoke a cascade of hormonal and synaptic changes to promote adaptation and reshape future responses. However, the plasticity mechanisms driving these adaptations following stress remain poorly defined. Hypothalamic corticotropin-releasing hormone (CRH) neurons orchestrate behavioral and endocrine responses to stress and are themselves highly sensitive to corticosteroid (CORT) stress hormones. Using fibre photometry in freely behaving mice, we recorded the CRH neuron activity dynamics to understand how their excitability and stress responses can be tuned. We observed that CRH neurons are tonically active and rapidly activated in response to stress (5min loud white noise). Stress-evoked CRH neuron activity robustly habituated to repeated presentations of the same stressor. This habituation was observed following inter-stress intervals as early as 30 min (65.4% of novel response) and lasting at least 24 hours (63.8% of novel response). This adaptation was dependent on stress familiarity as CRH responses failed to habituate against sequential presentations of unfamiliar threats (white noise vs footshock). To address the role of CORT feedback in tuning CRH neuron activity, peripheral administrations of CORT or metyrapone were used to manipulate feedback milieu. Negative feedback inhibited stress-induced ACTH release (Veh 178.8 ±29.7 pg/mL vs CORT 91.9 ±19.0 pg/mL, p=0.04) but had no discernable effects on the magnitude of CRH neuron activation in response to acute stress and the ability to
habituate to repeated stress remained surprisingly unchanged. Instead, CORT was found to slowly (>60 min) inhibit tonic CRH neuron activity in the absence of stress stimuli. These findings reveal how stress experience and stress hormones modulate distinct components of CRH neuronal activity to mediate stress-induced adaptations and challenge the notion that rapid CORT actions are involved in the CRH neuron “shut-off”.

Disclosures: J.S. Kim: None. K.J. Iremonger: None.

Poster

320. CRF in Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 320.15/U14

Topic: F.04. Stress and the Brain

Title: Ultradian and circadian rhythms in hypothalamic CRH neuron activity and their relation to home cage behavior

Authors: *C. M. B. FOCKE, K. IREMONGER; Ctr. for Neuroendocrinology, Univ. of Otago, Dunedin, New Zealand

Abstract: Corticotropin-releasing hormone (CRH) neurons control the hypothalamic-pituitary-adrenal (HPA) axis and thereby the circulating levels of corticosterone (CORT). CORT secretion occurs with a prominent circadian and ultradian rhythm. However, the neural activity patterns in the CRH neuron population which drive these rhythms are unknown. In addition, it is also unknown whether CRH neuron responsiveness to stress varies as corticosterone fluctuates across the circadian cycle.

Using GCaMP6s fiber photometry we have optically measured the activity patterns of CRH neurons in the paraventricular nucleus of awake behaving adult male mice. Photometry recordings were performed across the 24-hour day along with simultaneous monitoring of home cage behavior. In addition, we tested if the CRH neural responses to a 5-minute white noise stress differed at the circadian peak or nadir of CORT (evening versus morning respectively). CRH neuron population activity was found to be highly dynamic across the 24-hour day. However, we found no differences in event frequency or amplitude across the day-night cycle. Most notable however, was the presence of distinct ultradian oscillations (up-states) which occurred every 54 ± 7.8 min (n=7 mice). These up-states were significantly longer in duration over the night (active phase; 23.8 ± 1.2 min) as opposed to the day (inactive phase; 19.9 ± 1.0 min; p=0.01). Furthermore, behavioral analysis revealed that increases in CRH neuron activity were closely related to home cage activity (n=5 mice).

In response to a 5-minute white noise stressor, CRH neuron population activity quickly and robustly increased. However, both the average (AM: 26.2 ± 5.8%, PM: 30.1 ± 7.7%; p=0.1; n=7) and peak responses (AM: 63.6 ± 8.2%, PM: 71.6 ± 10.2%; p=0.1) were the same if the stress was
delivered in the evening versus the morning. Taken together, these data reveal ultradian and circadian rhythms in CRH neuron excitability across the day with a strong link to home cage activity. However, CRH neural responses to stress were not found to be different across the day.

Disclosures: C.M.B. Focke: None. K. Iremonger: None.

Poster

320. CRF in Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 320.16/U15

Topic: F.04. Stress and the Brain

Support: Health Research Council (HRC) of New Zealand
Royal Society of New Zealand Marsden Fund

Title: Sexually dimorphic corticosteroid induced plasticity in corticotropin-releasing hormone neurons

Authors: *K. J. IREMONGER*¹, A. SHERRINGTON², J. S. KIM², J. GOUWS², D. CHANDRASEKERA²;
¹Ctr. for Neuroendocrinology and Dept. of Physiol., ²Univ. of Otago, Dunedin, New Zealand

Abstract: Corticosteroid stress hormones drive a multitude of adaptations in the brain. Hypothalamic corticotropin-releasing hormone (CRH) neurons control the circulating levels of corticosteroid stress hormones in the body and are themselves highly sensitive to corticosteroids. CRH neurons have been shown to undergo various adaptions in response to acute stress hormone elevations. However, the changes in excitability under chronically elevated corticosterone are less clear. To address this, we have used in vitro electrophysiology and calcium imaging to study the changes in neural excitability in CRH neurons in the paraventricular nucleus of the hypothalamus following 14 days of corticosterone treatment (25µg/ml) in the drinking water. We find that prolonged corticosterone elevation in male mice reduces CRH neuron intrinsic excitability as measured by summation of subthreshold postsynaptic depolarisations and spiking output. Calcium imaging in brain slices from male mice revealed that CRH neurons fire with a bursting pattern of activity under control conditions, however, exposure to chronic corticosterone significantly reduced the number of neurons exhibiting this bursting pattern. While basal activity was found to be similar in brain slices from control female mice, corticosterone treatment failed to inhibit neural excitability and occasionally induced elevations in excitability. Blood corticosterone measurements revealed that while the drinking water treatment protocol elevated plasma corticosterone in males, it failed to elevate plasma corticosterone in females. Together these data reveal the functional adaptations in hypothalamic CRH neurons following
corticosterone excess and highlight important sexually dimorphic responses to corticosterone treatment regimes.

**Disclosures:** K.J. Iremonger: None. A. Sherrington: None. J.S. Kim: None. J. Gouws: None. D. Chandrasekera: None.

**Poster**

320. CRF in Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 320.17/U16

**Topic:** F.04. Stress and the Brain

**Support:** Marsden Fund

**Title:** Understanding sex differences in neural circuits controlling stress

**Authors:** *E. M. POWER*¹, K. J. IREMONGER²;
¹Physiol., ²Univ. of Otago, Dunedin, New Zealand

**Abstract:** Males and females respond differently to acute stress and have disparate risks for developing stress-related mental health disorders. Stress evoked release of adrenal corticosteroids are controlled by corticotropin-releasing hormone (CRH) neurons, located in the paraventricular nucleus of the hypothalamus. Corticosteroid release profiles are different between males and females as well as across the estrous cycle. However, it is currently unclear to what extent this is due to differences in CRH neuron intrinsic excitability. Estrogen regulates both the expression and function of multiple ion channels throughout the brain. Specifically, estrogen has previously been shown to modify I₁ and I₉ potassium (K⁺) currents. I₁ is a transient outward K⁺ current and I₉ is a subthreshold, slow delayed rectifier K⁺ current. Both I₁ and I₉ currents are important for CRH neuron function and an increase in either current corresponds to a decrease in neuronal excitability. Here we set out to test if differences in CRH neuron excitability exist between males and females as well as across the estrous cycle and whether these differences are due to disparate K⁺ channel function. To investigate this, we performed whole cell *in vitro* electrophysiological recordings of CRH neurons to record intrinsic excitability as well as I₁ and I₉ currents. Examination of frequency-current (F-I) curves revealed that the gain of CRH neuron firing was significantly higher in males compared to estrous and diestrous females (P<0.001 and P<0.001 respectively). Interestingly proestrous females had significantly higher F-I gain than estrous (P<0.001) and diestrous (P<0.001), but were not significantly different from males (P=0.3325). Despite the difference in excitability between the groups, action potential parameters, including halfwidth, rise time, amplitude and activation threshold, we remarkably similar. Consistent with the differences in excitability, both I₁ and I₉ currents were significantly larger in estrous and diestrous females compared to males and
proestrous females (2-way ANOVA, P=0.0006 and P=0.003 respectively, n>7 for each group). As estrous and diestrous females have lower circulating estrogen levels compared to proestrous, this may explain the differences in excitability and K+ channel function across the estrous cycle. Our findings suggest estrogen driven changes in K+ channel function may underlie variable CRH neuron excitability levels between stages of the estrous cycle. These differences may also contribute to sex differences in CRH neuron excitability and thus underlie sexually dimorphic responses to acute stress.

**Disclosures:**  
**E.M. Power:** None.  
**K.J. Iremonger:** None.

**Poster**

**320. CRF in Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 320.18/U17

**Topic:** F.04. Stress and the Brain

**Title:** Functional characterization of hypothalamic CRF neuron during exposure to appetite and aversive stimuli

**Authors:** *J. KIM, G. SUH;*  
Dept. of Biol. Sci., Korea Advanced Inst. of Sci. and Technol., Daejon, Korea, Republic of

**Abstract:** Corticotropin-releasing factor (CRF) neuron in the Paraventricular nucleus of hypothalamus secretes CRF when exposed to stress. CRF initiates a cascade of events that triggers the release of glucocorticoids from the adrenal cortex. The modulation of the hypothalamic-pituitary-adrenal (HPA) axis is essential for adjusting the neuroendocrine responses to stress, but it does not explain how PVN CRF neurons mediate behavioral changes in response to acute stress. Previous calcium imaging and optogenetic manipulation studies revealed that PVN CRF neurons are immediately activated by aversive cues and are rapidly suppressed by appetitive stimuli and that these neurons are essential for mediating behavioral responses to acute stress and appetitive stimuli (J. Kim, Nature Neuroscience 2019). These findings suggest that there exists a different mechanism independent of HPA axis. To further examine whether the response of PVN CRF neurons to acute environmental stimuli is independent of HPA axis, we surgically removed adrenal glands (adrenalectomy) in CRF-ires cre mice and tested these mice with optogenetically activating or inhibiting PVN CRF neurons in different behavioral paradigms.

**Disclosures:**  
**J. Kim:** None.  
**G. Suh:** None.
Depressive-related behaviors are modulated by crf neurons of the bnst

Authors: *S. E. HAYNES¹, H. SEO², D. G. RAINNIE³, M.-H. HAN⁴;
¹Icahn SOM At Mount Sinai, New York, NY; ²Emory, Atlanta, GA; ³Emory Univ., Atlanta, GA; ⁴Mount Sinai Sch. of Med., New York, NY

Abstract: Chronic stress is a key risk factor for the development of Major Depressive Disorder (MDD), yet it is unknown why some people develop MDD as compared to others, despite reporting similar levels of stress. Corticotrophin-Releasing Factor (CRF) has emerged as a molecular substrate that may play a critical role in the etiology of stress-related depression. While many preclinical and clinical studies implicate its role in depression etiology, the relationship between stress, MDD, and CRF neurotransmission is not well understood. The limbic forebrain region, Bed Nucleus of the Stria Terminalis (BNST), encodes the nature and chronicity of stressful stimuli and mediates behavioral/physiological adaptive responses and is thus thought to play a role in depression. Importantly, the oval nucleus of the BNST contains an enriched population of CRF (BNSTovCRF) neurons that are stress responsive and thereby may provide a source for the molecular link between CRF and depression. In this study, we seek to elucidate CRF neural mechanisms that underlie depression onset that arises from unmitigated exposure to chronic stress. We hypothesize that the time course to depression-onset is influenced by neuroadapative genetic changes in BNSTovCRF neurons, and that individual differences in crf gene expression may correlate with an individual’s vulnerability to depression. To test this, we employed the Chronic Social Defeat Stress paradigm in mice that reliably produces stress-resilient (RES) or depressive-like (DEP) phenotypes on the basis of social interaction and sucrose preference. We have discovered a discrete time window where mice rapidly diverged from baseline behavior to RES or DEP phenotypes, unmasking a temporal dimension to stress-susceptibility. Importantly, gene expression, electrophysiological, and in vivo fiber photometry measurements have been conducted to correlate circuit level neuroadaptive changes with those observed behaviorally. Optogenetics and chemogenetics have been used to show necessity and sufficiency for BNSTovCRF neurons for the timing, onset, and severity of depressive behavior. Altogether, these findings provide evidence of a temporal domain to stress adaptation where alterations in CRF neurotransmission may shift the predisposition of mice toward either stress-
resiliency (RES) or stress-induced depressive-like (DEP) phenotypes. This discrete period of neuroadaptation may present a window of opportunity for targeting molecular mechanisms with the possibility of preventing the onset of depression in stress-susceptible populations.

**Disclosures:** S.E. Haynes: None. H. Seo: None. D.G. Rainnie: None. M. Han: None.

**Poster**

320. CRF in Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 320.20/U19

**Topic:** F.03. Neuroendocrine Processes

**Support:** KAKENHI 18K08499

**Title:** A comparison of the fluorescent protein expression between different mouse lines generated by corticotropin-releasing factor gene targeting

**Authors:** *K. ITOI*¹, A. H. TALUKDER¹, K. UCHIDA¹, T. SATO¹, S. MURASAWA¹, M. KAWAMURA², R. NATSUME², M. ABE², K. SAKIMURA²;
¹Lab. Information Biol., Tohoku Univ., Sendai, Japan; ²Brain Res. Ins. Niigata Univ., Niigata, Japan

**Abstract:** Corticotropin-releasing factor (CRF) is a neuroendocrine peptide produced in the paraventricular nucleus of the hypothalamus (PVH), but CRF is also expressed in brain regions outside the PVH and may function as a neurotransmitter or neuromodulator. We generated previously the CRF-modified yellow fluorescent protein (Venus) knock-in mouse (CRF-Venus, CRF-Venus/wt), and then the CRF-VenusΔNeo mouse (CRF-VenusΔNeo/wt) was generated by deleting the pgk-1 promoter-driven neomycin phosphotransferase gene (Neo cassette) from the former genome using the Actb-FLPe mouse; the FLP recombinase expressed in the Actb-FLPe recognizes the Neo cassette flanked by the flippase recognition target sites and excises it from the CRF-Venus genome. We reported that the intensity of Venus expression in the CRF-VenusΔNeo was much more prominent than that in the CRF-Venus. In the present study, we sought to examine whether the presence or the absence of the Neo cassette in CRF driver mouse genome may also affect expression of a reporter gene. First, the morphological features of the EGFP-expressing neurons in the CRF-iCre;EGFP, which was generated by crossing the CRF-iCre (CRF-iCre/wt) with an EGFP-reporter mouse (TgCAG-floxed CAT-EGFP/wt), was similar to those of the Venus-expressing neurons in the CRF-VenusΔNeo, even in the presence of the Neo cassette in the CRF-iCre driver mouse genome. Next, distribution and intensity of EGFP expression were not different clearly between the CRFiCre;EGFP and the CRFiCreΔNeo;EGFP, which was generated by crossing the CRF-iCreΔNeo (CRF-iCreΔNeo/wt) with the EGFP-reporter. Thus, deletion of the Neo cassette from the CRF-iCre driver mouse genome did not affect expression of the
EGFP reporter gene explicitly. Finally, intensity of the Cre immunofluorescence was much more prominent in the CRF-iCreΔNeo than that in the CRF-iCre. From these results, it was assumed that the presence of the Neo cassette may have interfered with the CRF promoter, and reduced the expression of Venus or iCre, in either the CRF-Venus or the CRF-iCre. However, iCre-induced recombination may have been efficient enough for the full EGFP expression in the CRF-iCre;EGFP, even with much smaller amount of iCre expressed in the CRF-iCre compared with that in the CRF-iCreΔNeo.


Poster

320. CRF in Stress-Modulated Pathways: Hypothalamus, Amygdala, and Bed Nucleus

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 320.21/U20

Topic: F.03. Neuroendocrine Processes

Support: National Science Foundation IOS #1656734

Title: Corticotropin releasing factor (CRF) does not influence basal or depolarized GABA or glutamate release from a midbrain defense area

Authors: *J. A. CARR, C. M. PRATER;
Biol. Sci., Texas Tech. Univ., Lubbock, TX

Abstract: The 41 amino acid peptide CRF reduces food intake and related behaviors by acting on tectal CRFR1 receptors in the African clawed frog Xenopus laevis. Precisely how CRF acts within the optic tectum to inhibit food intake is unclear but may involve inhibitory GABAergic interneurons. We predicted that if tectal CRFR1-induced inhibition of food intake is mediated via inhibitory GABAergic interneurons, that exogenous CRF would increase basal and evoked GABA release from tectal explants in-vitro. Tectal explants from X. laevis were depolarized with 60 mM K⁺ and GABA was measured in the medium after derivatization using high-performance liquid chromatography coupled to electrochemical detection. GABA secretion from X. laevis tectal explants increased under depolarizing conditions and this evoked release was eliminated in calcium-free medium. Exposure of tectal explants to ovine CRF doses ranging from 1 nM to 1 micromolar (AnaSpec, Inc., Fremont, CA; 0, 0.001, 0.01, 0.1, 1 μM) had no effect on either basal or depolarization-induced GABA release. We conclude that our in-vitro assay measures calcium-dependent evoked GABA release from tectal explants and that CRF does not appear to influence basal or evoked GABA release in-vitro. These findings do not support a role for
GABAergic interneurons in mediating tectal CRFR1 inhibition of food intake. Supported by a grant from National Science Foundation IOS #1656734.

**Disclosures:**  
J.A. Carr: None. C.M. Prater: None.

**Poster**

321. Stress-Modulated Pathways: Brainstem and Others

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 321.01/U21

**Topic:** F.04. Stress and the Brain

**Support:**  
Rita Allen Foundation Scholar Award  
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William and Bernice E. Bumpus Young Innovator Award  
NARSAD Young Investigator and P&S Fund Grant  
NIH T32 AG20506

**Title:** Ketamine rescues behavior and plasticity through dopamine systems

**Authors:** *M. Wu*1, Y. KOZOROVITSKIY2;  
1Neurobio. Dept., 2Neurobio., Northwestern Univ., Evanston, IL

**Abstract:** Major depressive disorder (MDD) is a prevalent, recurrent mental illness linked to diminished quality of life. Ketamine and its S-enantiomer esketamine, which act as antagonists of the glutamatergic N-methyl-D-aspartate (NMDA) receptors, demonstrate rapid onset antidepressant effects in clinical trials. However, which neural circuit dynamics mediate the effects of ketamine is still currently unknown. Here, we demonstrate that activity of dopaminergic (DA) neurons in the ventral tegmental area (VTA) changes in an aversive learning paradigm. Ketamine normalizes the impaired dynamics of VTA activity after aversive learning, leading to behavioral symptom relief. The behavioral effects of ketamine require DA signaling, which modulates cortical synaptic plasticity across behavioral states. Together, our data demonstrate a link between DA activity dynamics, synaptic plasticity, and ketamine’s behavioral effect in a context of aversive learning.

**Disclosures:** M. Wu: None. Y. Kozorovitskiy: None.
**Poster**

321. Stress-Modulated Pathways: Brainstem and Others

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 321.02/U22

**Topic:** F.04. Stress and the Brain

**Support:** U.Albany Faculty Research Award Program

**Title:** Prolactin reduces chronic stress-induced depression-like behavior and increases tyrosine-hydroxylase expression in ovariectomized female rats

**Authors:** *J. MEDINA, D. BESWICK, R. M. DE GUZMAN, J. L. WORKMAN; Psychology, Univ. at Albany, State Univ. of New York, Albany, NY

**Abstract:** Postpartum depression (PPD) afflicts approximately 15% of new mothers with debilitating symptoms. The lack of or early cessation of breastfeeding is a unique risk factor for PPD. For instance, women who bottle-feed exclusively are more likely to develop PPD and prospective studies suggest cessation of breastfeeding often precedes depressive symptoms. Thus, certain aspects of lactation may buffer against the development of PPD. Breastfeeding promotes the release of the peptide hormone, prolactin (PRL). Beyond its role in supporting lactation, PRL influences multiple aspects of physiology and behavior including social behavior, neural plasticity, and stress responses in rodent models. Stress is a significant risk factor for depression and abnormalities in the hypothalamic-pituitary-adrenal (HPA) axis have been implicated in the etiology of depression, including PPD. We sought to determine whether prolactin reduces stress-induced depression-like behavior in female rats. Ovariectomized rats were either non-stressed or received chronic variable stress (CVS) and received daily subcutaneous injections of ovine PRL (1 mg/kg) or saline vehicle. Anhedonia was assessed with the sucrose preference test (SPT) at 3 time points during the study: the first day of PRL treatment, 9 or 10 days after the initiation of CVS, and after 20 days of CVS. Passive-coping behavior was assessed with the forced swim test (FST) across two consecutive days (days 23 and 24 of CVS): FST 1 for 15 minutes and FST 2 for 5 minutes. Blood samples from the tail vein were taken 30 minutes after FST 2 to assess stress-induced corticosterone concentrations. Chronic prolactin treatment prevented the development of stress-induced sucrose anhedonia in CVS females. In conjunction with these data, sections of the ventral tegmental area (VTA), a region critical in anhedonia, were labelled for tyrosine hydroxylase (TH) expression to determine whether CVS or PRL alter dopamine-producing cells. Regardless of stress condition, chronic PRL treatment significantly increased TH-immunoreactive cell density in the rostral VTA. Taken together, these findings have critical implications for neuroendocrine mechanisms by which lactation confers resilience against depression.
**Disclosures:** J. Medina: None. D. Beswick: None. R.M. De Guzman: None. J.L. Workman: None.

**Poster**

321. Stress-Modulated Pathways: Brainstem and Others

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 321.03/U23

**Topic:** F.04. Stress and the Brain

**Support:** NIMH Grant RO1MH115016

**Title:** Ultrastructural analysis of corticotropin-releasing factor regulation of dopaminergic signaling in the midbrain of the macaque

**Authors:** *E. A. KELLY*¹, J. L. FUDGE²;

**Abstract:** Dopamine (DA) is involved in stress and stress-related illnesses, including many psychiatric disorders. Corticotropin-releasing factor (CRF) plays a role in acute and prolonged stress responses by integrating endocrine, autonomic, and neural systems. DA is a key target of CRF in the ventral midbrain, providing a fundamental link between stress and altered monoamine function. In the primate, it is now recognized that DA neurons are physiologically heterogeneous with respect to both intrinsic firing and coding properties. Mapping DA subpopulations onto rodent systems is difficult because of a number of differences including: 1) the anatomical expansion in main subpopulations (A10, A9, A8) in primates, 2) relative changes in the size of DA subpopulations, and 3) varying ratios of DA to non-DA cells across A10, A9 and A8 groups. We recently found that a key source of CRF to the DA subpopulations, the central extended amygdala, sends terminals to DA subpopulations that lie mainly outside the midline VTA, preferentially targeting DA subpopulations in the parabrachial pigmented nucleus (PBP) of A10 and the A8 group. We therefore sought to characterize CRF terminals on DA and non-DA cells in the PBP and A8 regions at the ultrastructural level as a first look at understanding CRF dynamics in these subpopulations. A10-PBP and A8 DA neurons are calbindin (CaBP)-positive. Using CaBP and the distribution of CRF-containing fibers in adjacent sections, we prepared blocks from the PBP and A8 subpopulations in a 3-year-old male macaque and performed ultrastructural analysis following double immunoperoxidase (CRF) and immunogold reactivity (tyrosine hydroxylase, TH) for electron microscopy (EM). We then determine the synaptic interactions between CRF-containing axons and TH positive (DA+) and TH negative (NON-DA+) neurons in each region. We found CRF immunoreactivity present mostly in axon terminals establishing either symmetric or asymmetric synapse onto dendrites within both the PBP and A8. CRF axons made preferential connections onto NON-DA+ cells in
both regions that were largely symmetric (inhibitory) in nature. Among the DA+ cells, CRF+ contacts were mostly symmetric in PBP while asymmetric (excitatory) in A8. Our preliminary findings suggest similar CRF regulation of non-DA cells in PBP and A8. DA+ cells, however appear to be differently regulated across region.

Disclosures: E.A. Kelly: None. J.L. Fudge: None.

Poster

321. Stress-Modulated Pathways: Brainstem and Others

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 321.04/U24

Topic: F.04. Stress and the Brain

Support: NIH Grant R01MH099505

Title: Inactivation of the deep layers of the superior colliculus disrupts prepulse inhibition in macaques

Authors: *H. F. WAGUESPACK¹, B. L. AGUILAR², L. MALKOVA¹, P. FORCELLI¹; ¹Pharmacol., Georgetown Univ., Washington, DC; ²Univ. of California, Davis, Davis, CA

Abstract: Sensorimotor gating as measured by prepulse inhibition of the acoustic startle (PPI) is impaired in various disorders, including autism, schizophrenia, and PTSD. The acoustic startle is an unconditioned motor response to a startling stimulus. This response can be reduced when a prepulse of smaller intensity precedes the startling pulse stimulus, a paradigm known as PPI. While the circuitry mediating PPI is well characterized in rodents, it is almost completely unexplored in the primate brain. A recent study from our lab found opposite effects of inhibition of substantia nigra pars reticulata (SNpr) on PPI in rodents and primates (Aguilar et al., 2018). Inactivation of SNpr in rodents disrupted PPI, whereas inactivation in primates facilitated PPI. The SNpr sends direct inhibitory projections to the deep layers of the superior colliculus (DLSC) in both the rodent and the primate. In rodents, lesions to the DLSC reduce PPI and stimulation of the DLSC has the same effect as a prepulse to dampen startle (Fendt & Schnitzler, 1994; Li & Yeomans 2000). The goal of the present study was to compare the effects of DLSC manipulations in macaques to those previously done in rats, to determine if our prior finding was due to divergence at the level of the DLSC. We transiently inhibited the DLSC in four rhesus macaques by bilateral microinfusion of the GABA₆ agonist, muscimol (9nmol). Whole-body startle responses were measured with startle pulses between 95-105dB and prepulses between 4-12dB above background noise. Bilateral inhibition of the DLSC significantly disrupted PPI without altering baseline startle amplitude. In contrast to the divergent roles for SNpr between the two species, here we found a similar effect for the DLSC inhibition to that in rodent. These results suggest that the species divergence at the level of the SNpr is not due to downstream
differences at the level of the DLSC. These data also underscore the role of the DLSC as a multimodal integrator of sensory information and an important modulator of sensorimotor gating across species.

**Disclosures:** H.F. Waguespack: None. P. Forcelli: None. L. Malkova: None. B.L. Aguilar: None.

**Poster**

**321. Stress-Modulated Pathways: Brainstem and Others**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 321.05/U25

**Topic:** F.04. Stress and the Brain

**Support:** AALAS Grants for Laboratory Animal Science (GLAS)

**Title:** Effects of altitude on depressive-like behavioral responses in adult male and female rats

**Authors:** K. NGUYEN¹, C. A. GATES², *J. E. HASSELL, Jr¹, C. L. CHO¹, A. ARNOLD¹, A. ELSAYED¹, S. SALAZAR¹, A. LUTHENS¹, M. LEBLANC⁴, S. C. ADAMS⁴, C. A. LOWRY³, J. D. REUTER²;
¹Integrative Physiol., ²Dept. of Integrative Physiol. and Ctr. for Neurosci., ³Univ. of Colorado Boulder, Boulder, CO; ⁴Salk Inst., La Jolla, CA

**Abstract:** Major depression is one of the most prevalent mental illnesses in the United States with an estimated 16.1 million people suffering from a depressive episode every year. The prevalence of major depressive disorder (MDD) among females (8.5%) is nearly twice that of males (4.8%). In addition, states at high altitude in the Intermountain West report higher suicide rates compared with the national average. However, little is known about the effects of acute vs chronic exposure to high altitude on the risk of development of depression or the persistence of depressive symptoms, and there are no previous studies that examine the effects duration of acclimation to high altitude on anxiety- and depressive-like behavioral responses in animal models. We hypothesize that exposure to hypobaric hypoxia at high altitude can increase vulnerability to depression in a gender-specific manner. To determine if high altitude has effects on depressive-like behavioral responses, males and females of two different strains of rats (Sprague Dawley and Long Evans) were tested in the sucrose preference test. Testing took place at sea level or 5,328 feet above sea level (mid- to high altitude) following acclimation for 1, 2, 3, 4, or 5 weeks (n = 8 per group; using a 2 (high altitude versus sea level) x 2 (male versus female) x 2 (Sprague Dawley versus Long Evans) x 5 (1, 2, 3, 4, or 5 weeks) experimental design; N=320). Rats were euthanized 24 hours after behavioral testing. Here we demonstrate that rats acclimated to 5,328 ft displayed lower sucrose preference, relative to rats housed at sea level, at 1, 2, and 5 weeks of acclimation. Additionally, male rats acclimated to 5,328 ft displayed lower
sucrose preference than female rats acclimated to 5,328 ft, while no sex differences were observed at sea level. Physiologically, using data from both male and female rats, rats tested at 5,328 ft had increased plasma hemoglobin and red blood cell concentrations compared to rats tested at sea level. For red blood cell concentrations, the effect of altitude was evident after 1 and 2 weeks of acclimation. In contrast, for hemoglobin concentrations, the effect of altitude was evident after 1, 2, 3, 4, and 5 weeks of acclimation. These data are consistent with the hypothesis that hypobaric hypoxia at higher altitudes increases vulnerability to development of anhedonic-like behavioral responses.


**Poster**

**321. Stress-Modulated Pathways: Brainstem and Others**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 321.06/U26

**Topic:** F.04. Stress and the Brain

**Support:** UF CVM Research Grant

**Title:** Manganese enhanced MRI (MEMRI) imaging and changes in cardiovascular reactivity implicate the dorsal raphe in modulating anxiety like behavior in DOCA/salt hypertension

**Authors:** J. M. WATKINS¹, M. FEBO², M. POMPILUS², *L. F. HAYWARD¹; ¹Physiol Sci., ²Psychiatry, Univ. of Florida, Gainesville, FL

**Abstract:** Changes in the brain's serotonin and corticotropin-releasing factor (CRF) systems are strongly associated with anxiety and depression. Anxiety/depression and the development of high blood pressure (HBP) are also thought to be interconnected. In the current study, we evaluated whether chronic HBP alters regional baseline brain activity and anxiety related behavior in adult male Sprague Dawley rats. HBP was induced by subcutaneous implantation of a slow-release pellet containing the aldosterone precursor DOCA (150 mg/21 days) and 13 days of elevated salt consumption via voluntary intake of a 1%NaCl/0.2%K+ solution (182 ± 31 vs 22 ± 6 ml/day, DOCA vs control, respectively). One group of rats underwent MEMRI imaging (4.7 T Magnex MR scanner; 25 mg/kg manganese, ip, n=5-7/group). Imaging identified basal activity was significantly reduced in three specific brain regions in the DOCA/salt versus control, including the dorsal raphe, dorsomedial hypothalamus and nucleus accumbens shell (P=0.05). A second group of animals were chronically instrumented with arterial pressure (AP) telemetry probes (Stellar TSE). Resting AP was 151± 10 mmHg in the DOCA/salt group compared to 110 ± 5 mmHg in the controls (P=0.005; n=5/group). On day 13, DOCA/salt and control animals
were placed on an elevated plus maze (EPM) for 5 min. DOCA/salt rats spent significantly more time transitioning between the closed arms and center zone compared to controls (P=0.01). During EPM testing both AP and heart rate (HR) increased, but the rise in both AP and HR was attenuated in the DOCA/salt group, paralleling the reduction in anxiety-like behavior. The average change in AP during EPM testing in the DOCA/salt group was 11±2 vs 28±3 mmHg in the controls (P=0.001). The rise in HR was 80±4 in the DOCA/salt group vs 103±14 bpm in the controls (P=0.07). These findings support previous work in a genetic model of hypertension, the spontaneously hypertensive rat (SHR) which was identified to have both reduced anxiety and cardiovascular reactivity, and suggest that chronic HBP can attenuate anxiety related responses in rodents. Furthermore, a comparison of whole brain MEMRI imaging from the SHR (Zubcevic J et al., 2018) and the current DOCA/salt model identifies one common brain region potentially involved in attenuating anxiety related behavior in hypertension, the dorsal raphe. Preliminary data shows DOCA/salt hypertension modulates gene expression of both tph2 (serotonin system) and CRF-R1 in the dorsal raphe. The results of this study demonstrate a novel link between tonic dorsal raphe dysregulation and reduced measures of anxiety in chronic HBP.

**Disclosures:** J.M. Watkins: None. M. Febo: None. M. Pompilus: None. L.F. Hayward: None.

**Poster**

**321. Stress-Modulated Pathways: Brainstem and Others**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 321.07/U27

**Topic:** F.04. Stress and the Brain

**Support:** CIHR - Doctoral Foreign Study Award

**Title:** Identifying glucocorticoid receptor mediated mechanisms of polygene-environment interactions involved in stress sensitivity

**Authors:** *S. PENNER-GOEKE, S. RÖH, M. JAKOVCEVSKI, E. B. BINDER; Max Planck Inst. of Psychiatry, Munich, Germany

**Abstract:** Despite the significant social and economic costs of stress related psychiatric disorders, the molecular mechanisms underlying these diseases remain poorly understood. Using an eQTL approach, previous research from our group successfully identified common functional genetic variants mediating stress sensitivity by altering the transcriptional response of stress-related genes (Arloth, 2015, Neuron). These variants are enriched in long-range enhancer regions in genes targeted by the glucocorticoid receptor (GR), a key transcription factor in the stress response system. Using the variants, a genetic risk score (PRS) was calculated describing transcriptional sensitivity to glucocorticoids (GR-PRS). Importantly, a derivative of this score
could predict risk for major depressive disorder (MDD) and changes in neural circuit activity related to emotion-processing, highlighting the clinical importance of understanding the molecular mechanisms by which these variants alter transcriptional activity at GR target genes. We speculate that under stress conditions, these variants alter a number of different molecular mechanisms, namely GR binding and chromatin interactions between transcriptional start sites and GR-driven enhancer regions, resulting in transcriptional changes in stress relevant networks. MDD patient and control derived lymphoblastoid cell lines harboring high and low GR-PRSes were selected. RNA-sequencing was performed to assess whether there were transcriptional differences in stress relevant networks in LCLs with high vs. low GR-PRSes upon treatment with the GR agonist, dexamethasone. Clustering of samples into high and low polygenic risk was observed when stress relevant genes were analyzed, but not when all differentially expressed genes were included. Next, the mechanisms by which these variants cause this altered transcriptional response upon activation of the stress response system will be identified, using GR-ChIP and a high throughput enhancer screen (STARR-seq). Identification of specific mechanisms mediating stress sensitivity may represent new therapeutic targets for stress-related disease, such as MDD.


Poster

321. Stress-Modulated Pathways: Brainstem and Others

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program#/Poster #: 321.08/U28

Topic: F.04. Stress and the Brain

Support: PTE AOK KA-2019-12

Title: TRPA1 KO mice show increased HPA axis sensitivity to chronic variable mild stress

Authors: *V. KORMOS¹, J. FARKAS², T. GASZNER², A. KECSKES¹, Z. HELYES¹, B. GASZNER², E. PINTER¹;
¹Pharmacol. and Pharmacotherapy, ²Anat., Pécs Univ., Pécs, Hungary

Abstract: Transient receptor potential cation channel subfamily A member 1 (TRPA1) is an ion channel expressed primarily in the sensory nervous system. The receptor is sensitive for numerous chemical substances and for temperature changes, therefore, its involvement in nociception and inflammatory responses is in the center of interest. TRPA1 knockout mice were shown to display reduced response to nociceptive stimuli. This phenomenon was attributed to functional changes in the sensory peripheral nervous system. Little is known however about the possible role of TRPA1 receptors in longer term environmental challenges that would require
complex adaptation response. Therefore, our aim was here to test the stress adaptation response of the TRPA1 knockout (KO) mice in chronic variable mild stress (CVMS) model. We hypothesized that mice lacking the functional TRPA1 will show altered response to the stressor. Sixteen TRPA1 KO mice and wildtype (WT) counterparts were subjected to CVMS for three weeks. The paradigm consisted of a short term daytime stressor (i.e. 30 mins restraint, shaker, tilted cage or behavioral test) and an overnight mild stress exposure (i.e. wet bedding, social isolation). Control TRPA1 KO and WT mice were not exposed to stress, except behavioral testing by marble burying (MBT), tail suspension (TST) and forced swim (FST), sucrose preference (SPT) tests. Trunk blood samples were collected, and also thymus and adrenal glands were removed and weighed. Control TRPA1 KO mice showed reduced weight gain compared to WT animals. CVMS attenuated the bodyweight gain in both genotypes. Both control and CVMS exposed KO mice showed slightly reduced anxiety levels in MBT. A similar tendency was observed in TST for depression level also, however without statistical power. In FST, control TRPA1 KO mice exerted increased depression level which did not increase upon CVMS, in contrast to that of WT mice. The anhedonia level in SPT was increased by CVMS exposure, but the genotype did not influence the consumption of sweetened fluid. Similarly, blood corticosterone was elevated by CVMS exposure in both genotypes. Control relative adrenal weights did not differ, but upon CVMS, TRPA1 KO mice had larger relative adrenal weights. Interestingly, the relative thymus weight was reduced by CVMS in WT mice only. These data suggest that TRPA1 knockout mice show altered behavioral phenotype in depression and anxiety tests. Altered dynamics of thymus- adrenal- and bodyweight in TRPA1 KO mice upon CVMS exposure suggests the long-term change in the activity of hypothalamus-pituitary-adrenal axis. In our ongoing experiments we study the underlying central mechanism in the brain.


Poster

321. Stress-Modulated Pathways: Brainstem and Others

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 321.09/U29

Topic: F.04. Stress and the Brain

Support: F32DA043924

Title: Stress-dependent plasticity of glutamatergic afferents to the locus coeruleus

Authors: *K. BARCOMB¹, C. FORD²;
¹Univ. of Colorado Anschutz Med. Campus, Aurora, CO; ²Pharmacol., Univ. of Colorado, Aurora, CO
Abstract: The locus coeruleus (LC) is the primary source of norepinephrine (NE) to the brain and acts as a major regulator of arousal and attention. The LC is strongly activated by stress and LC dysfunction can lead to psychiatric disorders such as post-traumatic stress disorder. Because of its prominent role in brain function and dysfunction, it is essential to understand the circuitry of the LC. However, while the projection targets of LC-NE neurons have been well studied, little is known about the excitatory LC afferents driving LC activity. In particular, it is unknown if and how glutamatergic inputs are mediated by stress and whether or not they exhibit stress-dependent plasticity. These questions were addressed here using an optogenetic approach combined with whole cell electrophysiology in mice. First, the overall glutamatergic synaptic strength was assessed by recording spontaneous excitatory postsynaptic currents (sEPSCs) either basally or after a 30 minute restraint stress. No change was found after stress in either the frequency or amplitude of events. This lack of an effect of stress on global glutamatergic strength was verified using a reporter mouse that expressed channelrhodopsin-2 (ChR2) in all excitatory (CaMKII-expressing) neurons. In these animals, EPSCs were optically evoked (oEPSCs) and glutamatergic strength was measured using AMPAR/NMDAR ratios and paired pulse ratios (PPRs). Neither measure differed between control and stress conditions. Having observed no change in global synaptic strength post-stress, we finally asked whether stress-dependent modulation may occur at isolated LC afferents by expressing ChR2 in specific regions. A cre-dependent ChR2 was stereotaxically injected into the prefrontal cortex (PFC), periaqueductal grey (PAG), or lateral hypothalamus (LH) of CaMKII-Cre mice, allowing for the expression of ChR2 in just the glutamatergic neurons in those regions. Again, AMPAR/NMDAR ratios and PPRs were measured using oEPSCs in LC-NE neurons of control or stressed mice. While no change was found in the inputs from PAG and LH, there was a significant reduction in the PPRs of PFC inputs. These results suggest a presynaptic increase in the strength of PFC afferents to the LC after stress, which is not observed in other afferents or when looking globally at all excitatory inputs. Further experiments will determine the physiological relevance of this stress-dependent plasticity.

Disclosures: K. Barcomb: None. C. Ford: None.

Poster

321. Stress-Modulated Pathways: Brainstem and Others

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 321.10/U30

Topic: F.04. Stress and the Brain

Support: NIH Grant MH106757
Young Investigator Award from the Brain and Behavior Research Foundation

Title: Regulation of VTA GABAergic microcircuitry by chronic variable stress
**Authors:** *C. BOUARAB, B. THOMPSON, N. FERN, A. POLTER;*  
The George Washington Univ., Washington, DC

**Abstract:** Chronic exposure to stress interferes with hedonic processing and plays a significant role in the development of mood and anxiety disorders. While numerous studies have shown the post-hoc consequences of stress, very little is known about the processes that occur during the transition from acute to chronic stress and how they contribute to the emergence of anhedonic behavioral sequelae over the course of stress. GABAergic neurons of the ventral tegmental area (VTA) are uniquely situated to be a powerful regulator at the intersection of stress and reward. VTA GABA neurons exert strong control over the activity of VTA dopamine neurons. Inputs from throughout the brain that carry neuroendocrine, homeostatic, stress-related and social information synapse onto VTA GABA neurons. Thus, these neurons receive diverse information about an animal’s internal and external state and integrate this information to tune dopaminergic neuron activity and thus reward-seeking behavior. Moreover, VTA GABA neurons are robustly activated by aversive stimuli or acute stress, perfectly positioning them to regulate changes in reward-related behavior in response to stressful or aversive experiences. However, few studies have measured the effects of chronic stress on VTA GABAergic neurons. In this study, we set out to characterize changes in the activity of VTA GABA and dopamine neurons following subchronic and chronic variable stress and uncover synaptic mechanisms mediating these changes. We find that subchronic variable stress, which induces behavioral changes in females but not males, is associated with an increase in inhibitory tone in both female and male mice. However, we observe a decrease in the firing rate of dopamine neurons in female, but not male mice. This suggests that a parallel protective mechanism prevents stress-induced decreases in dopaminergic neuron firing in males. Likewise, we find that chronic variable stress induces changes in inhibitory transmission onto dopamine neurons in both males and females. Our future studies will use fiber photometry and slice recording to characterize the sex-specific changes in VTA inhibition that occur over the course of chronic stress. Taken together, our data indicate a significant role for VTA GABA neurons in mediating the switch from physiology to pathology in depression-like behavior.

**Disclosures:** C. Bouarab: None. N. Fern: None. B. Thompson: None. A. Polter: None.

**Poster**

321. Stress-Modulated Pathways: Brainstem and Others

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #: Poster #:** 321.11/U31

**Topic:** F.04. Stress and the Brain

**Support:** NIH Grant MH106757  
Young Investigator Award Brain and Behavior Research Foundation
Title: VTA GABA neurons mediate sex-specific social interaction deficits after subchronic variable stress

Authors: B. THOMPSON1, C. BOUARAB3, *A. M. POLTER2;  

Abstract: Women are roughly twice as likely as men to be diagnosed with a mood or anxiety disorder. Given that stress plays a significant role in the development of these disorders, sexual dimorphisms in the response to stress are likely to be a critical factor in the enhanced vulnerability of females to mood and anxiety disorders. Across both human populations and animal models, males and females exhibit divergent responses to stress at all levels, from molecular signatures to behavioral adaptations. Subchronic variable stress (SCVS) is a model of depression and anxiety in which female mice develop anhedonia and anxiety, but males do not (LaPlant et al, Biological Psychiatry, 2009; Hodes et al, J. Neuroscience, 2015). In this study, we use this model to investigate the role that VTA GABA neurons play in sex-specific social interaction and in the response to stress. 

Dysregulation of the mesolimbic reward circuitry is implicated in the pathophysiology of stress-related illnesses such as depression and anxiety. VTA GABAergic neurons are poised to be a critical node in the regulation of female-specific maladaptive behavior following SCVS. These neurons regulate activity of the mesolimbic dopaminergic pathway, both by gating the activity of neighboring dopamine neurons and through a projection to the NAc. VTA GABA neurons modulate reward and anxiety-related behaviors and are activated by acute stressors; however, little is known about neuroadaptations in these neurons in response to chronic or repeated stressors. We hypothesize that SCVS increases activity of GABAergic neurons in the VTA in female animals, and that reversing this will decrease SCVS-induced social interaction deficits. Here, we show that SCVS causes sex-specific social deficits in female mice. Stressed female mice spend less time interacting with a naïve male mouse, however, they show no decrease in interaction with another female. In contrast, stressed males show no social deficits. Chemogenetic inhibition of VTA GABAergic neurons after stress reverses this deficit in females, but has no significant effects on sociability in males. Future studies will address the ability of inhibition of GABAergic neurons during stress to prevent stress induced neuroadaptations. Taken together, our data shows that regulation of the VTA microcircuitry in response to stress is sexually dimorphic and may play a role in the development of maladaptive behavioral responses.

Disclosures: B. Thompson: None. C. Bouarab: None. A.M. Polter: None.
**Poster**

321. Stress-Modulated Pathways: Brainstem and Others

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 321.12/U32

**Topic:** F.04. Stress and the Brain

**Support:** KAKENHI 18K10852

**Title:** Stress drives deliberative tendency via influences on vicarious trial-and-error and monoaminergic systems

**Authors:** *S. AMEMIYA*\(^1\,\,2\), M. ISHIDA\(^2\), N. KUBOTA\(^2\), T. NISHIJIMA\(^2\), I. KITA\(^2\);
\(^1\)RIKEN CBS, Saitama, Japan; \(^2\)Tokyo Metropolitan Univ., Tokyo, Japan

**Abstract:** Extensive evidences have indicated that stress influences decision making even under familiar choice situations. However, how stress affects decision-making processes is still unclear. Previous studies have reported that vicarious trial-and-error (VTE), rat's head-orienting behavior toward possible options at choice points, reflects decision-making processes. Other series of studies have reported that monoaminergic neurons, including dopamine neurons in the ventral tegmental area (VTA DA), serotonin neurons in the dorsal raphe nucleus (DRN 5-HT), noradrenaline neurons in the locus coeruleus (LC NA), play roles in decision making by regulating reward evaluation, impulse control and attentional control. The monoaminergic neurons are also known to be sensitive to various stressors. Here, in order to examine effects of stress on decision making, we examine the effects of restraint stress on subsequent decision-making behaviors and neural activity of DRN 5-HT, LC NA, and VTA DA neurons in T-maze task in rats. In the T-maze choice task, an arm has 3 pellets (high-reward side) and the other arm has 1 pellet and the high-reward side was constant throughout the maze test for each rat. Rats ran the task under the same reward condition three consecutive days. Before starting the 3rd day session, rats in the stress-treated group received mild restraint stress for 30 min, and rats in the control group were left in the home cage. On the 3rd day session, we recorded the number of choice of high-reward side, and spending time and VTE at the T-shaped choice point. In addition, we quantified neural activity of the monoaminergic neurons, using c-Fos/5-HT or c-Fos/TH (tyrosine hydroxylase) immunohistochemistry. Acute restraint stress did not affect the number of choice of high-reward side, but increased spending time and the number of VTE at the choice point. The DRN 5-HT and LC NA, but not VTA DA neurons, activated in the stress-treated group compared to the control group. These results suggest that stress increases a deliberate tendency in decision making via increasing VTE and activity of monoaminergic neurons related to impulse control and attentional control.

Poster
321. Stress-Modulated Pathways: Brainstem and Others

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 321.13/U33

Topic: F.04. Stress and the Brain

Support: ERC-2015-CoG 682591

Title: Noradrenergic modulation of large-scale brain networks in response to acute stress

Authors: *M. KRENTZ, H. MURALI MAHADEVAN, Z. REPPMANN, R. TOUTOUNJI, F. KRAUSE, E. J. HERMANS;
Donders Inst. For Brain, Cognition and Behaviour, Radboud Univ. Med. Ctr., Nijmegen, Netherlands

Abstract: Introduction
Acute stress elicits adaptive cognitive and behavioral changes. These are hypothesized to be supported by rapid changes in the interplay between large-scale brain networks. While the salience network (SN) is upregulated after stress, executive control network (ECN) and default mode network (DMN) appear to be downregulated. This shift is thought to be triggered by rapid noradrenergic activation originating from the locus coeruleus (LC), the sole source of cortical norepinephrine. Here, we combined the assessment of large-scale brain network dynamics with functional imaging of LC, which is highly challenging given its small size. Due to increased LC activity under stress, we expect (1) stronger LC-SN coupling, and (2) stronger negative LC-ECN and LC-DMN coupling, after acute stress.

Methods
In the first of three sessions, high-resolution structural imaging, including an LC (neuromelanin)-sensitive T1-weighted TSE sequence, was performed. Two subsequent sessions consisted of resting-state functional assessments following a standardized stress-induction (SECPT) or control procedure. Time courses for network activity were extracted using individualized network masks based on Shirer et al. (2012). LC time courses were extracted using individual segmentations, created by interleaving differently positioned TSE-sequence acquisitions to increase vertical resolution while maintaining SNR. Connectivity measures were corrected for movement, physiological noise (RETOICOR), and compartment signals (e.g., ventricular noise).

Results
Pre-liminary data (n=16) does not suffice to test connectivity hypotheses, but shows that using an interleaved TSE sequence with neuromelanin contrast allows for accurate LC segmentation.
Furthermore, LC BOLD signal exhibits reasonable SNR and resting connectivity, using a set of carefully selected covariates. Moreover, network templates allowed for successful identification of individualized core networks of interest for connectivity analysis. Finally, physiological measures point toward robust stress induction effects.

Conclusion
Preliminary results show that our interleaved TSE sequence can successfully localize LC and data quality and validity assessments point toward testability of the hypotheses upon completion of data acquisition.

Disclosures: M. Krentz: None. H. Murali Mahadevan: None. Z. Reppmann: None. R. Toutounji: None. F. Krause: None. E.J. Hermans: None.

Poster
321. Stress-Modulated Pathways: Brainstem and Others

Location: Hall A
Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM
Program #: Poster #: 321.14/U34
Topic: F.03. Neuroendocrine Processes
Support: NIH NINDS R01NS053978
University of Michigan EBS initiative

Title: Electrophysiological characterization of the stress response in the intact adrenal medulla

Authors: *J. R. LOPEZ RUIZ*, N. PALLAS, S. ERNST, R. W. HOLZ, E. L. STUENKEL;  
1Mol. & Integrative Physiol., 2Cell and Developmental Biol., 3Pharmacol., Univ. of Michigan, Ann Arbor, MI

Abstract: The stress response has a major role in survival and prepares the body to deal with different challenges, however if prolonged, often drives adverse health consequences. A key pathway of the stress response is through the adrenal medulla, in which architecturally clustered and electrically coupled chromaffin cells secrete catecholamines to the general circulation affecting multiple distant target organs. Nonetheless, the in vivo dynamics and interactions within the intact adrenal medulla still remain unknown. Therefore, the goal of this project is to continuously and precisely monitor the electrophysiological responses from the adrenal medulla in the living animal during normal and under stress conditions, to topographically dissect the chromaffin cells population and their interactions. To achieve this, a high density silicon probe was slowly inserted into the immobilized left adrenal gland while recording, then the system was challenged first by electrically stimulating the splanchnic nerve with different intensities, frequencies and train durations, and thereafter by inducing hypoxia to assess the evoked stress response at a cellular level. During basal conditions, spontaneous single as well as compound action potentials with distinct firing rates that ranged from 0.3 to 4.5 Hz were recorded from
multiple anesthetized subjects, >90% of the recorded cells displayed a stable firing rate of under 3Hz. By locally applying TTX to the splanchnic nerve we were able to reversibly block this activity. When lined up and correlated with adjacent channels, the action potential’s waveforms, revealed submillisecond interactions that most likely reflect the activity from highly synchronized neighboring chromaffin cells that fire in a specific sequence, each cluster or group of cells fired independently from one another. The electrical stimulation of the splanchnic nerve evoked the firing of the same units and clusters that were spontaneously recorded during basal conditions, with latencies from 2 to 20 ms after the stimulus. The recorded units were able to follow up to 40Hz trains. To assess the physiological stress response, hypoxia by respiratory arrest was induced through an anesthesia overdose. The result was a generalized 1.5-3x increase in the firing rate of the recorded units at the moment breathing stopped, reaching a maximum several seconds after and then gradually dropping to a complete silence. For the first time we were able to document the internal dynamics of the intact adrenal medulla, showing the extend and interactions of the local circuitry that drives the adreno-medullary stress response in the living animal.


Poster

321. Stress-Modulated Pathways: Brainstem and Others

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 321.15/U35

Topic: F.04. Stress and the Brain

Support: FWO grant G.0287.16
Geneeskundige Stichting Koningin Elisabeth (GSKE)

Title: Neuromedin U-8 involvement in stress modulation

Authors: *W. ALLAOU1, A. DE PRINS, A. VAN EEEKHOUT, S. BALLET, I. SMOLDERERS, D. DE BUNDEL;
Vrije Univ. Brussel, Brussels, Belgium

Abstract: Neuromedin U (NMU) is a neuropeptide that is highly conserved across mammals, suggesting strong evolutionary pressure to maintain its structure and function. The C-terminal amidated octapeptide NMU-8 exerts the same biological effects as its longer endogenous isoforms NMU-23 and NMU-25. NMU interacts via two G-protein coupled receptors known as NMUR1 and NMUR2. Studies have shown the involvement of NMU in the stress response, demonstrating increased stress-related behavior following central NMU administration in rodents.
In the present study we evaluated the effects of NMU-8 on stress-related behavior in male C57BL/6J mice. Mice received intracerebroventricular (i.c.v.) administrations of NMU-8 or saline and were evaluated on stress-related behavior in a safe environment, being their home cage, or after forced swim stress. We subsequently evaluated c-Fos expression in the paraventricular nucleus of the hypothalamus (PVH) and arcuate nucleus (ARC) to assess neuronal activation. Additionally, we measured plasma corticosterone using an enzyme-linked immunosorbent assay.

I.c.v. administration of NMU-8 resulted in increased grooming in a safe environment, while no effects on digging or locomotor activity were observed. When mice were exposed to the forced swim test, NMU-8 increased immobility compared to control mice. Furthermore, when mice were pre-exposed to additional forced swim stress one day prior to the forced swim test, NMU-8 was still able to significantly increase immobility. In line with these observations, we observed elevated c-Fos immunoreactivity in the PVH and ARC in naïve mice. When mice were subjected to a single forced swim test, NMU-8 did not significantly increase c-Fos levels in the PVH but increased c-Fos levels in the ARC. However, NMU-8 increased c-Fos expression in both PVH and ARC when mice were pre-exposed to forced swim stress. Interestingly, central NMU-8 administration lowered plasma corticosterone significantly in both mice subjected to a stressful experience and in naïve mice. However, when mice were pre-exposed to forced swim stress, NMU-8 did not significantly decrease plasma corticosterone.

In conclusion, i.c.v. administration of NMU-8 increases neuronal activity in the hypothalamo-pituitary-adrenal axis in baseline and stressful conditions, in line with what we observed at a behavioral level. The exact mechanism through which central NMU-8 administration lowers corticosterone plasma concentrations remains unclear. We suggest that central NMU-8 administration elicits a negative feedback signal towards peripheral corticosterone release, while stress-related signaling in the brain increases.


Poster

321. Stress-Modulated Pathways: Brainstem and Others

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 321.16/U36

Topic: F.06. Brain Blood Flow/ Metabolism/ and Homeostasis

Support: GVSU Department of Psychology
        GVSU CSCE
        GVSU OURS
**Title:** Predator odor stress on stress and metabolic endocrine outcomes in male and female c57bl6 mice

**Authors:** *M. L. BLOCK*¹, K. WILK¹, E. I. M. FLANDREAU²;
²Psychology, ¹Grand Valley State Univ., Allendale, MI

**Abstract:** It has long been known that the brain and gut ‘talk’ to each other and this bidirectional communication is important for both physical and mental health as demonstrated by associations between metabolic syndrome and mental illness in humans. Neuropeptides found in both the gastrointestinal tract and the central nervous system are instrumental in brain / gut communication and may mediate links between stress, metabolism, and psychopathology. The present study is designed to determine whether a single exposure to predator odor is sufficient to produce longer-term changes in the endocrine system as measured by plasma concentrations of the “stress hormone” corticosterone (CORT) and the “hunger hormone” ghrelin. While numerous studies implicate CORT in stress-induced psychopathology, we are just beginning to appreciate the influence of ghrelin in this process. Male and female mice were exposed to one hour of predator stress. Blood was collected 48 hours, 7 days, or 14 days later to assess plasma concentration of these hormones. In addition, body weight and food intake were recorded throughout the study to determine the impact of stress on metabolic efficiency (body weight gained per gram of food intake). Results from this study are important to better understand how stress can contribute to both metabolic syndrome and / or mental illness.

**Disclosures:** M.L. Block: None. K. Wilk: None. E.I.M. Flandreau: A. Employment/Salary (full or part-time); GVSU.

**Poster**

321. Stress-Modulated Pathways: Brainstem and Others

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 321.17/U37

**Topic:** F.06. Brain Blood Flow/ Metabolism/ and Homeostasis

**Support:** GVSU Psychology Department
             GVSU OURS
             GVSU CSCE
             McNair

**Title:** Timeline for impact of high sucrose diet on plasma corticosterone and ghrelin and caloric efficiency in male and female mice

**Authors:** *T. N. DESJARLAIS*¹, E. I. M. FLANDREAU²;
²Psychology, ¹Grand Valley State Univ., Allendale, MI
Abstract: An unhealthy diet is known to increase risk for metabolic syndrome but may also be a risk factor for mental illness. The present study examined the influence of a high sucrose diet (50% sucrose) on two hormones that are both implicated in stress and metabolism: corticosterone, and ghrelin. Control mice were fed a matched diet with less than 1% sucrose or standard chow. We’ve previously found that 10 days of a high sucrose diet increased negative-valence behavior related to anxiety and also increased plasma corticosterone and ghrelin concentrations 30 days later. The present study measures caloric efficiency weekly and collected blood 24 hours, 7 days, 14 days, or 21 days after starting the special diet to establish a timeline for changes in these endocrine systems, which may explain the links between metabolic and mental illness.

Disclosures: T.N. Desjarlais: None. E.I.M. Flandreau: A. Employment/Salary (full or part-time); Grand Valley State University.

Poster

321. Stress-Modulated Pathways: Brainstem and Others

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 321.18/U38

Topic: F.04. Stress and the Brain

Support: JSPS KAKENHI 16K09269
JSPS KAKENHI 17K09331

Title: Inhibitory effect of glucose infusion on restraint stress-induced elevation of plasma adrenaline level in rats

Authors: *N. YAMAGUCHI1, Y. KAKINUMA3, K. MIMURA1, T. YAKURA2, M. NAITO2, S. OKADA1;
1Dept. of Pharmacol., 2Dept. of Anat., Aichi Med. Univ., Aichi, Japan; 3Dept. of Physiol., Nippon Med. Sch., Tokyo, Japan

Abstract: Exposure to stressor causes various systemic responses such as activation of the sympathetic nervous system and the hypothalamus-pituitary-adrenocortical (HPA) axis. Stress-induced sympathetic activation increases plasma levels of catecholamines (noradrenaline and adrenaline), resulting in elevations in blood pressure and heart rate. In addition, facilitated secretion of adrenaline from the adrenal medulla and pancreatic glucagon associated with sympathetic activation increase blood glucose level. We have previously reported that stress-related neuropeptides such as corticotropin-releasing factor and acute exposure to restraint stress increase the plasma levels of catecholamines, and that prostanoids such as prostaglandin E2 and thromboxane A2 in the brain, especially in the paraventricular hypothalamic nucleus (PVN), regulate the elevation of plasma catecholamine levels. Therefore, there is a high possibility that
brain prostanoids are involved with stress-related changes in glucose dynamics. In the present study, we examined effects of intravenous glucose infusion on sympathetic activation, that is elevations of plasma catecholamine levels and changes in brain prostanoid levels, under restraint stress exposure in male rats. There were no significant differences in blood glucose levels between vehicle-treated stressed group and glucose-treated stressed group. However, intravenous infusion of 5% glucose solution effectively suppressed stress-induced elevation of plasma level of adrenaline, but not noradrenaline. Similarly, intracerebroventricular infusion of glucose solution also prevented the stress-induced elevation of plasma level of adrenaline, but not noradrenaline. Furthermore, we found that exposure to restraint stress increased thromboxane B$_2$, a stable metabolite of thromboxane A$_2$, in PVN dialysates, and the increase in thromboxane B$_2$ level was suppressed by intravenous glucose infusion. Our results suggest that glucose infusion can inhibit stress-induced sympathetic activation, and that prostanoids in the PVN are involved with this mechanism.


Poster

321. Stress-Modulated Pathways: Brainstem and Others

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 321.19/U39

Topic: F.04. Stress and the Brain

Support: JSPS KAKENHI 17K09331
JSPS KAKENHI 16K09269

Title: Thromboxane A$_2$ in the paraventricular nucleus of the hypothalamus participates in 2-deoxyglucose-induced sympathetic activation in freely moving rats

Authors: *S. OKADA, M. TACHI, N. YAMAGUCHI;
Depatment of Pharmacol., Aichi Med. Univ., Aichi, Japan

Abstract: Glucose is the major source of energy for the cells in higher organisms and its homeostasis is essential for maintaining physiological activities. 2-deoxyglucose (2-DG), a non-metabolizable glucose analog, competitively inhibit glucose utilization, resulting in a state of intracellular glucoprivation. Several lines of evidence indicate that 2-DG activates markedly the sympathetic adrenergic system, in contrast with relatively mild sympathetic noradrenergic responses. In addition, 2-DG stimulates noradrenergic system in the brain. We previously reported that release and/or secretion of plasma adrenaline and noradrenaline are separately regulated in brain prostanoid- and adrenoceptor-dependent manners. In this study, we examined the possibility that intravenously administered 2-DG stimulates noradrenaline release in the
PVN, the major integrative center for sympathetic activation, and then released noradrenaline and produced prostanoids in the PVN are involved with the 2-DG-induced elevation of plasma catecholamine levels in freely moving rats. Intravenously administered 2-DG dose-dependently elevated noradrenaline release in the PVN and plasma levels of catecholamines. Central pretreatment with phentolamine suppressed the 2-DG-induced elevation of plasma level of adrenaline, but not noradrenaline. In contrast, central pretreatment with propranolol suppressed the 2-DG-induced elevation of plasma level of noradrenaline, but not adrenaline. Pretreatment with indomethacin attenuated the 2-DG-induced elevation of both noradrenaline and adrenaline levels. Pretreatment with furegrelate, a thromboxane synthase inhibitor, attenuated the 2-DG-induced elevation of both noradrenaline and adrenaline levels, while pretreatment with L-798106, a prostaglandin E2 receptor subtype EP3 antagonist, did not alter the 2-DG-induced elevation of plasma catecholamine levels. Furthermore, 2-DG infusion elevated thromboxane B2, a metabolite of thromboxane A2, but not PGE2 in the PVN microdialysate. Our results suggest that glucoprivation stress increases noradrenaline release in the PVN and then causes TxA2 production in the PVN, which are essential for the 2-DG-induced elevation of both of plasma adrenaline and noradrenaline.

Disclosures: S. Okada: None. M. Tachi: None. N. Yamaguchi: None.

Poster

321. Stress-Modulated Pathways: Brainstem and Others

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 321.20/U40

Topic: F.04. Stress and the Brain

Support: PAPIIT IN306918
PAPIME PE306318

Title: Evaluation of systemic corticosterone and VEGF in a diabetes-CUSB model

Authors: *D. B. PAZ-TREJO*¹, K. M. NAVARRETE-VELÁZQUEZ², P. ZARATE GONZALEZ³, H. SANCHEZ-CASTILLO³, R. ZAMORA ALVARADO⁶, L. D. OCHOA DE LA PAZ⁷,⁸
¹Sistema Univ. Abierta, ³Psychobiology and Neurosci. Dept., ²Univ. Nacional Autonoma de Mexico, Facultad de Psicologia, Mexico City, Mexico; ⁵PTSD traslational research, ⁴Sociedad Iberoamericana de Neurociencia Aplicada A.C., Mexico City, Mexico; ⁶Unidad de investigación Hosp. para evitar la ceguera en Mexico IAP, Hosp. Dr. Luis Sanchez Bulnes, Mexico City, Mexico; ⁷Biochem. Dept., Univ. Nacional Autonoma de Mexico, Facultad de Medicina, Mexico City, Mexico
**Abstract:** Diabetes is a metabolic disease with several health complications mainly related to poor glucose control and time with the disease, however it’s possible that stress serve as a modulator for them. Stress has been defined as a trigger for many mental diseases including depression and anxiety, also diabetic patients present more depression symptoms than no diabetic population. Besides metabolic alterations, it has been described that diabetes induces endocrine deregulation including disturbance in the HPA axis, one of the main regulators of stress response. In those findings, cortisol has been proposed as an indicator of health complications severity. The aim of the present work was to evaluate effects of exposure to a chronic unpredictable stress battery (CUSB) to subjects with induced diabetes. Ablation of pancreas is commonly use as type 1 diabetes model. Streptozotocin (STZ) induces chemical partial ablation of pancreas reducing insulin production and generating hyperglycemic conditions.

We administered a single dose of i.p. 65 mg/kg of STZ to 200-250g male Wistar rats. Subjects were divided in 4 groups: vehicle, vehicle+CUSB, STZ, STZ+CUSB. Six weeks after STZ administration, rats were sacrificed and trunk blood was collected for corticosterone and VEGF-A analyses. After diabetes induction subjects were exposed to a 10 days CUSB. Body weight was daily measured, meanwhile glucose levels were measured after CUSB and near to the end of the experiment. Results didn’t show statistical differences in corticosterone nor VEGF-A levels. Not according to other findings, corticosterone levels of the STZ (hyperglycemic) group were similar to both normoglycemic groups; however there was a great increase in variability in corticosterone levels of the STZ+CUSB group. This data suggests that in early stages of diabetes, the negative feedback regulating corticosterone levels may not be affected and its impairment could be modulated by stress. This can be interpreted as an accelerated deterioration in the presence of stress and could be proved with a continuous measurement of corticosterone and evaluation of other elements of the HPA axis.

Under stress conditions both groups of CUSB decreased their weight and a lower glucose level in the STZ+CUSB was obtained compared to STZ group. The absence of effect in VEGF could be due to the fact that evaluation was systemic and previous evidence shows that VEGF levels variate differentially in particular structures of the system in diabetes and stress conditions. We observe a relationship between stress and diabetes, however more data is needed in order to establish stress as a risk factor in diabetes for health complications.

Title: Life course contribution of prenatal stress in modulating the prepulse inhibition of the acoustic startle reflex in an AD mouse model

Authors: *Z. JAFARI*\(^1,2\), B. KOLB\(^3\), M. MOHAJERANI\(^3\);
\(^1\)Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada; \(^2\)Basic Sci. in Rehabil., Iran Univ. of Med. Sci., Tehran, Iran, Islamic Republic of; \(^3\)Neurosci., Univ. of Lethbridge, CCBN, Lethbridge, AB, Canada

Abstract: The prepulse inhibition (PPI) of the acoustic startle reflex (ASR), as an index of sensorimotor gating, is one of the most extensively used paradigms in the field of neuropsychiatric disorders. Few studies have examined how prenatal stress (PS) regulates the sensorimotor gating during the lifespan, and also how PS modifies the development of Aβ pathology in brain areas underlying the PPI formation. We followed alternations in corticosterone levels, learning and memory, and the PPI of the ASR measures in APP\(^{NL-G-F/NL-G-F}\) offspring of dams exposed to gestational noise stress. In-depth quantifications of the amyloid-beta (Aβ) plaque accumulation were also performed at six months. The results indicated an age-dependent deterioration of sensorimotor gating, long-lasting PS-induced abnormalities in PPI magnitudes, as well as deficits in spatial memory. The PS also resulted in a higher Aβ aggregation predominantly in brain areas associated with the PPI modulation network. The findings suggest the contribution of a PS-induced HPA-axis hyperactivity in regulating the PPI modulation substrates leading to the abnormal development of the neural protection system in response to disruptive stimuli. The long-lasting HPA axis dysregulation appears to be the major underlying mechanism in precipitating the Aβ deposition, especially in brain areas contributed to the PPI modulation network.

Keywords: Prenatal stress, Alzheimer's disease, amyloid-beta, HPA axis, prepulse inhibition, sensorimotor gating

Disclosures: **Z. Jafari:** None. **B. Kolb:** None. **M. Mohajerani:** None.
Poster

322. Stress, Cognition, and Behavior: Animal Studies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 322.02/V2

Topic: F.04. Stress and the Brain

Support: AA017991
AA07680
AA022448

Title: Therapeutic effects of dihydromyricetin on anxiety disorders induced by social isolation in mice

Authors: A. SHAO¹, X. M. SHAO², Y. OUYANG³, J. SILVA³, D. L. DAVIES³, R. W. OLSEN¹, *J. LIANG³;
¹Dept. of Mol. & Med. Pharmacol., UCLA, Los Angeles, CA; ²Neurobio., David Geffen Sch. Med. at UCLA, Los Angeles, CA; ³USC, Los Angeles, CA

Abstract: Anxiety disorders are one of the most common mental illnesses in the US. Many patients exhibit treatment-resistance or substantial side effects to available therapies. New treatment strategies are critically needed. Using a mouse model of social isolation stress, we examined the anxiolytic effects of dihydromyricetin (DHM) with elevated plus-maze (EPM) and Open Field (OF) tests. Mice were singly housed with opaque walls for 2 or 4 weeks (Iso2w or Iso4w). Control mice of group housing (social-group) spent 126± 5 seconds (sec) of total 300 sec in open arm of EPM, 153± 2 sec in closed arm. Iso4w-group spent substantially shorter time (37± 1 sec) in open arm, 242± 10 sec in closed arm. DHM (2mg/Kg, oral, daily) treated-iso4w group spent 94± 3 sec in open arm, 183± 5 sec in closed arm. DHM treated-iso2w group spent 146± 10 sec in open arm, 137± 10 sec in closed arm. Diazepam (DZ, 10 mg/Kg oral, daily) treated iso4w group spent 50±19 sec in open arm, 217± 12 sec in closed arm. These EPM results suggest that social isolation increases anxiety levels and can be improved with DHM treatment. Meanwhile, DZ treatment displayed no effect. To quantify changes in locomotor activity, we analyzed the running distance of our mice models in the square OF apparatus. Social-group ran 3950 ± 58 cm during 10 min. iso4w-group ran 2800 ± 231 cm. DHM treated iso4w-group ran 3300 ± 58 cm. DHM treated Iso2w-group ran 3433± 88 cm, indicating DHM treatment improves social isolation-induced reduction in locomotor activity. In contrast, diazepam does not improve locomotor activity (2683 ± 148cm). Social-group showed frequent rearings (54 ± 3 times during 10 min), exploratory behavior and remained at the center area for 0.5 ± 0.3 min. Iso4w-group showed rearing 33 ± 4 times, and remained at the center for only 0.02 ± 0.01 min. DHM treated iso4w-group increased rearing to 55 ± 4 times and 63 ± 7 times for Iso2w-group. DZ-group did not change rearing frequency (35± 2 times). Interestingly, when iso4w-group mice got into OF,
they began with slow movements for 25 ± 2 sec, and then slowly started running. Iso4w-mice also showed lifting tail up after placement into OF for 168 sec. DZ-group took 26 ± 2 sec to start running and displayed a lifted tail for 72 sec. In contrast, Social- and DHM treated- groups did not show these signs. The results suggest that isolation decreases exploratory/locomotor activity and that DHM treatment improves changes in behavior of socially isolated mice. The data indicate that DHM ameliorates the behavioral deficits of anxiety induced by social isolation, providing pharmacological evidence that DHM can potentially be used to treat anxiety disorders.

Disclosures:  A. Shao: None. X.M. Shao: None. Y. Ouyang: None. J. Silva: None. D.L. Davies: A. Employment/Salary (full or part-time):; Full time, USC. 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.; AA022448. R.W. Olsen: A. Employment/Salary (full or part-time):; Full time, UCLA. 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.; AA07680. J. Liang: None.

Poster

322. Stress, Cognition, and Behavior: Animal Studies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 322.03/V3

Topic: F.04. Stress and the Brain

Support: NIH MH73136
       NS28912

Title: Low estradiol protects female mice from the memory-impairing effects of multiple concurrent acute stresses

Authors: *R. E. HOKENSON¹, A. K. SHORT², Y. CHEN², A. L. PHAM², S. P², T. Z. BARAM¹;
¹Anat. and Neurobio., ²Pediatrics, Univ. of California Irvine, Irvine, CA

Abstract: Rationale: When multiple, concurrent acute stresses, involving physical, emotional and social, stressors, simultaneously disturb an animal, lasting effects are seen in the brain. In males, this stress impairs spatial memory, reduces the number of thin spines on dendrites of dorsal CA1 of the hippocampus, and inhibits synaptic potentiation of these hippocampal synapses. Sex and estrous cycle differences have been acknowledged in stress responses and spatial memory. Here we investigate if sex and cycle influence the consequences of concurrent acute stresses on memory, and probe the molecular mechanisms.
Methods: Adult female C57BL6 mice were exposed to multiple acute stresses (MAS) for 2 hours. After a recovery period of 2 hours, they were trained for 10 minutes on an object location memory (OLM) task, then tested for memory 24 hours later. In a separate cohort, brains were perfused immediately after stress to determine estrous cycle effects on dendritic spine integrity or neuronal activation indicated by c-fos. For estrous cycle stage classification, vaginal smears were taken and stages were categorized by proportions of cell types. To quantify circulating estradiol levels, trunk blood was collected and serum was analyzed with a commercial estradiol enzyme-linked immunoassay (ELISA) kit.

Results: Females subjected to MAS during proestrus had impaired spatial memory in the OLM task, associated with reduced density of thin spines in CA1 (similar to findings in males). When stressed during estrus, however, memory and spine densities did not differ from those in control mice. Assessments of the circuitry activated by MAS also revealed striking differences between proestrus and estrus mice: the stress-vulnerable proestrus group had higher activation of the basolateral amygdala, a region with monosynaptic projections to CA1. Cell-type proportions of vaginal smears taken prior to serum collection were aligned with measured estradiol concentration, confirming that the proestrus female group had high levels of circulating estradiol and the estrus group had low levels.

Conclusions: In females during high estradiol cycle phases multiple concurrent acute stresses impair hippocampus-dependent memory through loss of spines/ synapses. This may be a result of stronger excitatory input to CA1 from stress-activated afferents including the amygdala. Ongoing experiments are looking to determine estrogen receptor contribution to both spine disruption and circuit activation in male and female mice.


Poster

322. Stress, Cognition, and Behavior: Animal Studies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 322.04/V4

Topic: F.04. Stress and the Brain

Support: MH 053851

Title: Chronic unpredictable stress results in decreases in dendritic complexity within the medial prefrontal cortex

Authors: *S. E. BULIN*, J. B. NERIOS, D. A. MORILAK;

1Univ. of Texas Hlth. At San Antonio, San Antonio, TX; 2Pharmacol. and Ctr. for Biomed. Neurosci., 3Univ. of Texas Hlth. at San Antonio, San Antonio, TX
Abstract: Stress-related mood and anxiety disorders, like depression and posttraumatic stress disorder (PTSD), are highly prevalent yet poorly treated. Relapse and residual symptoms remain problematic, and a poor understanding of the neurobiology underlying these illnesses has limited the development of new treatments. Imaging studies have shown reduced activity in the mPFC in both depression and PTSD, associated with deficits in executive functions, including impaired cognitive flexibility, that not only represent symptoms of these illnesses, but also contribute causally to their development and maintenance. Such changes presumably involve aberrant forms of neural plasticity in the mPFC. Similarly, chronic stress compromises cognitive flexibility, and is likely to disrupt the plasticity that normally underlies this executive process. Cognitive flexibility mediated in the mPFC can be measured using the attentional set-shifting test, and chronic unpredictable stress (CUS) induces a deficit of cognitive flexibility on this test. Further, we have previously shown that CUS attenuates the response of the mPFC to stimulation of the excitatory afferent from the mediodorsal thalamus (MDT). Alterations in evoked responses as well as reduced plasticity could be mediated by stress-induced changes in dendritic morphology. Chronic restraint stress has been shown to reduce dendritic complexity and synaptic spine density in the mPFC (Liston et al., 2006; Moench & Wellman, 2017.) However, effects of CUS on such morphological parameters in mPFC are not known. To test this, male and female rats were subjected to CUS and sacrificed via pericardial perfusion three days after their last daily stressor. Vibratome sections containing mPFC were transferred individually into the wells of a 6-well cell culture plate containing PBS. DiI-coated gold bullets were delivered ballistically into the tissue using a pressurized gene gun. Pyramidal cells in layers II/III in the mPFC were acquired via Z-stack confocal images and total dendritic length, branch number and complexity were analyzed by Sholl analysis. Spine density in both apical and basal dendrites was also measured via 100x images acquired through the confocal images and reconstructed with Neurolucida. We found that CUS treatment resulted in decreased dendritic length over distance from the soma in layer II/II mPFC neurons, the location of MDT afferent synapses. Spine density and phenotype analysis is ongoing

Disclosures: S.E. Bulin: None. J.B. Nerios: None. D.A. Morilak: None.

Poster

322. Stress, Cognition, and Behavior: Animal Studies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 322.05/V5

Topic: F.04. Stress and the Brain

Support: R01 MH072672
        R01 MH053851
        T32 NS082145
Title: Stress effects on neuronal connectivity in the orbitofrontal cortex

Authors: *S. M. ADLER*¹, M. GIROTTI¹, D. A. MORILAK²;
¹Univ. of Texas Hlth. At San Antonio, San Antonio, TX; ²Pharmacol. and Ctr. for Biomed. Neurosci., Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX

Abstract: Depressed patients often experience impairments in cognitive flexibility, a symptom dimension that may underlie illness maintenance and is poorly treated by classic antidepressants. One major risk factor for depression is chronic stress, which has frequently been utilized in rodent models to cause a depression-like phenotype. We have shown previously that chronic intermittent cold stress (CIC) induces a deficit in reversal learning on the attention set-shifting test (AST) in Sprague-Dawley rats, and this deficit can be corrected by antidepressants like citalopram, vortioxetine, and ketamine. We hypothesize that cold stress causes an aberrant potentiation of afferent-driven response in the OFC, leading to dysregulated reversal learning. We wanted to examine if increased activity in the OFC is necessary and sufficient to cause deficits in reversal learning. We used optogenetics by delivering the ChETA variant of channelrhodopsin in an AAV viral vector into the MDT. We first demonstrated that we were able to successfully induce long-term depression (LTD) and long-term potentiation (LTP) using a laser-induction protocol in rats expressing ChETA. Next, we examined the effects of LTD on reversal learning. In our experiment, we induced opto-LTD 1 hour prior to reversal learning. Our preliminary data indicate a trend for LTD induction to reverse a CIC-induced deficit in reversal learning. Furthermore, there was a significant interaction of virus and stress (p<0.05). We will also soon examine if induction of opto-LTP is able to mimic the CIC-induced deficit in reversal learning in non-stressed animals.


Poster

322. Stress, Cognition, and Behavior: Animal Studies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 322.06/V6

Topic: F.04. Stress and the Brain

Support: NIH grant MH053851
CBN pilot project grant from the HSC Center for Biomedical Sciences

Title: Effects of stress on impulsivity measured by the 1-choice serial reaction time test

Authors: *M. GIROTTI*¹, C. M. GEORGE³, F. R. CARRENO⁴, D. A. MORILAK²;
¹Pharmacol., ²Pharmacol. and Ctr. for Biomed. Neurosci., Univ. of Texas Hlth. Sci. Ctr. at San
Abstract: Impulsive behavior, or the tendency to act in response to internal or external stimuli without planning and without regard to potential negative consequences, is a symptom dimension shared by several psychiatric conditions such as bipolar disorder, ADHD, OCD and chronic substance abuse. Stress is known to exacerbate many symptoms in these psychiatric conditions, including impulsivity. However, in many tests of impulsive responding, it is difficult to discriminate pure behavioral inhibition/disinhibition from attentional processes, therefore the effects of stress purely on impulsive responding have not been well characterized. In this work we employed the one-choice serial reaction time (1-CSRT) task to measure the effects of stress on behavioral inhibition. The 1-CSRT task is a variant of the 5-CSRT task where only a single stimulus light and nose poke hole are used, therefore requiring less processing of visuospatial attentional cues. The rat needs only to wait a defined period of time for the stimulus to appear, after which a nose poke into the lit hole will provide a sugar pellet. Rats were food restricted and trained to respond to brief flashes of light presented in the center hole that is illuminated for 5 sec (the limited hold period, LH). Nose-poke responses in the center hole while the light is on are ‘correct responses’ rewarded by the delivery of a single pellet. A failure to respond within the limited hold period (‘omission’) is punished by the house light being extinguished for 5 s (timeout period, TO). Premature responses made during the inter-trial interval (5 sec) before the presentation of the visual stimulus are a measure of behavioral disinhibition/impulsivity. Premature responses are punished by a 5s time-out period. Rats were trained to a criterion of 80% correct responses and fewer than 20% omissions with ITI= 5s, LH= 5s and TO= 5s, maintained over the course of 5 days. Under these conditions a “baseline” impulsivity score was measured. Rats were then challenged in one session where the ITI was set to 8s. Following 4 days of reminder sessions at ITI= 5s, rats were subjected to 2 days of inescapable footshock, two weeks of chronic intermittent cold (CIC) stress, or two weeks of chronic unpredictable stress (CUS) and measures of impulsivity at baseline and after challenge (ITI = 8 s) were taken again. We found that impulsive responding was increased at baseline after footshock or after CIC stress, and was increased at baseline and challenge after CUS. These data suggest that stress directly increases impulsive responding in the absence of confounding attentional biases.

Disclosures: M. Girotti: None. C.M. George: None. F.R. Carreno: None. D.A. Morilak: None.

Poster

322. Stress, Cognition, and Behavior: Animal Studies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 322.07/V7

Topic: F.04. Stress and the Brain
Title: Gut-brain actions underlying comorbid neuropsychiatric disturb associated with inflammatory bowel disease

Authors: *S. CUZZOCREA, E. GUGLIANDOLO, M. CORDARO, R. SIRACUSA, R. D'AMICO, A. PERITORE, R. FUSCO, R. CRUPI, D. IMPELLIZZERI, R. DI PAOLA; Univ. of Messina, Messina, Italy

Abstract: Inflammatory bowel disease (IBD) is a chronic relapsing and remitting disorder characterized by inflammation of the gastrointestinal tract and its associated with different neurological and psychiatric disorders, which are integrated among the extra-intestinal manifestation of this disease. In fact, epidemiological studies have shown an increased incidence of depression in patients with colitis, in which strong inflammation in the gut occurs. The possible mechanisms connecting gut inflammation and neuroinflammation in the brain could be via an increase in peripheral cytokines or via damaged nerve terminals in the gut. Recent evidence demonstrated that the gut-brain-axis has a central function in the perpetuation of IBS, and, for this reason it can be considered as a possible therapeutic target. N-Palmitoylethanolamine-oxazoline (PEA-OXA) possesses anti-inflammatory and potent neuroprotective effects. Although, recent studies have explained the neuroprotective properties of PEA-OXA, nothing is known about its effects on gut-brain axis during colitis. The aim of this study is to explore the mechanism and the effect of PEA-OXA on gut brain axis in rats subjected to experimental colitis induced by allowing rats to a free access to drinking water containing 5% DSS for 7 days. Daily oral administration of PEA-OXA 10 mg/kg daily o.s. was able to decrease body weight loss, macroscopic score and colon length and histology alteration after DSS induction. Additionally, we demonstrate that PEA-OXA administration significantly promoting neurotrophic grow factor release and decrease astroglial and microglial activation DSS-induced. Moreover, PEA-OXA, was able to restore tight junction in both hippocampus and colon. In conclusion, in our work, we demonstrate for the first time the action of PEA-OXA on gut-brain axis during in a model of DSS induced colitis.


Poster

322. Stress, Cognition, and Behavior: Animal Studies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 322.08/V8

Topic: F.04. Stress and the Brain

Title: Olfactory bulbectomy induces learning and memory deficits associated with impaired hippocampal structural plasticity in the rat
**Authors:** *J. C. MORALES MEDINA*¹, G. GALINDO-PAREDES¹, A. VÁZQUEZ HERNÁNDEZ², P. SÁNCHEZ-TEOYOTL¹, R. A. VAZQUEZ², P. AGUILAR-ALONSO², D. L. CORONA QUINTANILLA³, G. FLORES⁴;

¹Ctr. de Investigación en Reproducción Animal, Ctr. for Res. and Advanced Studies, Tlaxcala, Mexico; ²BUAP, Puebla, Mexico; ³Univ. Autónoma de Tlaxcala, Tlaxcala, Mexico; ⁴Univ. Autonoma de Puebla / Inst. de Fisiologia, Puebla, Mexico

**Abstract:** Major depression induces memory deficits which have been associated with neuronal alterations in the hippocampus. Neural alterations could be observed at three levels: dendritic arborization, spine density and type of spine. Moreover, olfactory system dysfunction have been associated with major depression in humans and olfactory bulbectomy (OBX) is a well-known model of depression-related behavior in rodents. OBX also induces major neurochemical alterations in various brain regions including the hippocampus and prefrontal cortex (PFC). In the present study, we aimed to investigate whether OBX induced memory and learning deficits and evaluate possible neuronal rearrangement in the hippocampus. To measure spatial memory, we used the classical Morris Water Maze (MWM) task. To characterize neuronal remodeling, we used the Cox-Golgi staining to evaluate possible modifications in dendritic arborization, spine density as well as dynamics of dendritic spines in the CA1 and CA3 region of the dorsal hippocampus and PFC. The present results show that OBX rats required longer time to find the hidden platform of the MWM during the phases of learning and reverse memory. Moreover, the OBX rats also presented a retention deficit as the OBX rats performed less crosses when the platform was removed. OBX induced dendritic atrophy in pyramidal neurons of the CA1 subregion of hippocampus and PFC. These results add further support to the validity and usefulness of the OBX rat as a model of depression.


**Poster**

**322. Stress, Cognition, and Behavior: Animal Studies**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 322.09/V9

**Topic:** F.04. Stress and the Brain

**Support:** Mizzou Research Council Grant
Mizzou Mission Enhancement Funds

**Title:** Anxiety and exploratory behaviors in heterozygous SERT knockout mice exposed to enriched vs. standard housing
**Authors:** *B. M. KILLE*¹, T. WOO², E. SOLIDUM¹, J. CUI³, C. M. GREENLIEF⁴, D. Q. BEVERSDORF⁵;
¹Psychological Sci., ²Interdisciplinary Neurosci. Program, Univ. of Missouri, Columbia, MO; ³Path & Ana Sci. Dept., U of Missouri-Columbia Sch. of Med., Columbia, MO; ⁴Dept Radiol, Neurol, Psychol Sci, DGS of INP, ⁵Univ. of Missouri Columbia, Columbia, MO

**Abstract:** Housing conditions for mouse models greatly affect behavior. Mice, which are social creatures, housed in isolation show increased anxiety when presented with a novel environment. Social housing decreases these anxiety behaviors. There is also evidence that enriched home cages, environments that encourage exploration and novel interaction, decrease anxiety behaviors when compared to typical barren home cage environments. However, the interaction of genetic variability and housing in susceptibility to stress is not known. Knockout of one copy of the SERT gene in mice have similar alterations in stress reactivity to humans with a deletion in the promoter region of SLC6a4, which results in increased risk for mood disorders in association with stressors in humans. Therefore, we wished to examine the effects of enriched environment in mice with increased stress reactivity due to heterozygous KO of SERT (SERT(+/-)). SERT KO mice have previously shown benefits of enriched housing. However, the mice most commonly used for this research are homozygous knockouts (SERT(-/-)). SERT(-/-) mice present a much more extreme stress-reactive phenotype both behaviorally and physiologically when compared to the heterozygous SERT(+/-). Since SERT(+/-) is most similar to humans with the short SLC6a4 variant, the current study set out to explore the potential benefits of enriched housing for SERT (+/-) mice. 24 SERT(+/-) and 18 wildtype C57bl6 mice were assigned to two housing types at weaning—half in standard/barren housing, half in enriched housing. Anxiety behaviors were evaluated using an open field test and exploratory behaviors using a novel object paradigm. Preliminary analysis shows a main effect of housing environment, but no main effect of or interaction with genotype on anxiety behaviors, with enriched housing decreasing anxiety behaviors. Exploratory behavior shows an interaction of gene x housing, with heterozygous animals showing no change in novel-object interaction, and enriched wild-type animals actually exploring the object less than their non-enriched counterparts. This project was supported by the University of Missouri Research Council Grant and Mission Enhancement Fund.

**Disclosures:** B.M. Kille: None. T. Woo: None. E. Solidum: None. D.Q. Beversdorf: None. J. Cui: None. C.M. Greenlief: None.

**Poster**

322. Stress, Cognition, and Behavior: Animal Studies

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 322.10/V10

**Topic:** F.04. Stress and the Brain
Support: AFOSR Grant 14RH08COR

Title: Inheritable vs. acquired genetic effects of chronic unpredictable variable stress on cognition and the relationship to stress resilience and vulnerability

Authors: *S. H. JUNG, N. BECHMANN, R. MOORE, C. HATCHER-SOLIS;
Applied Neurosci., U.S. Air Force Res. Lab., Wright-Patterson AFB, OH

Abstract: Stress affects cognitive processes, but the degree of stress-induced changes in cognitive performance is dependent on an individual’s genetics and the environments that they have been exposed to. In general, chronic distress induces negative changes in neuronal morphology that are associated with negative effects on cognition, especially memory performance. However, chronic distress can have no effect or even enhance cognition. Thus, individuals can present vulnerability, resilience, or enhancement by stress. However, no scientific explanations for these phenomena are available yet. In this study, we used recombinant inbred BXD strains (64 strains, total n = 3805 mice) to elucidate the molecular mechanisms of these phenomena by identifying genetic candidates that are associated with inheritable and acquired effects of chronic unpredictable variable stress (CUVS; 4 weeks) on cognitive performance measured by the Morris water maze (MWM) test. Quantitative trait loci (QTL) analysis was conducted to identify cognition-associated, inheritable genetic candidates from the MWM performance of sham mice. Our analysis resulted in no significant QTL genetic region. Genetic factors acquired by stress environments were identified in CUVS mice. QTL results from sham and CUVS groups were compared to identify potential interactions of genetic factors of cognition and stress environments. Results show that likelihood ration statistics (LRS) in some genetic regions are greatly increased (chromosomes 1, 2, 5 & 18) and decreased (chromosomes 7, 9 & 19) by CUVS. Moreover, all strains were ranked based on their average latency to the platform during the MWM test, and their ranks were compared between sham and CUVS strains to identify which strains showed negative (susceptibility), no (resilience) and positive (enhancement) changes by CUVS. Seven strains showed positive changes in their ranks (range of rank change: -47 to -19 ranks; range of time change: -16.1 to -8.9 seconds), and 13 strains showed minimal changes (-3 to +3 ranks; -5.5 to 0.01 seconds). Eight strains showed negative changes (20 to 31 ranks; 2.7 to 5.4 seconds). Hippocampal transcriptomes of selected mice were analyzed and we identified signaling pathways and genes that are significantly associated with cognitive performance and stress susceptibility, resilience and enhancement. Therefore, our results provide a genetic explanation for inheritable factors for cognition, acquired factors for stress environment and their interacting factors. Moreover, we suggest that certain hippocampal genes may underlie stress resilience, susceptibility and enhancement of cognition.

Cognitive flexibility and mood disorders after chronic stress exposure


1 Psychology Sch., 3 Sistema de Univ. Abierta, Facultad de Psicología, 4 Neuropsychopharm. Lab., Psychology Sch., 5 Univ. Nacional Autónoma de México, Mexico City, Mexico; 5 Sociedad Iberoamericana de Neurociencia Aplicada, A.C., Mexico City, Mexico

Abstract: Deficits in cognitive flexibility and emotional regulation as seen in mood disorders are associated with stress-related neuropsychiatric disorders such as depression and anxiety disorders. Specifically, impaired cognitive flexibility contributes to the onset and maintenance of these illnesses. The chronic unpredictable stress battery (CUSB) consists of repeated and unpredictable exposure to stressors over a period of time that could modulate behavioral deregulations as anxiety-like and depression-like behaviors. The aim of this study was to examine the phenotypic behaviors of mood disorders and cognitive flexibility in rats. Twenty two male Wistar rats between 250-300 g were divided into control group and CUSB group. CUSB stressors include 45°cage tilt, wet bedding, lights on overnight, water deprivation, cold water immersion and movement restrictor. The behavioral evaluation started the next day after the last stressor. The sequence of the behavioral tests were randomized. The CUSB group was exposed to the sequence of mild stressors for 10 days. While the control group was left undisturbed. Cognitive flexibility was evaluated by the attentional set shifting task (ASST), depressive-like behaviors were evaluated by the saccharine preference test and anxiety-like behaviors were assessed by the open field test. Rats exposed to the CUSB exhibit an impairment on the extra dimensional (ED) set-shifting phase at the ASST and not in other phases of the task. The impairment was seen as an increase in the number of trials required to achieve six consecutive correct responses. CUSB-exposed animals presented a reduction in locomotor activity and in crosses at the center area of the open field compared with the non-stressed group. Besides, CUSB group had a reduction of consumption of saccharine and more consumption of
water in comparison with the non-stressed group. In conclusion, the chronic unpredictable stress battery induced anhedonia, anxiety-like behaviors and impairments in cognitive flexibility. These results could be related with the pro-inflammatory profile that has been described as one of CUSB main effects. Therefore, a non-steroidal anti-inflammatory drug (NSAID) with anti-inflammatory properties could be used as a therapeutic alternative to prevent the behavioral changes seen after stress and could be used as an alternative for the treatment of depression and mood disorders diseases.


Poster

322. Stress, Cognition, and Behavior: Animal Studies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 322.12/V12

Topic: F.04. Stress and the Brain

Support: PAPIIT IN 306918
         PAPIME PE 306318

Title: Interaction prenatal and postnatal stress in anxiety-like behavior and recognition memory of male and female offspring

Authors: *C. ORIZABA-HUERTA1, D. B. PAZ-TREJO1,2,3, H. SÁNCHEZ-CASTILLO1,3;
1Facultad de Psicología, Univ. Nacional Autónoma de México, Ciudad de México, Mexico;  
2Sistema Univ. Abierta, Facultad de Psicología, Univ. Nacional Autónoma de México, Ciudad de México, Mexico; 3Sociedad Iberoamericana de Neurociencia Aplicada A. C., Ciudad de México, Mexico

Abstract: It has been described that offspring exposed to prenatal stress present long-term disturbances and reexposure to stressors promote behavioral changes with greater ease and magnitude. Previous researches focused on evaluating males, their results showed that stress reexposure decrease anxiety-like behaviors and improve the memory deficit. However, no data is available for females. The aim of this study was to evaluate effects of pre and postnatal stress interactions in male and female animals. The subjects were 102 Wistar rat offspring (51 females and 51 males) of 10 pregnant females divided in five groups (a) control, (b) prenatal stress, (c) prenatal and postnatal stress, and (d) postnatal stress. Predator odor (PO) exposure (bobcat pee) was used as stressor. Prenatal stress took place during the second half of rats pregnancy and postnatal stress occurred in PND90, corresponding to adulthood. Open Field (OF) and Elevated Zero Maze (EZM) were used to assess anxiety-like behaviors and Novel Object Recognition
(NOR) test was used to evaluate recognition memory in adult rats. Behavioral markers for anxiety-like behaviors in OF and EZM indicate that prenatal stress increases anxiety in offspring, however, it attenuates anxiety-like behaviors when stress was limited to adulthood. This indicates that *in utero* stressful environment could cause long-term changes in the fetus, these changes remain in adult life and may function as adaptations for environment. The recognition memory in NOR test didn’t show differences between experimental groups although males obtained higher discrimination index. In some parameters, differential effects were observed between males and females. These effects could be a result of differences in the hypothalamic-pituitary-adrenal (HPA) axis function which involves the activation of gonadal hormones linked to sexual dimorphism in susceptibility to stress effects. 2 out of 3 pregnant stressed rats presented decrease or absence of affiliative care towards their pups. This generated, in the offspring, a decrease in anxiety-like behaviors in adulthood. These findings indicate that absence of affiliative care may increases “resistance” to stress for generating anxiety-like behaviors. This data gives us information of the importance of taking maternal care into account since exposure to stress not only disrupts fetal development but maternal caring; affiliative care also have an impact on the development of the offspring.

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**Poster**

322. Stress, Cognition, and Behavior: Animal Studies

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 322.13/V13

**Topic:** F.04. Stress and the Brain

**Support:** DGAPA-PAPIIT IN306918

PAPIIT PE306318

**Title:** Sex differences in recognition memory after stress exposure

**Authors:** *P. TORRES-CARRILLO*¹, M. VARGAS-GÓMEZ¹, M. D. VERGEL-MUNGUÍA¹, J. E. RAMÍREZ-SÁNCHEZ², D. B. PAZ-TREJO³, L. D. OCHOA-DE LA PAZ⁶, H. SANCHEZ-CASTILLO⁴;

¹Lab. de Neuropsicofarmacología, ³Sistema de Univ. Abierta, ⁴Dept. de Psicobiología y Neurociencias, ²Univ. Nacional Autónoma de México, Facultad de Psicología, Ciudad de México, Mexico; ⁵Sociedad Iberoamericana de Neurociencia Aplicada AC, Ciudad de México, Mexico; ⁶Dept. de Bioquímica, Univ. Nacional Autónoma de México, Facultad de Medicina, Ciudad de México, Mexico

**Abstract:** Stress impairs recognition memory, whereas acute stress seems improvement recognition memory. On the other hand, studies have demonstrated sex differences in
neuroendocrine and behavioral responses to stress, where females have showed more impairment than males, which could explain the higher prevalence in females to develop stress related disorders. Additionally, most neural and behavioral studies not include females, however, the pattern suggest that stressed males have down-regulation on neural activity while stressed females have not alter or have up-regulation of neural activity. The above supports the idea of sex specific stress effects on behavior. The aim of this study was to compare female and male responses to different stressors vs. no-stress condition: Chronic Unpredictable Stress Battery (CUSB), Predator Scent Stress (PSS), and control group. Male and female Wistar rats 12 weeks old were used (n=10 per group). Animals of CUSB group were exposed to a battery of stressors for ten days. The stressors that made up the battery consisted of 1) put animals in movement restrictors for 20 min. (3 times per day), 2) swimming in cold water for five minutes (16°C), 3) overnight light exposure (12 hours), 4) placing the rats for 12 hours (overnight) or 3 hours (on day) in their home cages with wet bedding, 5) placing the rats for 3 hours in their home cage that was tilted at 45°, and 6) overnight water deprivation (12 hours). The exposure to each stressor was randomized according to the CUSB protocol. Animals of the PSS group were exposed for 10 minutes in an exposure box that contained a bottle with scent tag impregnated with predator urine. Behavior was assessed with Novel Object Recognition Test 24 hours after the final stressor exposure. The results obtained were CUSB-exposed females had negative values in the recognition index than control females and CUSB-exposed males. PSS-exposed females also had negative values in the recognition index but more higher than CUSB-exposed females. Furthermore, CUSB- and PSS-exposed males had a lower recognition index than control males but they had not negative values. The above suggests that CUSB and PSS induced impairment on recognition memory in females and males but not in the same way. That impairment could be associated with sex-specific alterations in areas important for learning and memory like the prefrontal cortex and hippocampus and for areas mediating mood and anxiety such amygdala.


Poster

322. Stress, Cognition, and Behavior: Animal Studies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 322.14/V14

Topic: F.04. Stress and the Brain

Title: Brain atlas of the red Mayan octopus, Octopus maya

Authors: *J. F. VERGARA-OVALLE1, D. GONZÁLEZ-NAVARRETE1, F. AYALA-GUERRERO1, D. B. PAZ-TREJO2, H. SANCHEZ-CASTILLO3;
Abstract: The use of cephalopods for research in neurosciences goes back to almost a century. This model has attracted the attention of several researchers thanks to the great complexity of behaviors, comparable even with those of some vertebrates, as well as the anatomical and physiological difference when compared with vertebrates, proof of a very different evolutionary history that diverged from ours about 550 million years ago. These characteristics offer us an opportunity to study and compare completely different nervous systems but that converge in some capacities such as learning, decision making, use of tools and even emotions. Despite the great advantage of neuroscience research in cephalopods and its great progress in recent years, it has not grown to the level of other models such as arthropods or vertebrates. This delay in research is due to several problems, one of which is the lack of a standardized species for daily use and shared by several laboratories. In the present project we propose the use of Octopus maya, a species reared in captivity, in the facilities of the UNAM in Sisal, Yucatán. As the first part of this proposal, the atlas of the O. maya brain ganglion is presented. To this end, we extracted the “brains” of 4 adult specimens. Sagittal and coronal sections were made every 80µm to make Hematoxylin-Eosin stains. From the coronal slices, 42 lamellae were obtained, while from the sagittals 75 were obtained. In addition, the lobe connectivity was tracked by the injection of DiL (1,1'-Dioctadecyl-3,3,3 ', 3'-Tetramethylindocarbocyanine). It was determined that the brain of O. maya has a structure similar to that of sister species such as O. vulgaris and O. mimus, with 14 main structures, divided into more than 60 subregions. However, O. maya presents differences regarding the size and communication of the structures. Considering the plasticity of the nervous system, associated with the different behaviors observed along phylum Mollusca, it is feasible to hypothesize that these differences in the brain of O. maya are related to the development of particular behaviors in the habitat of the species (shallow waters with grasses and rocky bottoms).

supported by PAPIIT IN 306 918 y PAPIME PE 306 318


Poster

322. Stress, Cognition, and Behavior: Animal Studies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program#/Poster #: 322.15/V15

Topic: F.04. Stress and the Brain

Support: PAPPIT IN306918
PAPIME PE306318
Title: Outcomes of chronic stress in memory and depression-like behavior


1Lab. de Neuropsicofarmacología, 2Psicobiología y Neurociencias, Univ. Nacional Autónoma De México, Facultad de Psicología, CDMX, Mexico; 3Sistema de Univ. Abierta, Univ. Nacional Autónoma De México, CDMX, Mexico; 4Sociedad Iberoamericana de Neurociencia Aplicada AC, CDMX, Mexico; 5Bioquímica, Univ. Nacional Autónoma De México, Facultad de Medicina, CDMX, Mexico; 6Unidad de Investigación Hosp. para Evitar la Ceguera en México IAP, Hosp. Dr. Luis Sánchez Bulnes, CDMX, Mexico

Abstract: Chronic stress induces several alterations in cognition, behavior and physiological function. After chronic unpredictable stress exposure, animals may develop depression-like behaviors and cognitive deficits. These negative effects of stress are related to alterations in sensitive-stress structures like the hippocampus. Patients with depression and stress-related pathologies have deficits in hippocampus-dependent tasks besides several hippocampus alterations such as neuronal atrophy, reductions in size and volume. There have also been described inflammatory and neuroinflammatory dysregulations in these patients. The aim of this experiment was to evaluate stress-induced depression-like behaviors and cognitive deficits. Stress and control groups were constituted with male Wistar rats with 200-250 gr bodyweight at the beginning of the experiment. Stress exposure took place with a 10-day Chronic Unpredictable Stress Battery (CUSB). This battery consisted in six stressors (restraint, wet bed, water deprivation, tilt cage, cold water immersion and lights over the night) randomly presented. Behavioral phenotype was assessed after stress. Barnes maze and Novel object recognition test were used for memory assessment. Meanwhile, forced swim test and saccharin preference were used to explore depression-like behavior. Stressed animals showed decreased bodyweight gain. Depression-like behavior was more prevalent in contrast to non-stressed animals as data have shown in forced swim test and saccharin preference. However, adipsia was observed in stressed animals. This last effect could explain the absence of statistical significance in saccharin preference. Memory deficits were observed in stress group since rats had higher escape latencies and made more total errors in Barnes Maze. In accordance to previous data, chronic unpredictable stress induces depression-like behaviors and deficits in cognition. After 10 days in CUSB, rats exhibited depression-like behaviors and deficits in hippocampal-dependent tasks. These results support the hypothesis of stress as a risk factor to mood disorders development and hippocampal-dependent cognitive alterations. Depression-like behaviors may be modulated by chronic and higher levels of proinflammatory markers and reactive microglia in the central nervous system.

Abstract: The increase in alcohol consumption in adolescents has become an important public health concern. The adolescence is a crucial stage, in which occur several plasticity processes in the central nervous system, that bring to the ability to adapt to the environmental demand, however, they also make the organism vulnerable to other factors, such as drug abuse or stress. It is known that the alcohol consumption at high doses during adolescence causes damage to brain areas undergoing maturation. Previous studies have suggested that alcohol consumption starts as a form of self-medication to stressful events. In addition, it is known that acute alcohol consumption produces anxiolytic effects, however, with prolonged use of alcohol, levels of anxiety and depression rise. Nowadays, the long-term effects of adolescent exposure to alcohol in combination with stress in the behavioral response, and particularly in the development of depression and anxiety remain largely unknown. For this reason, the purpose of this study was to investigate the effects of exposure to alcohol and chronic unpredictable stress during adolescence in the development of anxiety and depressive like behaviors in adult stage. Male Wistar rats at the postnatal day 21 were used under standard laboratory conditions and divided in 3 control conditions (No Alcohol + No Stress, No Stress + No Alcohol and Naive) and 4 experimental groups (Alcohol + Stress, Stress + Alcohol, Alcohol and Stress). The Alcohol + Stress group was administered with alcohol at 10% v/v for 21 days and then exposed to Chronic Unpredictable Stress Battery (CUSB), the Stress + Alcohol group was exposed to CUSB and then alcohol consumption; the Stress group was exposed to CUSB only and the Alcohol group was exposed to alcohol only. In all conditions, depressive effects were evaluated with forced swimming test and anxiogenic effects with open field test. Also, alcohol consumption of the Stress + Alcohol,
Alcohol + Stress and Alcohol groups was measured, as well as the water consumption in control groups. The results indicate that the order of exposure to alcohol and stress has repercussions in the development of depressive and anxiety like behaviors. Furthermore, alcohol consumption was also affected by a consequence of this exposure order. This research highlights the importance of early life events in the proper development of subjects in the adult life.


Poster

322. Stress, Cognition, and Behavior: Animal Studies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 322.17/V17

Topic: F.04. Stress and the Brain

Support: PAPPIT IN306918
       PAPIME PE306318

Title: Behavioral changes in octopus maya produced by acute stress exposure

Authors: *D. A. GONZÁLEZ-NAVARRETE1, J. F. VERGARA-OVALLE2, F. AYALA-GUERRERO3, D. B. PAZ-TREJO4,5, H. SANCHEZ-CASTILLO6;
1Univ. Nacional Autónoma De México, CDMX, Mexico; 2Biochem., UNAM, CDMX, Mexico; 3Univ. Nacional Autónoma de México, CDMX, Mexico; 4Univ. Nacional Autonoma de Mexico, Facultad de Psicología, Sistema de Univ. Abierta, Mexico City, Mexico; 5Sociedad Iberoamericana de Neurociencia Aplicada A.C., Cdmx, Mexico; 6Univ. Nacional Autonoma De Mexico. Fac Psicologia, Mexico City, Mexico

Abstract: The stress response allows individuals to increase the probability of survival in the face of a real or perceived situations that may put in danger an individual, its offspring or the entire species, therefore, the stress response is conserved throughout different phyla. The study of the stress response has focused mainly on the murine model, where studies have shown that chronic and acute stress exposure causes changes in the neurobehavioral response. The research model in octopus is a model that is gaining strength in Neurosciences due to the complexity of the behaviors that these animals presents, nevertheless, the stress response in this model as well as the effects that different stressors cause in octopuses behavior have not yet been understood and is usually known only by anecdotic descriptions. The absence of a standardization in the evaluation of the stress response in the octopus model causes that the welfare of the specimens influences the behavioral results obtained in the research. So, the aim of this study was to behaviorally assess the changes in the octopus behavior during and after acute exposure to an environmental stressor. Four specimens of Octopus maya were used from the Multidisciplinary
Unit of Teaching and Research UNAM, located in Yucatan, Mexico. They were divided into 2 groups: a group exposed to a low intensity red light (Control) and a Group exposed to high intensity light (Experimental) for 24 hours. The acute stress exposure causes an increase in activity and exploration behavior with respect to the control group during the stressor. The above suggests that acute exposure to an environmental stressor, modified the behavior of the octopuses, being a precedent for future investigations where the environment is considered in the welfare of the specimens as well as their influence on the behavioral results of the model.

Key Words: Octopus, stress, behavior, response, cephalopod

Supported by: PAPPIT IN306918, PAPIME PE 306318


Poster

322. Stress, Cognition, and Behavior: Animal Studies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 322.18/V18

Topic: F.04. Stress and the Brain

Support: DGAPA-PAPIIT IN306918
PAPIME PE 306318

Title: Behavioral effects of exposure to stress in different life times

Authors: *K. B. VALENCIA¹, Y. VIDAL-DE LA O¹, O. GALICIA-CASTILLO², D. PAZ-TREJO³, H. SANCHEZ-CASTILLO¹;
¹Lab. de Neuropsicofarmacologia, UNAM, Ciudad de Mexico, Mexico; ²Facultad de Psicologia, UNAM, Ciudad de Mexico, Mexico; ³Sistema de Univ. Abierta; Sociedad Iberoamericana de Neurociencia Aplicada, Univ. Nacional Autonoma de Mexico, Facultad de Psicologia, Ciudad de Mexico, Mexico; ⁴Sociedad Iberoamericana de Neurociencia Aplicada AC, Mexico City, Mexico

Abstract: Stress is a systemic response for a real or perceived threat, that enhance the survival probability of an organism. Several mechanisms have been proposed as modulators of this response, however, when these mechanisms are not enough, and the stress response is sustained over time (chronic stress), that response could be nocive. Research in humans has demonstrated that a history of adverse experiences specifically during childhood, adolescence (both considered critical development periods) or adulthood could be a risk factor to develop psychiatry disorders as anxiety or depression. In basic research, it has been suggested that exposure to chronic stress during adolescence could vulnerability the system, and the negative effects remains to adulthood. On the other hand, exposure to chronic stress during adulthood induce anxiety-like behaviors (increased thigmotaxis, decreased rearing, etc.), depression-like behaviors (decrease in
saccharine intake, increased immobility) or cognitive deficits (specifically on spatial and recognition memory). Therefore, animal models mostly explore the effects of chronic stress during adolescence or adulthood alone, but there are few reports in which the exposure to stress take place during different moments of life course, as occurs in most cases. Due this, the aim of this research was to evaluate the behavioral effects of a double exposure to chronic stress (first in adolescence and after in adulthood) in order to study a possible incubation, potentiation or adaptation effects. For that, we use a chronic unpredictable stress battery (CUSB) and we evaluate depression-like behaviors with saccharine preference (SP) and anxiety-like behaviors with open field test (OFT). Results showed a decreased saccharine intake in SP in groups exposed to stress only in adulthood (CUSB alfa) and in adulthood and adolescence together (CUSB beta) compared to control suggesting an anhedonic state. In OFT CUSB beta showed a decreased time spend in center, decreased rearing, increased immobility and grooming compared to control, suggesting an anxiety behavioral phenotype. CUSB alfa only showed a decreased rearing. This data suggests that an exposition to stress during adolescence could potentiate a second exposition during adulthood resulting in a more intense anxiety behavioral phenotype. This project was supported by PAPIIT IN 306 918 y PAPIME PE 306 318.


Poster

322. Stress, Cognition, and Behavior: Animal Studies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 322.19/V19

Topic: F.04. Stress and the Brain

Support: Knowledge Enterprise Development

Title: Investigation of second-order conditioning as a potential mechanism of the maintenance of fear memories in a rodent model of post-traumatic stress disorder

Authors: *J. M. JUDD, E. A. SMITH, J. KIM, D. PEAY, B. S. BADARUDDIN, F. SANABRIA, C. D. CONRAD;
Psychology, Arizona State Univ., Tempe, AZ

Abstract: Post-Traumatic Stress Disorder (PTSD) is characterized by the presence of robust and intrusive fear (American Psychiatric Association 2013). In PTSD, second-order conditioning may contribute to the maintenance of traumatic memories into environments where the trauma did not occur (Wessa and Flor 2007). We use chronically stressed rats to produce a vulnerable phenotype that, when combined with fear conditioning, leads to overly robust fear memories to model PTSD (Conrad et al. 1999; Hoffman et al. 2014; Miracle et al. 2006). Recently, we found
that implementing a delay between chronic stress and fear conditioning improves fear extinction (Judd et al. 2018). In the current study, we tested whether second-order conditioning contributes to impairments in fear extinction in rats that do not experience the post-stress delay. Male Sprague Dawley rats were chronically restrained by wire mesh (6hr/d/21) or assigned to a non-stressed control group (CON). Fear conditioning occurred within a day (i.e., immediately, STR-I) or 6 weeks (i.e., delay, STR-D) after chronic stress ended. Prior to the start of fear conditioning, rats were acclimated to two different contexts (A & B) for 10 min/day 3 days/context to attenuate the likelihood of chronic stress potentiating context fear learning during the training session. Day 1 of fear conditioning involved the presentation of 3 tone (20 s, 75 dB)-footshock (1 s, 0.8 mA) pairings in context A. On day 2, half of the rats were exposed to 3 tone presentations in context B, while the other half were exposed to context B for an equal duration without the tones. On day 3, rats were returned back to context B and their freezing to context was measured. All groups (CON, STR-I-STR-D) that received the tone in context B on Day 2 froze more to context B on day 3 than did their counterparts who never received a tone presentation in context B. These results suggest that CON, STR-I, and STR-D all show some level of second order conditioning, and this fails to explain the heightened freezing during extinction by STR-I in past work.

Disclosures:  
J.M. Judd: None.  
E.A. Smith: None.  
J. Kim: None.  
D. Peay: None.  
B.S. Badaruddin: None.  
F. Sanabria: None.  
C.D. Conrad: None.

Poster

322. Stress, Cognition, and Behavior: Animal Studies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 322.20/V20

Topic: F.04. Stress and the Brain

Support:  
ASU Knowledge Enterprise and Development  
BUILDing Scholars Summer Research Program

Title: A novel chronic unpredictable intermittent restraint stress paradigm disrupts spatial memory in male, but not female rats

Authors: *D. N. PEAY1, B. S. BADARUDDIN1, P. A. PARADA2, J. M. JUDD1, M. E. DONNAY1, C. D. CONRAD1;  
1Psychology, Arizona State Univ., Tempe, AZ; 2Univ. of Texas at El Paso, El Paso, TX

Abstract: In rodents, chronic stress typically impairs spatial memory in males, but has no effect or can even enhance spatial memory in females. Recently, a study found that an intermittent restraint (IR) paradigm over 9 days produced more robust effects on the stress response and anxiety profile than did a daily restraint paradigm. We investigated whether such an IR paradigm
could be applied to an extended duration over several weeks or more to impair spatial memory in both sexes. Results presented at last year’s meeting showed that IR for six hours/day for three weeks was potentially effective in impairing spatial memory in male rats. Here, we tested whether the IR paradigm would impair spatial memory in both male and female rats (IR-M, IR-F) when extended to six weeks before spatial memory testing commenced. After six weeks of IR, rats began behavioral testing on days when IR was not administered. Consequently, IR allowed for a continuation of the stressor, while permitting multiple behavioral assessments on the Y-maze, object placement (OP), novel object recognition (NOR), and open field (OF). We found that the IR paradigm failed to impair spatial memory in either males or females at 6 weeks and 9 weeks, suggesting that when extended, the IR paradigm may have become less stressful. Therefore, we modified the IR to be unpredictable (UIR) in the following ways: changing the time of day for restraint (ranging from 7:00 to 19:00), the number of consecutive days of IR before a day off (ranging from 2 to 6), and changing the duration of restraint combined with gentle orbital shaking (30min, 60 min). Despite these changes, UIR still impaired Y-maze performance in males, but not in females. All groups (males, females, CON, UIR) demonstrated memory on the 1-min and 1-hr NOR task. We also included an additional stressor seven days after the last UIR session to determine whether this novel stressor would impact Y-maze performance, but spatial memory remained intact. Together with other reports, these findings support the interpretation that chronic stress negatively impairs hippocampal-dependent function in males, but not in females. This and other reports raise the question as to whether females are resilient to chronic stress-induced spatial memory deficits.


Poster

322. Stress, Cognition, and Behavior: Animal Studies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 322.21/V21

Topic: F.06. Brain Blood Flow/ Metabolism/ and Homeostasis

Support: Hayward Foundation

Title: Stress-induced multi-system metabolic reprogramming in mice vulnerable to post-traumatic stress disorder (PTSD)-like behavior

Authors: *G. PRESTON, E. MORAVA-KOZICZ, T. L. KOZICZ;
**Abstract:** Mitochondrial dysfunction has been increasingly implicated in several psychopathologies, including post-traumatic stress disorder (PTSD), a debilitating psychiatric disorder induced by exposure to a traumatic event. We recently showed that mitochondrial function was significantly impaired in the brains of mice affected by PTSD-like symptomatology. Here we investigate multi-system metabolic effects of trauma exposure and PTSD-susceptibility in these animals. We previously induced PTSD-like symptomatology in a cohort of 48 WT FVB mice, identifying 7 PTSD-vulnerable animals and 16 PTSD-resilient animals. These animals displayed disrupted mitochondrial energy metabolism in the brain, which is often associated with diverse metabolic disruptions. To elucidate the metabolic and biochemical disruptions associated with PTSD vulnerability in the animals, as well as to identify novel biomarkers and potential therapeutic interventions, we subsequently performed targeted high-performance liquid chromatography and gas chromatography/mass spectrometry, and nuclear magnetic resonance for metabolites including acylcarnitines, amino acids, TCA metabolites and organic acids. Trauma-exposed animals and PTSD-vulnerable animals displayed metabolic changes associated with increased oxidative stress, disrupted fatty acid oxidation, and impaired phospholipid synthesis in the brain, as well as disrupted fatty acid oxidation in the liver. Conversely, PTSD-resilient animals displayed evidence of increased consumption of branched chain amino acids (BCAA’s). Ultimately, metabolites associated with energy metabolism, including ketone body production, glucose metabolism, and BCAA consumption were sufficient to fully delineate stress-naive, PTSD-resilient, and PTSD-vulnerable animals. Subsequent LASSO analysis identified concentration of plasma acetylcarnitine (C2) as the most significant predictor of PTSD-vulnerability within this cohort of animals. Our data illustrate a profound stress-induced metabolic rewiring in mice susceptible to PTSD-like behavior. The metabolic changes observed are consistent with the mitochondrial dysfunction identified in these animals, as well as with epidemiological evidence observed in human patients with PTSD. Furthermore, these data identify several novel biomarkers for stress exposure and PTSD-vulnerability, as well as potential therapeutic interventions for the prevention and treatment of PTSD. Further studies will focus on confirming the predictive power of these novel biomarkers, and investigating the efficacy of the potential therapeutic interventions identified.

**Disclosures:** G. Preston: None. E. Morava-Kozicz: None. T.L. Kozicz: None.

**Poster**

**322. Stress, Cognition, and Behavior: Animal Studies**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 322.22/V22

**Topic:** F.05. Neuroimmunology

**Support:** CONACyT CB-2014 -243419
IMSS - FIS/IMSS/PROT/1386
Title: Comparison of single or combined early life stress - Immune challenges on long term behavioral outcomes in male and female rats

Authors: L. SAAVEDRA¹, M. G. HERNÁNDEZ¹, A. OCHOA - ZARZOSA², *L. TORNER³; ¹Biomed. Res. Ctr., Inst. Mexicano del Seguro Social, Morelia, Mexico; ²Ctr. de Estudios Multidisciplinarios en Biotecnología, Univ. Michoacana de San Nicolás de Hidalgo, Morelia, Mexico; ³Biomed. Res. Ctr. of Michoacan, Inst. Mexicano Del Seguro Social, Morelia, Mexico

Abstract: Early life stress (ELS), including maladaptive family environments, low socioeconomic status or childhood maltreatment, creates a greater risk to suffer psychopathologies such as major depression and anxiety in adulthood. Early life severe infections can also alter affective and cognitive processes. Here we compared the effects of a neonatal single or combined stress-immune challenge on several behavioral parameters in adult male and female rats. Four groups of male and female Sprague Dawley rats were used: 1) control + vehicle, 2) maternal separation (MS, 3 hours/day on postnatal days [PN] 1 to 14) + vehicle, 3) control + Lipopolysaccharide (LPS, 0.5mg / kg, PN14), 4) MS + LPS. Starting at PN120, the emotional state of the groups was analyzed utilizing elevated plus maze (EPM), open field (OF) and forced swimming (FS) tests. To evaluate spatial and non-spatial memory, the object recognition (OR) and object placement (OP) tests were used following the emotional tests. Only female rats in diestrous were selected before each test. LPS, but not MS, increased anxiety like-behavior in the EPM and OF tests in male rats. In females both LPS and MS increased anxiety like-behavior. Combined MS+LPS increased anxiety in both males and females. MS, but not LPS, resulted in increased depressive like-behavior in the FS test in males. LPS or MS increased depressive like-behavior in females. Combined MS+LPS increased depressive like-behavior in both genders. MS and LPS challenges had no effects on spatial (OP) and non-spatial (OR) learning in males; in females, LPS decreased their performance in the OP test. Combined MS+LPS did not affect the execution of male and female rats in OP and OR tests. We conclude that behavioral responses to early life challenges depend on gender, suggesting a sexual dimorphism, and also on the nature of the adverse event faced.


Poster

322. Stress, Cognition, and Behavior: Animal Studies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 322.23/V23

Topic: F.04. Stress and the Brain

Support: IU Northwest Faculty Grant-in-aid of Research
IU SUBMIT Grant
Title: Basal and stress-induced cortisol and anxiety-like behavior after cannabidiol (CBD) injection in male and female zebrafish (Danio rerio)

Authors: *N. A. OPIOLA, M. L. PETRUNICH-RUTHERFORD; Dept. of Psychology, Indiana Univ. Northwest, Gary, IN

Abstract: Anxiety disorders are becoming more prevalent every year, with almost 19% of the adult population suffering from some sort of anxiety disorder within the past year. Due to a variety of biological and social factors, females are more likely to be diagnosed with anxiety disorders than males. Thus, it is imperative that investigations into therapeutic options examine possible sex-dependent differences in treatment efficacy. Cannabidiol (CBD) has recently gained popularity for treatment of various disorders, including stress-induced conditions like anxiety. CBD is the one of the main non-hallucinogenic components found in the Cannabis sativa plant, and acts on the endogenous cannabinoid (eCB) system in the body. The eCB system plays a role in many brain and behavioral functions, including the stress response. A key factor in the stress response is the activation of the hypothalamic-pituitary-adrenal (HPA) axis. Anxiety disorders are often associated with dysfunction of the HPA axis, leading to unpredictable responses to stress. Although CBD elicits anxiolytic effects in human and animal models, more studies are needed to determine whether these effects are mediated by HPA normalization. Very few studies have examined the effects of CBD on stress-induced neuroendocrine responses, namely cortisol. Vertebrates such as zebrafish (Danio rerio) share a high degree of functional and genetic homology with mammals, and thus are appropriate research subjects when studying the eCB system and neuroendocrine pathways governing stress. In the current study, an intraperitoneal injection paradigm was employed to expose zebrafish to either CBD (0.2 μg/1 μl) or a vehicle (1 μl 2% Tween-80 in saline). One hour after CBD or vehicle injection, zebrafish were exposed to either an acute net stressor or control condition. Acute stress was induced using a 9-minute net stressor while control subjects were placed into 1L fresh water for 9 minutes after injection. Immediately after the stressor or control condition, anxiety-like behavior was recorded for 15 minutes in the light-dark test. Subjects were then sacrificed to assess whole-body cortisol levels. Acute stress increased whole-body cortisol levels in both male and female fish; this stress-induced increase in cortisol was not as pronounced in the CBD-treated animals. CBD treatment elicited generally anxiolytic behavior in both males and females. The results from the current study suggest that CBD does not appear to influence male and female zebrafish differently; however, these results support the use of CBD as a general anxiolytic compound that has the potential to influence neuroendocrine stress regulation.

**Poster**

**322. Stress, Cognition, and Behavior: Animal Studies**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 322.24/V24

**Topic:** F.04. Stress and the Brain

**Support:** IU Northwest Faculty Grant-in-aid of Research
Minority Opportunity for Research Experiences (MORE) program

**Title:** Acute effects of N-acetylcysteine on basal and stress-induced anxiety-like behavior and whole-body cortisol levels in zebrafish (*Danio rerio*)

**Authors:** *M. L. PETRUNICH-RUTHERFORD, A. APONTE;* Dept. of Psychology, Indiana Univ. Northwest, Gary, IN

**Abstract:** Anxiety disorders are highly prevalent today; however, many of the common treatment options for anxiety-related disorders have an elevated risk for potential abuse. Thus, safer alternative treatment options are needed. N-acetylcysteine (NAC), a drug used to prevent liver damage resulting from acetaminophen overdose and often used as a cough suppressant, has recently surfaced as a potential compound that could be repurposed to treat anxiety disorders. Although promising, evidence supporting the anxiolytic potential and mechanism of action of NAC is limited. Animal models, such as the zebrafish (*Danio rerio*), are critical for investigations into pharmacological potential of anxiolytic compounds, as neural structures and biochemical pathways regulating stress responses are highly conserved between zebrafish and mammals. The aims of the current study were to determine whether NAC would mitigate the anxiogenic effects of an acute (9-minute) net stressor on (1) anxiety-like behavior and (2) the neuroendocrine stress hormone response in zebrafish. Adult zebrafish were exposed to a net stressor following an acute 10-minute NAC treatment (1 mg/L) and were subsequently subjected to the light/dark preference test. The light-dark test is a well-validated paradigm used to measure scototaxis behavior as a result of anxiety. It was expected that the acute stressor would increase anxiety-like behavior and whole-body cortisol levels, and that NAC pretreatment would prevent stress-induced increases in these measures. NAC blunted the increase in whole-body cortisol levels elicited by the acute stressor; however, NAC did not appear to influence stress-induced freezing and scototaxis behaviors observed in the light-dark test. The results of the current study provide evidence that brief NAC exposure has potential to regulate the neuroendocrine responses elicited by acute stress; however, chronic exposure of NAC may be required to have an impact on anxiety-like behavior. Thus, NAC may be of potential clinical use to treat neuropsychiatric conditions associated with significant neuroendocrine dysfunction. Further studies are necessary to examine the long-term impact of chronic NAC, particularly on behavioral and neuroendocrine alterations observed in response to chronic stress exposure.
Disclosures: M.L. Petrunich-Rutherford: None. A. Aponte: None.

Poster

322. Stress, Cognition, and Behavior: Animal Studies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 322.25/V25

Topic: F.04. Stress and the Brain

Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001
Fapemig Grant APQ-01420-14
CNPq Grant 447537/2014-8

Title: Cerebellar antioxidant capacity is improved and cognitive function preserved in wistar rats after six weeks of high-intensity interval training

Authors: *R. A. DE SOUSA1, D. A. FREITAS1, E. ROCHA-VIEIRA1, B. A. SOARES2, A. ROCHA-GOMES1, B. C. C. GARCIA1, R. C. CASSILHAS3, V. A. MENDONÇA1, A. C. R. CAMARGOS5, J. A. M. GREGORIO6, A. C. R. LACERDA4, H. R. LEITE1; 1Physiol., 2Physioterapy, 3Physical Educ., 4Physiotherapy, Federal Univ. of the Valleys' Jequitinhonha and Mucuri, Diamantina, Brazil; 5Physiotherapy, Federal Univ. of Minas Gerais, Belo Horizonte, Brazil; 6Musculoskeletal Hlth., The Univ. of Sydney, Sydney, Australia

Abstract: High-intensity interval training (HIIT) is associated with better physical performance, but there is limited information about the effects of HIIT on redox state of cerebellar tissue, cerebral cortex, and cognition. The aim of this study was to evaluate the effects of HIIT on redox state parameters in cerebellar tissue, cerebral cortex, and cognitive function of wistar rats. Forty-three young male wistar rats were housed under controlled environmental conditions with food, and water ad libitum. Animals were assigned to HIIT or Non-trained groups. HIIT protocol was performed during six weeks. Speed was determined through the assessment of the maximum oxygen consumption (VO2max). HIIT consisted of short bouts (1 minute) running on a treadmill at 10º inclination (85-100% of VO2max) with 2 minutes of active recovery (60% of VO2max, 10ºC inclination). Non-trained group was daily exposed to a disconnected treadmill for the same amount of time as HITT group. Both groups were submitted to the open field, and novel object recognition tasks after six weeks. Malondialdehyde concentration (MDA), superoxide dismutase (SOD) activity, and non-enzymatic antioxidant capacity (FRAP) were quantified to determine the redox state. HIIT presented increased levels of MDA, SOD, and FRAP (p<0.05) in the cerebellar tissue, but no differences were seen in cerebral cortex. These results indicated an improved antioxidant capacity, despite increased MDA levels in the cerebellar tissue. Both groups did not present impairment in locomotor activity, development of anxious behavior or
cognitive decline. HIIT enhanced the antioxidant defenses on cerebellar tissue with no deleterious effects on rats’ cognition.


**Poster**

**322. Stress, Cognition, and Behavior: Animal Studies**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 322.26/V26

**Topic:** F.04. Stress and the Brain

**Support:**
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- NIH grant MH091645
- NIH grant MH086633
- NIH grant U54 HD079124
- NIH grant MH100029
- NIH grant MH096773

**Title:** Effects of early life stress on development of the prefrontal cortex, amygdala, hippocampus and nucleus accumbens in Rhesus monkeys: Associations with psychostimulant escalation during adolescence?

**Authors:** *B. KOCHOIAN*¹,², A. G. WAKEFORD², A. RATLIFF³,², E. R. SIEBERT², K. KUITCHOUA¹,², E. L. MORIN³,², R. HONG¹,², M. H. KYLE¹,², S. COWAN¹,², B. R. HOWELL⁴, Z. KOVACS-BALINT², M. A. STYNER⁵, L. L. HOWELL³,², M. A. NADER⁶, M. SANCHEZ³,²;

¹Emory Univ., Atlanta, GA; ²Yerkes Natl. Primate Res. Center, Emory Univ., Atlanta, GA; ³Dept. of Psychiatry & Behav. Sci., Emory Univ. Sch. of Med., Atlanta, GA; ⁴Univ. of Minnesota, Inst. of Child Develop., Minneapolis, MN; ⁵Deparments of Psychiatry and Computer Sci., Univ. of North Carolina At Chapel Hill, Chapel Hill, NC; ⁶Dept Physiol Pharmacol, Wake Forest Univ. Sch. Med., Winston Salem, NC

**Abstract:** Early life stress (ELS) is a major risk factor for anxiety, mood and substance use disorders (SUDs). A form of ELS, infant maltreatment (MALT), occurs during rapid brain development, leading to long-term neurobehavioral effects. The developmental trajectory and underlying mechanisms are not well understood and difficult to address in humans due to limitations of prospective, longitudinal designs. Here, we used translational macaque model of
infant MALT and a cross-fostering design with random assignment to caregiving group at birth. Rates of MALT in macaques are similar to human populations, with similar long-term psychopathology outcomes as in children. We studied longitudinal brain structural alterations underlying increased stress/emotional reactivity and risk for SUDs, focusing on regions controlling these processes: amygdala, hippocampus, prefrontal cortex (PFC), and nucleus accumbens (NAcc). We collected (1) MRIs in 42 rhesus monkeys (20 CONT -11F, 9M-, 22 MALT -8F, 14M-) at 3 and 6 mos (infancy) and 12 mos (juvenile period), and (2) hair samples at 6 and 12 mos to measure cortisol (CORT) accumulation as index of stress exposure during infancy or the juvenile period, respectively. We used regression models to examine if higher CORT exposure predicted alterations in neurodevelopment. We also examined if MALTs were more vulnerable than CONTs to psychostimulant escalation during adolescence using a cocaine (COC) self-administration extended access (EA) paradigm. We used a fixed-ratio 20 schedule of reinforcement with daily 4hr sessions and two COC doses: a “high” dose of COC, 0.1 mg/kg/inj, and a second dose (ranging from 0.003 to 0.1 mg/kg/inj), which elicited the highest response rate in each subject (EDMax). Our findings suggest bigger amygdala (F_{1,30} =5.96, p=0.021) and NAcc (F_{1,29} =6.63, p=0.015) volume growth with age in MALTs compared to CONTs. Although higher CORT exposure did not explain these results, higher CORT levels predicted blunted PFC volume growth from 3 to 12 mos (total, grey and white matter (e.g. Total PFC: right: r=-0.604, p=0.003; left: r=-0.472, p=0.027). CONTs showed stable COC intake during EA and did not “escalate” intake at either COC dose. Our findings are consistent with previous reports in macaques and in contrast to rodent studies, which increase response rate (“escalation”) under EA compared to limited access (1hr sessions). We are currently analyzing if MALTs show differences in EA drug intake compared to CONTs. Our findings suggest ELS alters structural development of cortico-limbic circuits. We are currently examining if these neural changes underlie increased vulnerability for psychostimulant escalation.


Poster

322. Stress, Cognition, and Behavior: Animal Studies

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 322.27/V27

Topic: F.04. Stress and the Brain

Support: PAPIIT IN306918

PAPIME PE306318
Title: Simultaneous effects over the release of corticosterone on hippocampus, amygdala and prefrontal cortex after stress exposure


1Univ. Nacional Autonoma De Mexico. Fac Psicologia, CDMX, Mexico; 2Univ. Nacional Autónoma de México, Facultad de Psicología, Sistema Univ. Abierta, Mexico City, Mexico; 3Sociedad Iberoamericana de Neurociencia Aplicada. A. C., Cdmx, Mexico

Abstract: The stress response has been described as a systemic response in real or perceived conditions of treat. Current literature shown that the stress exposure alters the levels of corticosterone in different brain structures such hippocampus (Hip) amygdala (Am) and prefrontal cortex (PFC). However such changes not necessarily has been reported simultaneously. The aim of this research was to evaluate simultaneously the corticosterone levels in three brain structures related with the stress response (Hip, Am and PFC). 14 Wistar male twelve weeks old rats were exposed to a chronic unpredictable stress battery (CUSB). 30 minutes after the end of the stress exposure the brains were removed and the Hyp, Am and PFC were dissected and corticosterone levels were measured with ELISA protocol. Results showed great variability in the corticosterone levels of the control condition, by the other side, the stressed group showed a decrease in the variability. This data suggests that an exposition to stress alters differentially in different brain structures.


Poster

323. Reward: Neuropharmacology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 323.01/V28

Topic: G.02. Motivation

Title: Sigma-1 receptor antagonist effects on sucrose reinforcement driven by intra-accumbens opioid and dopamine agonist administration


Abstract: Feeding behaviors can be influenced by a multitude of factors including one’s demographic and socioeconomic status, biological tendencies, nutritional knowledge, food preference, and portion size (Scaglioni et al., 2018). As the intersectionality of these factors have important implications as it relates to the prevention and treatment of one’s risk of obesity, it is important to investigate pharmacological methods in which feeding behaviors can be altered. Sigma 1 receptors have been investigated for their involvement in learning, rewarding and motivational processes. PD144418 1,2,3,6-tetrahydro-5-[3-(4-methylphenyl)-5-isoxazolyl]-1-propylpyridine, a potent and selective sigma 1 ligand exhibiting a high affinity and selectivity for sigma 1 receptors, has been found to produce a dose-dependent attenuation of locomotor activity induced by cocaine. Moreover, PD144418 decreases motivational effort of food-reinforced operant behavior, without altering locomotion, appetite or food palatability in rats. However, it remains unknown whether PD144418 can alter the motivational effort of food-reinforced operant behavior in response to altered modification to hedonic or dopaminergic driven feeding behaviors. The present study examined the effects of PD144418 on hedonic and dopaminergic driven motivational aspects of feeding, via central administration of a mu opioid agonist, DAMGO, or d-amphetamine, a dopamine uptake inhibitor and releaser, in male and female rats using a progressive ratio (PR) schedule of reinforced operant task. Male and female rats (n=24) were first trained on a fixed ratio (FR) schedule of reinforcement. Following FR training, rats were tested under a PR schedule of reinforcement. 15-minutes prior to testing, each rat received a single dose of PD144418 (0, 3.16 or 10 µmol/kg, ip). Immediately prior to being placed in the operant chamber, each rat received an infusion of either DAMGO (0.25μg/0.5 µl/side bilaterally), d-amphetamine (10μg/0.5 µl/side bilaterally), or saline. Results determined that pretreatment with PD144418 did influence operant responding under these conditions of opioid and dopaminergic manipulations, raising intriguing possibilities and questions regarding the mechanism underlying the interaction of sigma 1 receptors and these motivational processes.


Poster

323. Reward: Neuropharmacology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 323.02/V29

Topic: G.02. Motivation

Support: NIH Grant AA018779

Title: The interaction of astrocytes and medium spiny neurons in dorsomedial striatum regulates adenosine-dependent reward-seeking behaviors
Authors: *S. KANG*¹, S.-I. HONG¹, H. LEE³, D.-S. CHOI¹,²;  

Abstract: The maladaptation in reward-value dependent shifting between goal-directed and habitual actions may underlie multiple psychopathologies such as addiction, obsessive compulsive disorders, impulsivity and some decision-making disorders. Dorsomedial striatum (DMS) has been spotlighted because of its role in goal-directed behaviors orchestrated by neurons within the DMS via adenosine signaling. However, how astrocytes, the major modulator of extracellular adenosine levels, contribute to the neuronal synaptic activities and the sequential behavioral shifts remains largely unknown.

Utilizing the Inscopix endomicroscopy/fiber photometry in vivo calcium imaging and the cell type specific activation of designer receptors exclusively activated by designer drugs (DREADDs), we confirmed that GFAP-driven activation of hM3Dq DREADDs increases intensity and frequency of calcium signaling in the DMS astrocytes of ALDH1L1-GCaMP6s expressing mice. We found that this chemogenetic activation of the DMS astrocytes increase and decrease neuronal excitability of direct pathway Medium Spiny Neurons (dMSNs) and indirect pathway Medium Spiny Neurons (iMSNs), respectively. Specifically, it reduced the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) in dMSNs, whereas it increased the amplitude of the sEPSCs and decreased the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) in iMSNs. The changes in sEPSCs and sIPSCs induced by astrocytic activation were significantly inhibited in the DMS of mice lacking ethanol-sensitive adenosine transporter, ENT1 (equilibrative nucleoside transporter 1, Slc29a1). NBTI, an ENT1-specific blocker, also reduced the chemogenetic-evoked synaptic events, suggesting that ENT1 is, at least partly, required for astrocytic adenosine release.

In the devaluation tests of reward seeking by within-subject instrumental nose-poking paradigm, which mice shift between goal-directed and habitual actions according to outcome value change, the chemogenetic activation of DMS astrocytes shifted the habitual behaviors to the goal-directed actions in sucrose-reward seeking in the ENT1 WT mice, but not in the ENT1 KO mice. When the ENT1 expression in the DMS is rescued by injection of GFAP-driven ENT1 expressing AAV into the DMS of the ENT1 KO mice, the behavioral shift was restored by the chemogenetic activation.

Together, our results indicate that astrocyte-mediated adenosine signaling in the DMS determines goal-directed and habitual actions by selectively regulating specific synapses suggesting that reward-seeking behavior results from the interaction of neurons and astrocytes.

Disclosures: S. Kang: None. S. Hong: None. H. Lee: None. D. Choi: None.
**Poster**

**323. Reward: Neuropharmacology**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 323.03/V30

**Topic:** G.02. Motivation

**Title:** Measurement of response vigor in a progressive ratio task in common marmosets

**Authors:** *T. ENOMOTO¹, N. KONOIKE², K. NAKAMURA², K. IKEDA¹;
¹Platform Technol. Res. Unit, Drug Res. Div., Sumitomo Dainippon Pharma Co., Ltd., Osaka, Japan; ²Primate Res. Institute, Kyoto Univ., Inuyama, Japan

**Abstract:** Motivational deficits are common symptoms in a wide range of neuropsychiatric disorders. However, there are no approved medicines for motivational deficits. In the drug discovery for this symptoms, translational methods to measure the motivation are essential. Since the prefrontal cortex has played a crucial role on motivation, non-human primates with the developed prefrontal cortex would be useful in this field. We previously developed a novel effort-based decision making task in common marmosets (*Callithrix jacchus*). In the present study, we measured response vigor and persistence, another aspect of motivation, in a progressive ratio task in common marmosets. We used the same touch-panel system and food reward (a piece of cakes) as the effort-based decision making task. Marmosets were trained to touch the picture in the panel to get the reward. The required numbers of touch responses to get one reward were progressively increased. A test session was terminated after 30 min or earlier if 3 min elapsed after a response. The breakpoint, which was the last ratio obtained, was measured as an index of response vigor and persistence. Animals were treated with compounds or vehicle in a counter-balanced way. A vesicular monoamine transporter-2 inhibitor tetrabenazine, which has been known to deplete the dopamine in the synaptic vesicles, was administered 120 min before the test. Tetrabenazine (1 and 3 mg/kg s.c.) decreased the breakpoint. Either a dopamine D₁ receptor antagonist SCH-39166 (0.01 and 0.03 mg/kg i.m.) or D₂ receptor antagonist raclopride (0.01 and 0.03 mg/kg i.m.) also decreased the breakpoint. Therefore, the decrease of D₁ and/or D₂ receptor signaling may be related with the effects of tetrabenazine on motivation. On the other hand, methamphetamine (0.1 mg/kg i.m.), which has been known to elevate the extracellular dopamine levels in the brain, increased the breakpoint. Clinical studies have shown that tetrabenazine induces some motivational deficits, while amphetamine elevates the motivation in humans. Therefore, our progressive ratio task in common marmosets would have predictive validity. This behavioral task could be a simple and useful assay to measure the motivation in translational research.

**Disclosures:**  
* T. Enomoto: None.  
* N. Konoike: None.  
* K. Nakamura: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and
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**Poster**

**323. Reward: Neuropharmacology**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 323.04/V31

**Topic:** G.02. Motivation

**Support:** NIAAA Division of Intramural Clinical and Biological Research Z1A AA 000466

**Title:** Varenicline, a nicotinic acetylcholine receptor partial agonist, alters reward network functional connectivity during an alcohol incentive delay task in heavy drinkers

**Authors:** *B. W. STEVENS*¹, A. L. COWAND¹, V. VATSALYA³, J. L. GOWIN⁴, S. E. BARTLETT⁵, M. HEILIG⁶, R. MOMENAN², V. A. RAMCHANDANI¹; ¹Section on Human Psychopharmacology, ²Clin. Neuroimaging Res. Core, Natl. Inst. on Alcohol Abuse and Alcoholism, Bethesda, MD; ³Dept. of Med., Univ. of Louisville, Louisville, KY; ⁴Dept. of Radiology and Psychiatry, Univ. of Colorado Denver, Denver, CO; ⁵Sch. of Clin. Sci., Queensland Univ. of Technol., Brisbane, Australia; ⁶Dept. of Clin. and Exptl. Med., Linköping Univ., Linköping, Sweden

**Abstract:** Varenicline is a nicotinic acetylcholine receptor (nAChR) partial agonist approved for smoking cessation. Clinical trials also suggest that varenicline can reduce alcohol drinking. Varenicline has been shown to reduce activation in brain reward regions to nicotine cues in smokers, and our lab found this effect for alcohol cues in heavy drinkers. These studies however did not examine the dynamics of these activations across brain networks. This analysis sought to extend these findings by studying the effects of varenicline on neural dynamics using functional connectivity in non-treatment seeking heavy drinkers (>14 standard drinks/week for females and >20 for males). Following randomization to varenicline (VR; N=17; Female=3) or placebo (PL; N=12; Female=1) for 2 weeks, participants underwent an MRI scan while performing the Alcohol-Food Incentive Delay (AFID) task which included alcohol (Alc), neutral (Neut) or food cues followed by a target response phase, and then feedback. After the task they rated how excited, happy, fearful and unhappy they were to each cue type. fMRI data were analyzed using seed to voxel generalized psychophysiological interactions including all the task phases as regressors. Seed regions included the left (L) and right (R) nucleus accumbens (NA), putamen, medial and lateral OFC. Analyses focused on comparing Alc and Neut trial types at p<0.05 FDR cluster-corrected. The VR group reported feeling less excited (p= 0.004) during Alc trials. In the Alc>Neut cue contrast, the VR group had greater connectivity between 2 seed regions (R NA and L putamen) with the right posterior cingulate (PCC). A similar pattern emerged for the
Alc>Neut winning feedback contrast, with the VR group showing higher connectivity between the R medial OFC and L NA with the L angular gyrus. Given the emerging role of PCC in regulating whether attention is internalized or externalized, these results suggest that VR may be interacting with the reward regions reducing internalization of alcohol cues. The angular gyrus has been shown to play a role in emotional regulation, helping signal the PFC to exert control, and therefore VR could be downregulating the emotional response to winning feedback. These findings provide additional insights into the neural dynamics underlying VR’s effect on alcohol drinking and efficacy in alcohol use disorder in that VR might influence regulatory mechanisms that act upon reward networks.


Poster

323. Reward: Neuropharmacology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 323.05/V32

Topic: G.02. Motivation

Support: 5RO1DA037257-05
5R21DA044486-02

Title: Histone demethylase KDM6B mediates heroin craving

Authors: *S. MITRA¹, P. H. GOBIRA³, J. A. MARTIN⁴, S. THOMAS⁵, C. T. WERNER², D. M. DIETZ⁶;
¹Pharmacol. & Toxicology, ²Dept. of Pharmacol. and Toxicology; Program in Neurosci., State Univ. of New York at Buffalo, Buffalo, NY; ³Univ. of São Paulo, Ribeirao Preto, Brazil; ⁴Dept. of Pharm and Tox; Res. Inst. on Addictions; Program in Neurosci., State Univ. of New York At Buffalo, Buffalo, NY; ⁵Pharmacol. & Toxicology, The State Univ. of New York at Buffalo, Buffalo, NY; ⁶State Univ. of New York, Buffalo, NY

Abstract: Opioid use disorder is a chronic relapsing disease. Following prolonged drug cessation, relapse events are often triggered by drug-associated cues. Nucleus accumbens (NAc) acts as a hub for convergence of reward-related pathways and therefore forms a critical region for enduring neuroadaptations implicated in cue-evoked maladaptive behaviors. One intriguing mechanism underlying neuroadaptation leading to relapse vulnerability is the epigenetic modification of DNA and histones. These unique epigenetic signatures constitute the backbone for chronic alterations in genomic landscape and cellular dysregulation leading to aberrant behaviors such as compulsive drug craving. Lysine-specific demethylase 6B (KDM6B) is a histone demethylase that modifies histone tails and is at the nexus of mechanisms known to be
dysregulated in the addicted state. Bone morphogenetic proteins (BMPs) are a member of the TGF beta superfamily of proteins that serve as important mediators for a wide array of cellular functions. With emerging evidence for overlapping roles of BMP pathway and KDM6B and limited understanding of their role in addiction, we aimed at investigating if there is a potential cross-talk between KDM6B and the BMP pathway underlying behavioral and cellular plasticity inflicted by drugs of abuse. Here, we show that following prolong abstinence (abstinence day 14 [AD14]) from heroin self-administration, a time point characterized by enhanced drug craving, both canonical BMP pathway intermediates SMAD 1/5/8 phosphorylation and histone demethylase KDM6B is potentiated in the NAc. Pharmacological manipulation of canonical BMP pathway and KDM6B by noggin and GSK-J4 respectively, reduced cue-induced heroin seeking with evidence of potential cross-talk between KDM6B and BMP pathway. Our findings demonstrate that KDM6B mediates cue-induced heroin seeking following prolong abstinence, potentially through a transcriptional mechanism involving canonical BMP pathway.


Poster

323. Reward: Neuropharmacology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 323.06/V33

Topic: G.02. Motivation

Title: Influence of physical activity on diet preferences in male and female rats

Authors: *J. R. LEE, M. A. TAPIA, V. N. WEISE, E. L. BATHE, V. J. VIEIRA-POTTER, F. W. BOOTH, M. J. WILL; Univ. of Missouri, Columbia, MO

Abstract: Palatability driven feeding and voluntary physical activity are mediated by and influence similar neural mechanisms, notably through the actions of opioids within the nucleus accumbens. Recent studies suggest that access to a voluntary running wheel results in sex dependent behavioral and physiological adaptations related to opioid mediated palatability-driven feeding. However, experimental parameters such as testing environment and composition of diet may also influence these results. To explore this relationship, male and female Wistar rats were given either access to a voluntary running wheel (RUN group) or no access (SED group) for one week prior to being stereotaxically implanted with bilateral cannulae targeting the nucleus accumbens. Following 7 days of recovery, with RUN or SED conditions continuing the duration of the experiment, all rats were assessed daily (2h/day) for feeding behavior of concurrently accessible high-carbohydrate and high-fat diet for one week. Following this week, all rats were administered the µ-opioid receptor agonist D-Ala2, NMe-Phe4, Glyol5-enkephalin
(DAMGO) (0.0025µg, 0.025µg, or 0.25µg/0.5µl/side) or the opioid antagonist naloxone (20µg/0.5µl/side) into the nucleus accumbens and given concurrent access (2h) to both diets.


Poster

323. Reward: Neuropharmacology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 323.07/V34

Topic: G.02. Motivation

Support: NIDA Grant R01DA002812

Title: Naltrexone does not alter willingness to exert effort in a monetary reward task

Authors: *J. K. HOOTS¹, M. C. WARDLE¹, H. DEWIT²; ¹Univ. of Illinois At Chicago, Chicago, IL; ²Univ. of Chicago, Chicago, IL

Abstract: Background: The opioid system contributes to the hedonic or “liking” experience of rewards such as foods and drugs. Opioidergic “hot spots” governing hedonic experience are important for reward learning and goal-directed behavior. Here, we used the mu-opioid receptor antagonist naltrexone to examine the role of opioids in determining effort expenditure for rewards in healthy adults. We hypothesized that naltrexone would reduce willingness to exert effort for monetary rewards. Specifically, we expected effort levels to be equivalent at the start of each session, but we predicted that effort expenditure would decrease over the naltrexone sessions, as individuals would experience less pleasure from “wins” during opioid blockade and alter their effort accordingly.

Methods: Healthy adults (N = 34) participated in a 3-session within-subjects double-blind study in which they received oral doses of placebo or naltrexone (25 and 50 mg). Subjects completed the Effort Expenditure for Rewards Task, a 50-trial computerized game in which they were given an opportunity on each trial to choose between two task difficulty levels. In the easy task, participants could receive $1 after completing a relatively easy button-pressing task. In the hard task, they could receive between $1.24 and $4.20 by completing a more difficult button-pressing task. Subjects were informed of the reward amount for the hard task and the probability of receiving any reward upon task completion (12%, 50%, or 88%) before choosing the easy or hard task for each trial. Participants received feedback about the amount of money won after each trial. The outcome variable was the number of times each subject chose the hard task.

Results: In a Generalized Linear Mixed Model, naltrexone dose (0, 25, and 50 mg) had no significant effect on willingness to exert effort for monetary rewards. Williness to exert effort decreased over the course of the 50 trials in each session in all conditions, but this decline in
effort was also not affected by naltrexone. Further, the effect size observed for even the highest dose of naltrexone was small ($d = 0.23$).

**Conclusions**: We demonstrated that acute opioid antagonism did not significantly alter willingness to exert effort for monetary rewards. Although individuals decreased their willingness to exert effort during the session, this did not vary across drug conditions. While naltrexone at these doses has shown an effect on evaluation of social stimuli, these findings suggest that blockade of the opioid system has little effect on willingness to exert effort for monetary rewards.

**Disclosures**: J.K. Hoots: None. M.C. Wardle: None. H. DeWit: None.

**Poster**

**323. Reward: Neuropharmacology**

**Location**: Hall A

**Time**: Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #**: 323.08/V35

**Topic**: G.02. Motivation

**Support**: Conacyt fellowship 339464

**Title**: Endocannabinoids are released in the VTA of male rats during copulation to satiety and activate CB1 receptors to induce a long-lasting sexual inhibition and drug hypersensitivity

**Authors**: *E. GONZÁLEZ-MORALES, R. GARDUÑO-GUTIÉRREZ, G. RODRÍGUEZ-MANZO;
Pharmacobiology, Cinvestav-Sede Sur, Mexico, Mexico

**Abstract**: A male rat with unlimited access to a receptive female will ejaculate repeatedly until becoming sexually satiated. This process induces the appearance of two long lasting (72h) phenomena: a sexual behavior inhibition and a generalized hypersensitivity to drug actions. During copulation to satiety, the mesolimbic dopaminergic system is constantly activated. The dopaminergic neurons of this system synthesize and release endocannabinoids (eCB) in the ventral tegmental area (VTA), in response to its repeated stimulation. Blockade of CB1 receptors during copulation to satiety, prevents the appearance of both the sexual inhibition and the drug hypersensitivity in sexually satiated rats. We hypothesized that eCBs, which have been associated to brain plasticity processes, might be involved in the induction of these phenomena by acting at the VTA. To test this hypothesis we examined the effects of the direct infusion of the CB1 receptor antagonist AM251 into the VTA of male rats prior to copulation to satiety on sexual behavior inhibition and hypersensitivity to the 5 HT1A receptor agonist, 8 OH DPAT, measured by the appearance of one sign of the serotonergic syndrome, the flat body posture. In addition, we searched for changes in VTA CB1 receptors’ density and activation of male rats that copulated to satiety 24h earlier. Results showed that antagonism of CB1 receptors in the VTA
blocked the appearance of the sexual behavior inhibition and the hypersensitivity to 8 OH DPAT in the sexually exhausted male rats. VTA CB1 receptor’s density did not differ between sexually experienced and sexually satiated rats. However, we found a significant increase in the CB1 receptors that co localized with beta arrestin2 in the sexually satiated males in comparison to the sexually experienced animals. CB1 receptor and beta arrestin2 co localization reflects receptor internalization, indicative of its activation. It is concluded that during copulation to satiety eCBs are released in the VTA, where they activate CB1 receptors. Blockade of this eCBs action at VTA CB1 receptors interferes with the appearance of the two plasticity phenomena that characterize sexually satiated male rats.

**Disclosures:** E. González-Morales: None. R. Garduño-Gutiérrez: None. G. Rodríguez-Manzo: None.

**Poster**

323. **Reward: Neuropharmacology**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 323.09/V36

**Topic:** G.02. Motivation

**Support:** NIMH73136

George E. Hewitt Fellowship to M.T.B

**Title:** The CRH-expressing projection pathway from the basolateral amygdala to the nucleus accumbens contributes to neurobiological mechanisms of anhedonia

**Authors:** *M. T. BIRNIE*¹,², A. K. SHORT¹,², Y. CHEN¹,², C. A. ITOGA², X. XU², T. Z. BARAM¹,²;

¹Pediatrics, ²Anat. and Neurobio., Univ. of California Irvine, Irvine, CA

**Abstract:** **Rationale:** Disruption to the operations of the reward circuitry underlies many aspects of affective disorders. A major manifestation of disrupted function of the reward circuitry is anhedonia, the reduced ability to experience pleasure. Anhedonia is an important dimensional entity linked to depression, schizophrenia, and other emotional disorders, yet its origins and underlying mechanisms remains unclear. We have previously identified augmented expression of the stress-related neuropeptide corticotropin-releasing hormone (CRH) within the amygdala as a cause of anhedonia in experimental models. More recently we identified a CRH-expressing projection pathway from the basolateral amygdala (BLA) to the nucleus accumbens (NAc).

Therefore, we aim to understand how this molecularly-defined CRH-expressing pathway impinging on the nucleus accumbens might contribute to anhedonia. **Methods:** We utilized novel viral-genetic approaches to map CRH⁺ pathways using Cre-dependent viruses injected into CRH-Cre mice. Further, we assessed chemogenetic targeting of CRH⁺ neurons using excitatory
and inhibitory Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), as well as excitatory optogenetic tools paired with behavior analysis. Results: We show that CRH-expressing neurons project from the basolateral amygdala to the nucleus accumbens. Preliminary data indicates anhedonia was observed when targeting these CRH$^+$ neurons with excitatory DREADDs and optogenetic stimulation, as measured by the sucrose preference, scent of a mouse and three-chamber social interaction tasks. Conclusions: These findings suggest that aberrant connectivity of the CRH$^+$ BLA-NAc pathway results in anhedonia and further identifies a mechanistic role for CRH-expressing neurons in stress-related major neuropsychiatric disorders.

Disclosures: M.T. Birnie: None. A.K. Short: None. Y. Chen: None. C.A. Itoga: None. X. Xu: None. T.Z. Baram: None.

Poster

323. Reward: Neuropharmacology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 323.10/V37

Topic: G.02. Motivation

Support: ZIA-AA000421

Title: Dopamine receptor independent actions of dopamine on medium spiny neuron synaptic transmission in the nucleus accumbens

Authors: *D. A. BURKE$^{1,2}$, V. A. ALVAREZ$^1$;
$^1$Lab. on Neurobio. of Compulsive Behaviors, Natl. Inst. on Alcohol Abuse and Alcoholism, Bethesda, MD; $^2$Neurosci., Brown Univ., Providence, RI

Abstract: The nucleus accumbens (NAc) is a brain region that subserves healthy motivated behaviors and is an important site of dysfunction in psychiatric diseases, such as addiction and depression. Acting as a “limbic-motor interface,” projection neurons in the NAc, medium spiny neurons (MSNs), integrate excitatory glutamate signals from cortex, amygdala, and hippocampus along with dopamine (DA) signals from midbrain to facilitate motivation and reward learning. However, NAc also contains an extensive network of inhibitory GABA synapses between MSNs, which remains understudied. It is currently unknown how DA interacts with this network of MSN GABA synapses. We hypothesize that by modulating synaptic strength within this network of lateral inhibition, DA influences NAc information processing by shifting the balance of excitation/inhibition at the micro circuitry level. MSNs express either Gs coupled D1 receptors or Gi coupled D2 receptors (D2MSNs). Due to the link between D2 receptor (D2R) signaling and multiple psychiatric disorders, we begin by focusing on D2MSNs. Here we ask: how does DA acutely affect GABA transmission from D2MSNs onto neighboring MSNs? We hypothesize that endogenously released DA from the midbrain can inhibit local GABA
transmission from D2MSNs to MSNs. Using a dual optogenetic approach to test this hypothesis in mouse brain slices, we show that DA neuron stimulation that precedes D2MSN GABA release can acutely depress GABA transmission from D2MSN synapses. To further probe this DA inhibition of D2MSN synaptic transmission, we applied exogenous DA at different concentrations and found the IC50 to be in the micromolar range, an order of magnitude higher than previous studies using heterologous systems. We next hypothesized that this DA depression of D2MSN GABA transmission is mediated presynaptically by D2Rs expressed on D2MSNs. We found that indeed this depression is driven by a presynaptic mechanism, but surprisingly it is only partially explained by D2R activation. Genetically deleting or pharmacologically blocking D2Rs only attenuates DA mediated depression by ~50%. Moreover, D1 receptor antagonists did not block this remaining depression, suggesting a novel target of DA. Taken together, these results shed light on the actions and mechanisms by which DA acutely modulates the lateral inhibitory network in the NAc. This insight is necessary for understanding how DA shapes NAc circuitry and information processing to promote motivated behaviors, both in health and disease.

Disclosures: D.A. Burke: None. V.A. Alvarez: None.

Poster

323. Reward: Neuropharmacology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 323.11/V38

Topic: G.02. Motivation

Title: Methadone administered immediately after ejaculation do not inhibit the sexual behavior of the male rat

Authors: *J. ROJAS-HERNÁNDEZ¹, J. JUAREZ²;
¹Univ. De Guadalajara, Guadalajara, Mexico; ²Univ. Guadalajara, Guadalajara, Jalisco, Mexico

Abstract: Release of endogenous opioids during the execution of male sexual behavior, particularly after ejaculation, has been related to the reinforcing and rewarding properties of sexual performance. On the other hand, the literature show evidence that opioids agonists administered before mating, suppress the sexual behavior in male rats. This supports the notion that opioids are responsible for the sexual inhibition that occurs after ejaculation. However, the literature indicates that sexual inhibition occurs when opioid agonists are administered before sexual intercourse, and there is no evidence of this effect when the opioid activity is enhanced immediately after ejaculation. On this basis, the aim of this work was to investigate whether the methadone, administered immediately after reaching ejaculation, facilitates or inhibits, both the subsequent copulatory event, and the sexual intercourse 24 h after its administration. In addition, qualitative changes in the copulatory pattern were studied. Methods: Three groups of sexually expert male rats were treated with either, 1) methadone 2.5 mg/Kg immediately after ejaculation;
2) saline solution 1 mL/Kg immediately after ejaculation, and 3) No any treatment. Thirty minutes after injection, all males were exposed to the same female with whom ejaculation was previously executed. The results showed that there was no difference in the proportion of males able to resume intercourse immediately after methadone injection, as well as 24 h after receiving the treatment. But there was an increase in the number of mounts, intromissions and in the ejaculatory latency of the copulatory serie following methadone injection when compared with control group. These results show that an increase in the opioid activity after ejaculation do not inhibit the forward progress of sexual execution, rather increases, apparently, the stimulation threshold to reach the next ejaculation. This finding contrast with the described sexual inhibition, when opioid agonists are administered before sexual intercourse.

Disclosures:  J. Rojas-Hernández: None. J. Juarez: None.

Poster

323. Reward: Neuropharmacology

Location: Hall A

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Program #/Poster #: 323.12/V39

Topic: G.02. Motivation

Support: NIH Grant DA045913
NCRG Seed Grant
UPMC Competitive Medical Research Fund Award
NARSAD Young Investigator Grant 25185

Title: Sign tracking is predictive of suboptimal behavior in a rodent gambling task

Authors: M. C. SWINTOSKY, J. T. BRENNAN, J. P. PAULUS, *S. E. MORRISON; Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Cues that are repeatedly associated with rewards can exert a powerful influence over behavior. They can elicit approach or interaction with the cue even when such behavior is maladaptive: e.g., the flashing lights of a slot machine might prompt approach by a patient in recovery from gambling disorder. Notably, the ability of reward-associated cues to produce approach and/or interaction varies widely among individuals. In a Pavlovian conditioned approach protocol, for example, if a cue (e.g., extension of a lever) predicts a reward in a different location (e.g. a sugar pellet delivered to a food cup), some rats will preferentially approach and interact with the lever - a behavior known as sign tracking (ST) - and others will approach the site of reward delivery, a behavior known as goal tracking. A propensity towards ST has been linked to susceptibility to addiction and relapse, as well as various forms of impulsive behavior.

The neurobiological basis of ST has much in common with brain processes underlying addiction
and relapse, including pathological gambling. Both ST and gambling disorder, among other forms of addiction, are dependent on dopaminergic transmission in the mesocorticollimbic circuit. However, little is known about the relationship between ST and risky decision-making. Therefore, we characterized the performance of sign trackers and goal trackers on a rodent gambling task (rGT) incorporating win-associated cues (modified from Barrus & Winstanley, 2016). We found that rats with a propensity towards sign tracking, as compared to goal trackers, collected significantly fewer rewards during the rGT, which resulted from two forms of suboptimal behavior: an increase in premature nosepokes (impulsive action) and increased choice of the high-risk, high-reward option. Administration of dopaminergic agents, including a D2 agonist (quinpirole) and a D3 agonist (PD128907) exacerbated this suboptimal performance in a manner that was dependent on the individual’s baseline behavior. These results suggest that individual differences in cue reactivity, as represented by sign tracking behavior, may be an important factor underlying individuals’ susceptibility to pathological gambling, perhaps arising from enhanced engagement of the mesolimbic dopamine system by reward-associated cues.

Disclosures: M.C. Swintosky: None. J.T. Brennan: None. J.P. Paulus: None. S.E. Morrison: None.

Poster

323. Reward: Neuropharmacology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 323.13/V40

Topic: G.02. Motivation

Support: NIH R00 MH106731
NARSAD Young Investigator Award, BBRF

Title: The role of the serotonin 1B receptor in impulsivity and related behavioral constructs

Authors: *S. S. DESROCHERS¹, V. M. MAGALONG², J. LEE¹, P. D. BALSAM², K. M. NAUTIYAL¹;
¹Psychological and Brain Sci., Dartmouth Col., Hanover, NH; ²Barnard Coll Columbia Univ., New York, NY

Abstract: The serotonin 1B (5-HT1B) receptor is implicated in a number of psychiatric disorders involving dysregulation of impulsive behavior, including ADHD and conduct, gambling, and substance use disorders. Prior work has shown that the absence of 5-HT1B receptor expression in adult mice (tetO1B model) causes increased impulsivity. Our studies here examine the effects of 5-HT1B on other phenotypes, theoretically related to impulsivity, including motivation, reward sensitivity, effort, and habitual-like behavior using operant paradigms. Mice lacking 5-HT1B expression in adulthood showed increased motivation assessed
on several reinforcement schedules, including random ratios (about 50% higher than controls) and progressive ratio (about 141% higher than controls). Interestingly, over repeated testing on a progressive ratio schedule, the effect of 5-HT1B on increased responding was no longer evident. This was not likely due to differences in extinction, as there were no group differences in a classic three day extinction test. To test the effect of 5-HT1B in a high-effort choice task, we used a concurrent choice paradigm; mice could freely consume milk reward or lever press on a random ratio 20 schedule for the same reward. An absence of 5-HT1B receptor expression resulted in increased lever pressing compared to controls. Though this potentially suggests habitual behavior, these mice showed normal decreases in lever pressing following satiety-induced devaluation, as well as normal contingency degradation. During free feeding, mice lacking 5-HT1B receptors consumed more reward but not more chow, suggesting 5-HT1B may influence reward sensitivity. Current work aims to assess the effect of 5-HT1B on reward sensitivity and additional measures of habit using lithium chloride devaluation. To examine how these various behaviors correlate with impulsivity, we performed an exploratory factor analysis using behavioral measures of impulsive action (Go/No-Go), motivation (progressive ratio), low and high-effort responding (random ratio 5 and 20 schedules), feeding (free chow consumption), and reward intake (free milk consumption) from one group of 41 mice. A two-factor model described the data well, accounting for almost 70% of the variability. Impulsivity, motivation, and high-effort responding loaded strongly onto one factor while feeding and low-effort responding loaded onto another factor. Reward intake loaded significantly onto both factors. These factors seem to differentiate between highly motivated and low-effort related behaviors. Together, these data point toward a more complex role of the 5-HT1B receptor in modulating motivated behavior.

**Disclosures:**  S.S. Desrochers: None. K.M. Nautiyal: None. J. Lee: None. P.D. Balsam: None. V.M. Magalong: None.

**Poster**

**323. Reward: Neuropharmacology**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 323.14/V41

**Topic:** G.02. Motivation

**Support:** NIH Grant P20 GM103425
ANPCyT, FONCYT, PICT 2015-2594
ANPCyT, FONCYT, PICT 2016-1728

**Title:** Distinct effects of psychostimulant drugs on the regulation of class IIa HDACs in the mouse mesocorticolimbic and striatal systems
Abstract: Some psychostimulant drugs cause neuroplastic changes leading to addiction and cognitive deficits while others enhance cognition. Epigenetic mechanisms are known contributory factors to drug-induced neuroadaptations. These epigenetic changes include dysregulation of histone deacetylases (HDACs) thought to participate in maintaining aberrant transcriptional programs associated with altered cognitive functions and behaviors. HDACs are divided into zinc-dependent [class I (HDAC1,2,3,8), class IIa (HDAC4,5,7,9), class IIb (HDAC6,10), and class IV (HDAC11)] and NAD-dependent [class III (sirtuins1-7)] enzymes. Our laboratories have previously reported that psychostimulants differentially influence the acetylation status of histones 3 and 4 at promoters of HDACs in the prefrontal cortex (Addiction Biology, 2019. doi: 10.1111/adb.12737). In the present study, we injected methamphetamine (1mg/kg), modafinil (90mg/kg), caffeine (10mg/kg) or methylphenidate (10mg/kg) in C57BL/6 mice to investigate potential changes on the mRNA expression of HDACs 4, 5 and 7 in the medial prefrontal cortex (mPFC) and dorsal striatum (DS). All psychostimulants increased locomotion at these doses. Modafinil decreased HDAC7 and HDAC5 mRNAs in DS but increased their levels in mPFC. Methylphenidate increased HDAC5 mRNA in mPFC but decreased it in DS. Methamphetamine increased HDAC4 and HDAC5 mRNA levels but decreased HDAC7 expression in mPFC. Caffeine did not effect HDAC expression. Interestingly, HDAC7, which is linked to inflammation, showed modafinil-dependent effects in both brain areas. Importantly, class IIa HDACs are phosphorylated and exported from the nucleus to cytoplasm, a process that is mediated by Ca2+-dependent signals via association with CaMKII via protein-protein interactions. We thus measured CaMKII-alpha mRNA levels after drug administration. Unexpectedly, only methylphenidate induced changes in CaMKII-alpha mRNA in the mPFC, suggesting that other phosphorylation pathways may be involved in stimulant-induced regulation of class IIa induced within the mesocorticolimbic and striatal systems.


Poster

323. Reward: Neuropharmacology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 323.15/V42

Topic: G.02. Motivation
Title: Endocannabinoid regulation of cue-induced incentive motivation in mesoaccumbal circuits of male rats

Authors: *M. P. K. LEIGH¹, M. FEJA², A. N. BAINDUR¹, K. T. WAKABAYASHI³, M. NIPHAKIS⁴, B. CRAVATT⁴, C. E. BASS¹;
¹Dept. of Pharmacol. & Toxicology, Univ. at Buffalo SUNY, Buffalo, NY; ²Dept. of Pharmacology, Toxicology, and Pharm., Univ. of Vet. Med. Hannover, Hannover, Germany; ³Res. Inst. on Addictions, Univ. at Buffalo, SUNY, Buffalo, NY; ⁴The Skaggs Inst. for Chem. Biol., The Scripps Res. Inst., La Jolla, CA

Abstract: The endocannabinoids (eCBs) 2-arachidonoyl glycerol (2-AG) and anandamide (AEA) are metabolized by the enzymes monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH), respectively. Our previous work shows that enhancement of 2-AG levels with a systemically active MAGL inhibitor, MJN110, leads to a robust increase in operant responding to reward predictive incentive cues (ICs) in male rats, while rimonabant dose-dependently decreases responding. The current study sought to determine brain regions that mediate these effects by microinfusing the drugs directly into the ventral tegmental area (VTA) or nucleus accumbens (NAc) before testing. Initially, free-fed rats are trained to nosepoke during discrete 8-sec audiovisual ICs to obtain 64µl of 10% sucrose. Once stable (average responding ~85-90%) the rats are switched to a decreasing reward IC task in which the sucrose volume delivered is progressively decreased every 15 min (from 64µl, 48µl, 32µl, to 16µl). During this task variant, overall response ratio decreases (~65-70%), with choice and vigor to respond to ICs and collect reward decreasing proportionately across the volumes of sucrose delivered. Intra-VTA MJN110 attenuates the decrease in IC responding, resulting in overall enhancement in the choice (response ratio) and vigor of responding (decreased latency to nosepoke, and enter the reward cup) to ICs. However, while intra-VTA infusions of rimonabant did not alter operant responding, intra-NAc infusions decreased responding to ICs. Intracranial infusions of the FAAH inhibitor PF3845 into either the VTA or NAc did not alter responding. Our data further suggests that intra-VTA infusions of the CB2 antagonist SR-144528 enhances the vigor of the response (e.g. decreases the latency to nosepoke) without affecting response ratio. Our results suggest normal endocannabinoid tone in the NAc, but not the VTA, can be suppressed to attenuate cue-induced reward-seeking behaviors. Increasing 2-AG, but not AEA, in either the VTA or the NAc, enhances cue-induced reward-seeking. In addition, our results indicate a potential specific role of CB2 receptors in mediating the vigor of responding to the cue. Together our data demonstrates heterogeneous regulation of reward seeking by eCBs in mesoaccumbal circuits.

Poster

323. Reward: Neuropharmacology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 323.16/V43

Topic: G.02. Motivation

Title: The effects of ketamine on sexual behavior and anxiety in female rats

Authors: *S. H. MEERTS*¹, F. A. GUARRACI², C. M. F. GONZALES², M. ALI², D. LUCERO², M. BROYLES²;
¹Psychology, Carleton Col., Northfield, MN; ²Psychology, Southwestern Univ., Georgetown, TX

Abstract: Most antidepressants disrupt sexual function. Ketamine administration is a new approach for treatment resistant depression and effects on sexual function are not known. The present study characterized the effects of chronic ketamine administration on sexual motivation and anxiety in female rats. To more closely mimic the treatment of ketamine in humans, ketamine was administered once per week for a total of 4 injections over 4 consecutive weeks. Sexual motivation was measured each week using the partner preference paradigm. Briefly, female subjects were first given the opportunity to spend time in the vicinity of a sexually receptive female stimulus or a sexually vigorous male stimulus, with restricted physical contact (NO-Contact). Immediately after, subjects were permitted unrestricted access to the stimulus animals (CONTACT). Female subjects received ketamine (10.0 mg/kg) or saline 30 min prior to each partner preference test. One week after the last partner preference test, subjects received ketamine or saline again, but then were tested for anxiety on the elevated plus maze (EPM). Ketamine consistently increased time spent with the male stimulus animal across the first three tests, with no differences observed during the last test. Effects were most robust during tests that permitted physical contact. On the first test, ketamine treated subjects were less likely to leave the male after mounts and faster to return to the male after intromissions than saline treated subjects. Ketamine had no effect on anxiety as measured by the EPM, although very low levels of anxiety were observed in both groups. These data demonstrate that rather than causing sexual dysfunction, ketamine exposure enhances sexual motivation as measured by time spent with the male during the partner preference test. Sexual motivation also increased across the 4 tests in saline treated rats to eventually match the levels of sexual motivation observed in the ketamine treated rats. Ketamine is a promising new treatment for depression particularly if, as shown here, effects on sexual function are positive and not disruptive.

Poster

323. Reward: Neuropharmacology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 323.17/V44

Topic: G.02. Motivation

Support: NIH Grant DA030618

Title: An examination of the effects of selective serotonin receptor stimulation or blockade of the ventral tegmentum on progressive ratio performance in the rat

Authors: *Z. PIERCE-MESSICK, R. VACA-TRICERRI, W. E. PRATT;
Dept. of Psychology, Wake Forest Univ., Winston Salem, NC

Abstract: Serotonin signaling has long been linked to satiety and motivation. Recent work from our laboratory has shown that stimulation of some, but not all, serotonin receptor subtypes within the meso-accumbens pathway affects food intake of both pabulum and palatable diets. In these experiments, we assessed whether ventral tegmental activation or blockade of selective serotonin receptor subtypes would impact the appetitive motivation for sugar pellets as assessed in a progressive ratio (PR) task. Separate groups of rats (N = 12/group) were food-deprived and trained to lever press for sugar pellets on a PR2 schedule, where the first lever press was reinforced and further reinforcement required the rat to increase responding by two additional lever presses for each subsequent pellet delivery. Once trained, standard aseptic surgical procedures were used to implant indwelling stainless steel guide cannulas (23 gauge) bilaterally above the VTA (with the skull flat; -5.6 mm posterior and 0.6 mm lateral to bregma, 7.3 mm ventral to skull surface). Upon recovery, rats were returned to the operant chambers and retrained on the task without food deprivation. Separate groups were then tested following stimulation or blockade of ventral tegmental serotonin 1A, 1B, 2A, 2B, 2C, or 3 receptors. Individual rats in each drug group were tested on all doses of a single drug across three test days, separated by at least 48 hours. Consistent with their effect on modulating food intake, significant effects on progressive ratio performance were observed following stimulation of ventral tegmental serotonin 1A receptors with 8-OH-DPAT [0, 2, 4, 8 micrograms/side; F(2,18)= 4.04, p = .036] or stimulation of serotonin 3 receptors with mCPBG [0, 10, & 20 micrograms/side; F (3,33)= 5.61, p = .003], with high doses of both agents generally decreasing break point. Additionally, stimulation of serotonin 2C receptors with RO-60-0175 (at 0, 2, and 5 micrograms/side) reduced total lever presses [F(2,16)= 3.89, p = .04] and demonstrated a trend towards reducing break point [F(2,16)= 3.09, p = .07]. There were no significant effects from stimulating ventral tegmental serotonin 1B, 2A, or 2B receptors on break point; neither was there any impact of antagonism of any of the serotonin receptor subtypes examined here. These data provide additional evidence of
a role for serotonergic signaling in the mesolimbic pathway on motivated behavior, and suggest that individual serotonin receptor subtypes serve separable roles within the ventral tegmentum.


Poster

323. Reward: Neuropharmacology

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program#/Poster#: 323.18/V45

Topic: G.02. Motivation

Title: An examination of the impact of nucleus accumbens cocaine- and amphetamine-regulated transcript on palatable food intake in rat

Authors: *W. E. PRATT, A. CARNEY;
Dept. of Psychology, Wake Forest Univ., Winston Salem, NC

Abstract: Cocaine- and amphetamine-regulated transcript (CART) is a neuropeptide that promotes satiety through its actions within hypothalamic and other neural circuitry that regulate energy homeostasis. Interestingly, CART-containing fibers are also found in the nucleus accumbens, which is a brain region that has been implicated in promoting food intake in the presence of palatable diets. Prior work from other laboratories have examined the impact of nucleus accumbens CART on food intake of rat chow in ad libitum or food-restricted animals, but to our knowledge there has been no investigation of the impact that nucleus accumbens CART signaling might have on feeding that is promoted by a palatable diet. This project compared the effects of nucleus accumbens injections of cocaine- and amphetamine-regulated transcript on the intake of a palatable diet in non-deprived animals. Male Sprague-Dawley rats underwent surgery to implant two stainless steel, 23 gauge guide cannulas bilaterally above the anterior medial nucleus accumbens shell (+3.1 mm anterior & 1 mm lateral to bregma; 5.0 mm ventral to skull surface; nose bar set at 5 mm above aural plane). After one week of recovery, all animals were presented with a high fat/sucrose diet for daily 2-hr feeding sessions. Food intake, water intake, and locomotor measures were monitored throughout the sessions. Once the animals were acclimated to the feeding chambers and the injection procedures, each rat (N= 7) received bilateral nucleus accumbens injections of 0, 0.1 µg, or 1.0 µg of CART in 0.5 µl of saline immediately prior to being placed into the chambers. On separate treatment days, the rats also received injections of the mu-opioid receptor agonist DAMGO (at 0.025 µg/side), with or without CART co-administration. All rats received each of the six drug combinations, with each test day separated by at least 48 hours. Injections of 1.0 µg of CART reduced feeding on the palatable diet, and caused a significant reduction in body weight after 24 hours. CART also inhibited the effects of mu-opioid receptor stimulation on palatable feeding, although there was no significant interaction between mu-opioid receptor stimulation and CART administration into
the nucleus accumbens. Injections of 1.0 µg of CART into the brain 2 mm anterior to the nucleus accumbens had no impact on palatable feeding in a separate group of animals, demonstrating that the effect was site-specific. These data suggest that CART signaling in the nucleus accumbens directly impacts the motivation to consume palatable diets.

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**Disclosures:** W.E. Pratt: None. A. Carney: None.

**Poster**

**323. Reward: Neuropharmacology**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 323.19/V46

**Topic:** G.02. Motivation

**Support:** NIH Grant UL1GM118979
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CSUPERB

**Title:** Administration of Zolmitriptan, a 5-HT1B receptor agonist, on the rewarding effects of methamphetamine in male and female adolescent rats

**Authors:** R. A. CABRERA¹, B. COYNE¹, N. PENTKOWSKI², *A. R. ZAVALA¹;
¹Psychology, California State Univ., Long Beach, CA; ²Univ. of New Mexico, Albuquerque, NM

**Abstract:** Preclinical research demonstrates the involvement of 5-HT₁B receptors in the rewarding effects of cocaine, alcohol, and methamphetamine in adult rats. The role of 5-HT₁B receptors in modulating drug reward has not been previously examined in adolescent rats. In the present study, we examined whether Zolmitriptan modulates the acquisition of methamphetamine-induced conditioned place preference (CPP), a validated model of reward, in male and female adolescent rats. We hypothesized that administration of Zolmitriptan will reduce the preference for methamphetamine in both male and female rats. Beginning on postnatal day (PD) 28, male and female adolescent rats underwent a 10-day methamphetamine CPP procedure. On days 1 and 10, rats were tested for their pre-conditioning and post-conditioning place preferences, respectively, during 20-min sessions. On days 2-9, rats were conditioned for 30-min with saline or methamphetamine (0, 0.125, 0.25, 0.5, 1.0 mg/kg), on alternating days. To examine the role of 5-HT₁B receptors in the acquisition of meth-induced CPP, Zolmitriptan (0 or 10 mg/kg) was administered 15 min before the start of every drug conditioning session. Results indicate that activation of 5-HT₁B receptors decreases the effects of methamphetamine in male and female adolescent rats in a dose-dependent manner. These
findings add to a growing body of literature that suggests the 5-HT$_{1B}$ receptor system may be a useful target for developing treatments for drugs of abuse.

**Disclosures:** R.A. Cabrera: None. B. Coyne: None. N. Pentkowski: None. A.R. Zavala: None.

**Poster**

323. **Reward: Neuropharmacology**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 323.20/W1

**Topic:** G.02. Motivation

**Support:** NIH Grant UL1GM118979  
NIH Grant TL4GM118980  
NIH Grant RL5GM118978

**Title:** Early-life ketamine administration attenuates the rewarding effects of ethanol in adolescent Sprague Dawley rats

**Authors:** *D. FRANCO, J. ZAMUDIO, A. R. ZAVALA;*  
California State Univ., Long Beach, CA

**Abstract:** The prevalence of depression in juvenile populations has increased in the past decades. Recently, ketamine, a non-competitive NMDA receptor antagonist, was approved by the Food and Drug Administration for the treatment of depression. Nevertheless, the long-term effects of early ketamine administration in juvenile populations remain unclear. Moreover, since ketamine is also a drug of abuse, ketamine exposure may inadvertently alter developing brain reward systems and thereby increase the vulnerability to abuse drugs that function within these circuits (e.g., ethanol). Thus, we examined the hypothesis that early ketamine administration increases the rewarding effects of ethanol in adolescent rats using the conditioned place preference (CPP) paradigm, a validated animal model of reward. Male and female rats received daily ketamine injections from postnatal day (PD) 21-30. One day after the last ketamine injection, rats were assessed for ethanol-induced CPP on PD31. CPP was established by conditioning rats with either ethanol (0.0-2.0 g/kg) or saline on alternating days during 15-min conditioning sessions across eight days, respectively. CPP was assessed on PD 40 in a drug-free state. Saline pretreated male rats demonstrated ethanol-induced CPP with the lowest dose of ethanol (0.125 g/kg), whereas saline pretreated female rats demonstrated ethanol-induced CPP with a moderate dose of ethanol (0.5 g/kg). In contrast, ketamine pretreated male rats no longer demonstrated ethanol-induced CPP at the lowest dose (0.125 g/kg). Similarly, ketamine pretreated female rats did not exhibit ethanol-induced CPP at any dose. Contrary to our hypothesis, early-life ketamine administration attenuated the rewarding properties of ethanol in
male and female adolescent rats. These findings suggest that early-life ketamine may have therapeutic potential for the treatment of alcohol use disorder. Future studies will need to be done to examine this hypothesis further.

**Disclosures:** D. Franco: None. J. Zamudio: None. A.R. Zavala: None.

**Poster**

324. Subcortical Circuitry: Reward Seeking and Reinforcement

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 324.01/W2

**Topic:** G.02. Motivation

**Support:** R01MH110391
NARSAD 25221

**Title:** Mice optimize switching behavior through cost-benefit evaluation

**Authors:** *N. A. SCHNEIDER, J. LISMAN, H. J. PI;
Brandeis Univ., Waltham, MA

**Abstract:** Avolition is a negative symptom of schizophrenia (SZ) characterized by a lack of motivation to initiate and sustain activities as well as an impairment of effort-choice evaluation. This symptom is debilitating in everyday life and few treatments are available. We have developed a quantitative behavioral paradigm for mice as an assay for motivation and effort-related choice behavior in order to mechanistically investigate neural circuits involved and their implication in avolition.

We hypothesized that mice can make economic decisions by evaluating cost and benefit of a given task and designed a neuroeconomic choice task by combining progressive ratio (PR) and fixed ratio (FR) lever pressing. Water-restricted mice can freely choose either PR or FR associated with a large and small volume of water respectively, and collect water reward by completing the required number of presses. Initially the mice prefer the large reward, but as the PR gets higher mice switch to preferring the small reward. A novel component of our design is a 2-dimensional parameter space of lever press ratio and reward amount which confers more quantitative and sensitive behavioral readouts. Based on the results from pilot experiments, we have chosen a 2x2 parameter space where large reward volume is either 2x or 5x larger than small reward and the FR schedule is either 6 or 12 (abbreviated as 2xFR6, 2xFR12, 5xFR6 and 5xFR12). By manipulating the FR requirement and large reward volume between sessions, we have been able to successfully influence effort-related choice behavior.

Seven adult mice (5 female, 2 male) of the strain C57BL/6J were trained on the task. The parameters and the sides of FR and PR were pseudo-randomly chosen. Consistent with our hypothesis, mice’s preference of larger reward progressed as the parameters changed. The
average percentages of large reward choices during a session were 6.8 ± 0.6%, 13.9 ± 1.5%, 7.7 ± 0.6% and 20.4 ± 1.6% for 2xFR6, 2xFR12, 5xFR6 and 5xFR12 respectively. We also determined the indifference point at which the subjective values of both sides becomes equal and the mouse switches from preferring the large reward to preferring the small reward. The indifference points also progressed with different parameters (31 ± 2 for 2xFR6, 40 ± 1 for 2xFR12, 33 ± 1 for 5xFR6 and 46 ± 2 for 5xFR12). Taken together, the results suggest that our mice are able to assess the effort and reward associated with the task given changing parameters and adjust decisions accordingly. We will collect data from more WT mice and are currently evaluating Df1/+ mice, a SZ model of the 22q11.2 deletion, to determine if this SZ model shows effort-cost evaluation deficits similar to patients with avolition.

**Disclosures:** N.A. Schneider: None. J. Lisman: None. H.J. Pi: None.

**Poster**

324. Subcortical Circuitry: Reward Seeking and Reinforcement

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 324.02/W3

**Topic:** G.02. Motivation

**Support:**
DA011289
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T32 MH20068-15

**Title:** Highly opioid-sensitive inhibitory afferents from the periaqueductal gray to the ventral tegmental area drive immobility

**Authors:** *R. ST. LAURENT*¹, A. C. TSUDA², J. A. KAUER²,³;  

**Abstract:** The ventral tegmental area (VTA) encodes information about both rewarding and aversive stimuli and is required for the addictive properties of drugs of abuse. In the VTA, opioids inhibit GABAergic nerve terminals that normally regulate dopamine cell firing, increasing their firing rate. The VTA receives inhibitory input from local interneurons, but also from numerous extrinsic brain areas. However, the synaptic physiology and behavioral output for all afferent connections have not been extensively characterized. The periaqueductal gray (PAG) sends both glutamatergic and GABAergic projections to the VTA. Longitudinal columns of the PAG dictate connectivity and behavioral outputs associated with pain, stress, and threats: dorsal subdivisions are associated with the generation of both active and passive responses while
ventral PAG subdivisions (vPAG) are generally associated with coordination of passive behaviors and opioid analgesia. Despite these intriguing and seemingly interrelated properties of the PAG and VTA, the functional relevance of PAGGABA to VTA synapses is unknown. We used an optogenetic approach to investigate synaptic plasticity, opioid effects, and behavior at the PAGGABA to VTA pathway in male and female mice. We found that an increase in synaptic strength was triggered by low frequency stimulation (LFS) of PAG GABAergic synapses in the VTA. This long term potentiation was not dependent on NMDA receptor or neurotensin receptor 1 activation. PAG inhibitory synapses were profoundly depressed by opioid receptor activation. Finally, activating PAGGABA to VTA synapses increased immobility in a real time place preference procedure. Preconditioning with LFS in vivo resulted in an increase in immobility. Additionally, exposure to morphine blocked the effects of photostimulation on immobility. In conclusion, we characterized an unusual form of synaptic plasticity that occurs at inhibitory PAG to VTA synapses that are also strongly depressed by opioids. We also report that activation of this projection in vivo induces a phenotype of immobility, a behavior that is typically associated with ventral portions of the PAG.


Poster

324. Subcortical Circuitry: Reward Seeking and Reinforcement

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 324.03/W4

Topic: G.02. Motivation

Support: 1R01DA045639-01A1

Title: Ventral pallidum GABAergic projections to the VTA support reinforcement that is attenuated by CB1 receptor blockade

Authors: *K. Z. PETERS, K. R. CRUZ, D. P. COVEY, J. F. CHEER; Anat. and Neurobio., Univ. of Maryland, Baltimore, MD

Abstract: Intra-cranial self-stimulation (ICSS) is reinforcing in rodents and involves activation of the mesolimbic pathway. Dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) are activated in response to natural rewards and drugs of abuse. Within the VTA, endocannabinoids and CB1 receptors modulate patterned dopamine release via disinhibition of presynaptic input. However, the afferent structures that are subject to CB1 receptor modulation and how this modulation influences reward and reinforcement is not clear. The VTA receives GABAergic inputs from the ventral pallidum (VP) that synapse onto dopamine and local GABA neurons. Here we report that GABAergic VP neurons projecting monosynaptically to VTA dopaminergic neurons express CB1 receptors on their terminals and
may be modulated by endocannabinoids. We assessed whether activation of GABAergic VP neurons projecting to the VTA supports ICSS and to what extent this is regulated by endocannabinoids. VGAT cre mice were injected with channelrhodopsin (ChR2) into the VP and implanted with optical fibers in the VTA. GABAergic terminals within the VTA originating from the VP were then stimulated using an ICSS protocol. We also assessed real-time place preference (RTPP) for optical stimulation of these terminals to further establish whether activation was reinforcing. We found that optogenetic stimulation of VP GABA terminals in the VTA supports ICSS, as stimulation was robustly reinforcing. This was corroborated by a significant place preference for the optically stimulated side in RTPP in all mice. In addition, ICSS was greatly reduced by the CB1 receptor antagonist rimonabant in a dose-dependent fashion. Thus, we demonstrate that optical stimulation of VP GABAergic terminals in the VTA is reinforcing and systemic CB1 antagonism attenuates ICSS supported by this projection. Future experiments will use intra-cerebral injections of rimonabant and cell type and pathway-specific genetic deletion of CB1 receptors to assess the contribution of endocannabinoids and CB1 receptors within the VTA specifically to these effects. We will also assess the downstream consequences of this pathway activation for dopamine release in the NAc using in vivo fast-scan cyclic voltammetry.


Poster

324. Subcortical Circuitry: Reward Seeking and Reinforcement

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 324.04/W5

Topic: G.02. Motivation

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Creative-Pioneering Researchers Program of SNU

Title: A hypothalamic circuit for behavioral thermoregulation

Authors: *S. JUNG¹, H.-G. RYU¹, M. LEE¹, D.-Y. KIM¹, G. HEO¹, M. KIM¹, S.-R. KIM¹, J. PARK², H.-E. PARK¹, D.-J. KOO¹, S.-Y. KIM¹;
¹Seoul Natl. Univ., Seoul, Korea, Republic of; ²Inst. of Mol. Biol. and Genet., Seoul, Korea, Republic of

Abstract: Maintaining the internal temperature within a narrow range optimal for cellular and molecular functions is critical for animals to survive. In face of a thermal challenge, the brain prompts an array of autonomic and behavioral responses to defend thermal homeostasis. Unlike
stereotyped autonomic responses, behavioral responses are flexible goal-directed behaviors that manifest in many forms, such as seeking warmth, building nests, or turning on heaters. While the circuit mechanisms of autonomic thermoregulation have been extensively studied, their counterpart in behavioral thermoregulation remains poorly understood. Here we identify a subpopulation of neurons in the lateral hypothalamus (LH), a region essential for diverse motivated behaviors, as a critical circuit node for behavioral thermoregulation. Chemogenetic inhibition of these neurons confirmed their role in diverse thermoregulatory behaviors. By recording in vivo calcium dynamics, we show LH neurons bidirectionally encode the motivational value of thermal stimuli. Furthermore, anatomical tracing revealed that the LH subpopulation receive projections from thermosensory relay structures. Our data establish the LH subpopulation as a key circuit node in thermoregulatory behaviors. Further investigations will uncover the neural mechanisms by which sensory information is integrated and transformed into the motivational value and trigger thermoregulatory behaviors.


Poster  
324. Subcortical Circuitry: Reward Seeking and Reinforcement  
Location: Hall A  
Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM  
Program #: Poster #: 324.05/W6  
Topic: G.02. Motivation  
Support:  
RO1 DA022340  
RO1 DA042595  
Title: Cocaine-induced increases in motivation require CB1 receptor activation in the ventral tegmental area  
Authors: *E. J. BEEBE, V. M. AYVAZIAN, J. M. WENZEL, N. E. ZLEBNIK, J. F. CHEER; Anat. and Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD  
Abstract: A large body of evidence supports an integral role for mesolimbic dopamine in motivation. Indeed, drugs that increase dopaminergic transmission, such as cocaine, increase motivation for a number of reinforcers as measured by elevated breakpoints under a progressive ratio (PR) schedule of reinforcement. Conversely, dopamine receptor antagonism decreases PR breakpoints. Our laboratory and others have shown that phasic mesolimbic dopamine is under the control of midbrain endocannabinoids (eCBs). Relatedly, antagonism of cannabinoid type-1 (CB1) receptors decreases PR breakpoints for food reinforcers, likely through downstream effects on dopaminergic function. However, it remains unclear if drugs that increase dopamine effectively increase motivation through eCB-dependent processes. To test this, we trained male
and female rats on a PR task for a food reinforcer. Once breakpoints stabilized, each rat underwent a series of test days in which they were pretreated with either cocaine (10mg/kg or 15mg/kg, IP), the CB1 receptor antagonist rimonabant (1 or 3mg/kg, IP), or both cocaine and rimonabant. Each test session was interleaved with non-drug baseline sessions until breakpoints re-stabilized. As expected, acute cocaine increased breakpoints, while rimonabant dose-dependently decreased breakpoints. Importantly, rimonabant, at a dose that on its own did not decrease breakpoints, blocked the ability of cocaine administration to increase breakpoints. These data suggest that cocaine-induced gains in motivation are dependent upon eCB signaling. However, because rimonabant was administered systemically, these effects could not be localized. Given the essential role of dopamine in motivated behavior, we hypothesized that rimonabant acts within the ventral tegmental area (VTA) to block phasic activation of DA cells and attenuate cocaine-potentiated motivation. Therefore, we implanted bilateral cannula in the VTA for rimonabant administration via intracranial (IC) injections. As expected, cocaine (IP) on its own increased breakpoints, and rimonabant (IC) dose-dependently decreased breakpoints. Crucially, rimonabant (IC), at a dose on its own that did not decrease breakpoints, when administered into the VTA, blocked the ability of systemic cocaine administration to increase breakpoints. These data suggest that cocaine-induced gains in motivation are dependent upon eCB signaling in the VTA.


Poster

324. Subcortical Circuitry: Reward Seeking and Reinforcement

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 324.06/W7

Topic: G.02. Motivation

Support: NIH Grant R00AA025384

Title: Ventral pallidum output pathways in cue-elicited reward seeking, reinforcement and choice

Authors: *C. A. CAYTON, D. M. FRAZEE, I. D. LIN, J. M. RICHARD; Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: The ventral pallidum (VP) is critical for both reward processing and responses to reward-related cues. We previously showed that excitations in many VP neurons are predictive of the latency and likelihood of cue-elicited reward seeking behavior, and that disruption of VP activity at cue onset disrupts reward-seeking. VP activity has also been shown to track the value of rewards during consumption, and to be reinforcing in the absence of other rewards. Here, we
aimed to determine whether these functions are assigned to distinct output pathways, focusing first on VP neurons projecting to the ventral tegmental area (VTA) versus the mediodorsal thalamus (mdThal). We used an intersectional virus strategy to express channelrhodopsin in VP-VTA or VP-mdThal neurons in rats and tested if stimulation of these populations was sufficient to invigorate cue-elicited reward-seeking behavior and alter reward value. Rats were trained with two auditory cues: a discriminative stimulus (DS) and a neutral stimulus (NS). Entering the reward port during the DS results in liquid sucrose delivery (10%), whereas port entries during the NS have no programmed consequences. After learning the task, rats received blue light stimulation in VP (20 Hz, 1s or 10s) during 50% of cue presentations. Stimulation of VP-VTA or VP-mdThal neurons during the cues failed to alter reward-seeking likelihood or latency. This suggests that activation of these neurons is not sufficient to invigorate cue-elicited reward seeking. To determine whether stimulation of these projection populations is reinforcing, we gave rats the opportunity to nose poke for stimulation (20 Hz, 1s). We found that, as expected, VP-VTA stimulation supported operant responding. In contrast, VP-mdThal stimulation did not. Finally, we tested whether stimulation of these projection populations would potentiate the value of a primary reward in a choice test. Presses on either of two levers resulted in delivery of equivalent liquid sucrose reward, but presses on one of the levers results in stimulation during sucrose consumption (20 Hz stimulation during continuous licking up to 8s). Rats receiving stimulation of VP-VTA neurons, but not VP-mdThal neurons, preferred the lever that produced sucrose paired with stimulation. Yet, when rats were tested in the absence of sucrose delivery they did not maintain responding, despite the continued availability of stimulation. Together these results suggest that while VP-VTA stimulation is capable of acting as a primary reinforcer and potentiating the value of other rewards, stimulation of these neurons is not sufficient to potentiate cue-elicited reward seeking.


Poster

324. Subcortical Circuitry: Reward Seeking and Reinforcement

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 324.07/W8

Topic: G.02. Motivation

Support: The Whitehall Foundation (2014-05-77) NIH (R01DA044199) NSF (IOS1557987)

Title: Circuit directionality for motivation: Lateral accumbens-pallidum, but not pallidum-accumbens, connections regulate motivational attraction to reward cues
**Authors:** *E. SMEDLEY*, A. DILEO, K. S. SMITH;

1Psychological and Brain Sci., Dartmouth Col., Hanover, NH; 2tufts Sackler Sch. of Grad. Biomed. Sci., Boston, MA

**Abstract:** Sign-tracking behavior, in which animals interact with a cue that predicts reward, provides an example of how incentive salience can be attributed to cues and elicit motivation. The nucleus accumbens (NAc) and ventral pallidum (VP) are two regions involved in cue-driven motivation. The VP, and NAc subregions including the medial shell and core, are critical for sign-tracking. Further, connections between the medial shell and VP are known to participate in sign-tracking and other motivated behaviors. The NAc lateral shell (NAcLSh) is a distinct and understudied subdivision of the NAc, and its contribution to the process by which reward cues acquire value remains unclear. The NAcLSh has been implicated in reward-directed behavior, and has reciprocal connections with the VP, suggesting that NAcLSh and VP interactions could be important mechanisms for incentive salience. Here, we use DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) and an intersectional Cre-dependent viral delivery strategy, in adult male Long Evans rats, to produce a biased inhibition of NAcLSh neurons projecting to the VP (NAcLSh to VP, N=8), and vice versa (VP to NAcLSh, N=10). Each of the pathway inhibition manipulation groups were compared to their own respective controls given a control virus lacking the DREADD receptor and CNO delivery. We find that disruption of connections from NAcLSh to VP reduces sign-tracking behavior while not affecting primary motivation to consume food rewards. In contrast, VP to NAcLSh disruption affected neither sign-tracking nor reward consumption, but did produce a greater shift in animals’ behavior more towards the reward source when it was available. These findings indicate that the NAcLSh to VP pathway plays an important role in guiding animals towards reward cues, while VP to NAcLSh back-projections may not and may instead bias motivated behavior towards rewards.

**Disclosures:** E. Smedley: None. A. DiLeo: None. K.S. Smith: None.

**Poster**

**324. Subcortical Circuitry: Reward Seeking and Reinforcement**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 324.08/W9

**Topic:** G.02. Motivation

**Support:** NIH R01MH106500
US-Israel BSF 2015239

**Title:** Translatome analysis of ventral pallidum projection neuron subtypes

**Authors:** *H. NAM*, M. ENGELN, R. CHANDRA, M. LOBO;
Anat. and Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD
Abstract: The ventral pallidum (VP) is a structure within the basal ganglia that is classically known to participate in the canonical indirect pathway. However, its targets are diverse, as the VP also sends dense projections to regions outside the basal ganglia, such as the lateral hypothalamus (LH), the mediodorsal nucleus of the thalamus (MDT) and its neighboring region of the lateral habenula (LHb). Therefore, the VP is not just an internal nucleus of the basal ganglia but also an important output structure of the system. As expected from its complex connectivity, the VP is important in many behaviors, such as the processing and execution of motivated behaviors. Despite its position within the basal ganglia and its involvement in many behavioral processes, the VP remains an understudied brain region, with limited knowledge about the cellular-level characteristics. In this study, we sought to provide knowledge into the circuitry and cell subtypes of the VP by identifying translatome profiles of discreet VP projection neurons. We used the RiboTag mice, which have a Cre-inducible HA-tagged ribosomal protein that allows for isolation of ribosome-associated mRNA. We injected a retrograde AAV5-Cre virus into the VP projection targets, the ventral tegmental area (VTA), the LH, the LHb, and the MDT. Additionally, added to our analyses was NPAS1-Cre crossed with RiboTag mouse, which identifies VP neurons that sends projections to the nucleus accumbens, VTA, and LHb. As a control, we used animals that were injected with Cre directly into the VP. RiboTag-isolated mRNAs were used for RNA-seq and clustering analysis confirmed that samples clustered well within its VP projection neuron subtype relative to controls. Out of about 16,000 genes analyzed, we found 1,628 genes to have at least 2-fold change in either direction in any of the VP neuron subtypes. 32 genes were commonly changed in all groups, and 1,036 genes were uniquely enriched or downregulated in each group. We observed changes in specific GABA, glutamate, and cholinergic receptor subunits, as well as neuronal transporters in subsets of VP neurons, which could serve as potential markers or be related to specific functions of the projections. We also saw upregulation of transcription factors in specific VP neuron populations, which may regulate transcripts that give rise to their neuron subtype identity. Results from our study will provide a comprehensive genetic signature for each VP neuron subpopulation which can have important ramifications to interpret studies of behavioral functions of VP and its functional role in disease.

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Poster

324. Subcortical Circuitry: Reward Seeking and Reinforcement

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 324.09/W10

Topic: G.02. Motivation

Support: NIDA R01 DA042206
NSF DGE-1110007
Title: Optogenetic stimulation of the lateral preoptic area drives apparent paradoxical reward and aversion

Authors: *A. G. GORDON¹, N. MITTAL², C. DUVAUCHELLE², M. MARINELLI¹;
¹Neurosci., ²Pharm., Univ. of Texas at Austin, Austin, TX

Abstract: The lateral preoptic area (LPO) is a hypothalamic brain region interconnected with limbic brain regions critical for reward and aversion. Despite previous research into the LPO, the emotional valence of LPO activity remains to be understood. We conducted a series of experiments to determine the emotional valence of LPO neuron stimulation using optogenetics and neuronal recording using fiber photometry in awake-behaving male Sprague-Dawley rats. In our first set of experiments, we found that optogenetic stimulation of the LPO (40 Hz, 1s, 15mW) supported intracranial self-stimulation (ICSS) on a fixed-ratio 1 schedule, and that optogenetic stimulation of the LPO-VTA projection also supported ICSS, but to a lesser extent. Furthermore, we found that optogenetic stimulation of the LPO supported ICSS in a progressive ratio test in which rats responded for stimulation periods up to 5 minutes in duration. The highest break-point was observed for stimulation durations of 10s and 60s, compared with shorter (1s) or longer (5 min) durations. In our second set of experiments, we unexpectedly found that optogenetic stimulation of the LPO and the LPO-VTA projection drove real-time place aversion (RTPA) in a real-time place testing procedure (RTPT) in the same rats that demonstrated ICSS. During the RTPA procedure, entry into the laser-paired side of the chamber was coupled with an increase in 50kHz ultrasonic vocalizations, which are typically associated with positive affect. To determine if rats that avoided the optically-paired side of the chamber were still willing to enter that side of the chamber in the face of adversity, we electrified the floor of the laser-paired side of the chamber (0.05-0.15 mA). We found that rats receiving LPO stimulation upon entering the electrified side of the chamber persisted entering the side of the chamber, whereas control rats stopped entering. This indicates that rats found LPO-stimulation rewarding enough to overcome adversity. Finally, we conducted an experiment to record the LPO during positive and negative Pavlovian conditioning. Preliminary results from these experiments indicate that LPO activity correlates with positive and negative unconditioned stimuli. Altogether, these results indicate that activation of the LPO is rewarding, despite producing an apparent real-time place-aversion; these results also challenge the assumption that time spent on each side in the RTPT procedure indicates reward or aversion.


Poster

324. Subcortical Circuitry: Reward Seeking and Reinforcement

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 324.10/W11
**Topic:** G.02. Motivation

**Title:** Lateral habenula projecting hypothalamic neurons govern food preference in a leptin dependent manner

**Authors:** *R. M. O. O'CONNOR¹, M. V. MICIONI DI BONAVENTURA², M. ISHIKAWA³, K. DEVARAKONDA⁴, V. MATHIS⁵, P. J. KENNY⁶;
¹Icahn Sch. of Medicine, Mount Sinai, New York, NY; ²Univ. of Camerino, Sch. of Pharmacy, Pharmacol. Unit, Camerino, Italy; ³Dept. of Pharmacol. & Systems Therapeut., ⁴Dept. of Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; ⁵Icahn Sch. of Med. at Mt Sinai, New York, NY; ⁶Dept. of Pharmacol. and Systems Therapeut., ICAHN Sch. of Med. at Mount Sinai, New York, NY

**Abstract:** Obesity rates are on the rise worldwide, resulting in a growing threat to public health. Pharmacotherapies that safely reduce body weight in obesity remain elusive, partially due to our incomplete knowledge of the complex neuronal mechanisms that control food choice (palatable high-calorie versus less palatable low-calorie food). The lateral hypothalamus (LH) is considered a critical node in the maintenance of energy homeostasis and prominently expresses the receptor for leptin, an adipocyte derived anorectic hormone. The development of obesity in rats is associated with deficits in LH sensitivity to rewarding stimuli, a switch in preference towards palatable calorically dense food items yet a seemingly paradoxical deficit in food-related motivation measured by the willingness of obese rats to deploy instrumental responding to receive food rewards. A major output of the LH terminates in the lateral habenula (LHb) which has been described as a “preference center”. We tested the hypothesis that leptin signaling on LHb innervating LH neurons plays an important role in food-related motivation. We found diphtheria toxin induced ablation of this pathway decreased levels of instrumental responding, and shifted preference towards palatable calorically dense food, phenotypes conspicuously similar to that seen in rats with diet-induced obesity. Interestingly, inhibition of leptin activity in LHb projecting LH neurons of obese rodents led to similar increases in preference for palatable food and rejection of standard chow. Furthermore, electrophysiological recordings revealed obesity induced disruptions to leptin mediated glutamatergic and GABAergic LH innervation of the LHb suggesting the involvement of leptin in communication between the LH and LHb. Based on these findings, we hypothesize that deficits in leptin mediated communication between the LH and LHb may emerge during weight gain contributing to obesity-associated behavioral abnormalities.

Poster

324. Subcortical Circuitry: Reward Seeking and Reinforcement

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 324.11/W12

Topic: G.02. Motivation

Title: Lateral habenula contributions to dynamic effort-based decision-making using a plus maze motivational task

Authors: E. N. BRYANT, J. P. SEVIGNY, *P. M. BAKER;
Psychology, Seattle Pacific Univ., Seattle, WA

Abstract: The lateral habenula (LHb) has been studied regarding memory and motivation especially in association with depression. Thought to be a modulator of motivation within the LHb-rostromedial tegmental nucleus circuit, the LHb likely affects an animal’s ability to make optimal survival decisions especially during dynamic tasks. Therefore the LHb may play a role in effort-based decision making by changing the way motivation is integrated into choices between high and low reward. To determine the role of the LHb in effort-based decisions, an ethologically relevant task was utilized to test choice patterns using male and female Sprague Dawley rats. The task measures the subjects’ willingness to climb up a ramp in order to receive a high reward (two 45mg sucrose pellets) when a low reward (one 45mg sucrose pellet), low effort option is available. Two arms of a plus maze were designated start arms with opposing arms holding rewards. Subjects encountered a series of effort-based stages presented in the form of progressively taller ramps (short = 15cm, medium = 20cm and tall = 30cm). They advanced to the next effort stage once two consecutive days of high arm preference above 80% were shown. The task proceeded using the highest ramp rats were willing to traverse. We conducted a within subjects inactivation experiment using an ABBA design with A being a saline treatment and B representing inactivation of the LHb by administration of the GABA agonist, muscimol (50ng/0.2µL) via cannula injection. After completing all trials with the ramp, rats ran two control trials, one with no ramp and drug treatment and another with no treatment and no ramp, to verify subjects’ ability to discriminate between low and high rewards without an effort component. Preliminary results showed that prior to surgery the average preference for high reward on the 30cm ramp was 71.8 ± 2.7%. This indicates that the reward to effort ratio was balanced between required effort and reward salience, demonstrating the validity of this task design to examine the LHb in effort-based decision making. Preliminary results suggest disruption of willingness to exert effort following LHb inactivation. These results support LHb involvement of weighing effort in choice behavior.

Disclosures: E.N. Bryant: None. J.P. Sevigny: None. P.M. Baker: None.
Novel population of nucleus accumbens direct-pathway MSNs express stress-associated neuropeptide corticotropin releasing hormone

Authors: *J. J. STOLLEY*¹, V. A. ALVAREZ², J. C. LEMOS¹;
¹Neurosci., Univ. of Minnesota, Minneapolis, MN; ²Lab. on Neurobio. of Compulsive Behaviors, Natl. Inst. on Alcohol Abuse and Alcoholism, Bethesda, MD

Abstract: The neuropeptide corticotropin-releasing hormone (CRH) has been shown to increase dopamine and acetylcholine transmission in the nucleus accumbens (NAc). These effects are accompanied by facilitation of appetitive behaviors. The endogenous source(s) of CRH to the NAc remain unclear. While recent evidence points to CRH containing afferents to the NAc from several limbic regions, there is also an uncharacterized population of CRH-expressing cells that resides in ventral NAc. Using fluorescent in situ hybridization, we characterized the cellular identity of neurons positive for Crh mRNA within the NAc of male mice using markers for both medium spiny projection neurons (Drd1, Pdyn, Drd2, Penk) and distinct interneuron classes (Chat, Npy, Th, Sst, Nos1, Pthlh). After analyzing the entire rostro-caudal and medial-lateral expanse of the NAc, we found that CRH-expressing cells make up 2.4% of all cells in the NAc and that there is a distinct rostro-caudal gradient, with greater numbers in the caudal aspect of the NAc. Interestingly, the results indicate that CRH-expressing cells in the NAc are predominantly direct pathway medium spiny projection neurons (dMSNs) (86.1% Drd1+, n=2338 Crh+ cells, 4 mice; 75.1% Pdyn+, n=1493 Crh+ cells, 4 mice). A smaller percentage were either Drd1/Drd2 co-expressors or only positive for Drd2 indicating that they were indirect pathway medium spiny projection neurons (iMSNs) (25.2% Drd2+, n=2234 Crh+ cells, 3 mice; 33.5% Penk, n=1493 Crh+ cells, 4 mice). Our findings suggest that interneurons account for little of NAc CRH expression (% of Crh+ cells: 0.13% Chat+, 0.10% Npy+, 0.93% Th+, 1.22% Sst+, 1.79% Nos1+) with the exception of Pthlh+ interneurons (10.3% of Crh+ cells). This work adds insight into the conditions in which endogenous CRH is released to regulate NAc circuitry. Next steps underway include examining the role these cells play in motivation and exploration using behavioral assays with conditional knockout mice.

Disclosures: J.J. Stolley: None. V.A. Alvarez: None. J.C. Lemos: None.
Optogenetic stimulation of nucleus accumbens circuits elicits behavioral and synaptic plasticity

**Authors:** *A. Taylor*¹, K. M. Manz¹, J. C. Becker², B. A. Grueter¹,²;

**Abstract:** The nucleus accumbens (NAc) is important for integrating sensory information and coordinating motor output. The main neuronal cell types in the NAc are GABAergic medium spiny neurons (MSNs). Stimulation of either dopamine 1 (D1) receptor expressing or dopamine 2 (D2) receptor expressing MSNs result in activation of distinct pathways that differentially modulate reward behavior. In addition to MSNs, GABAergic interneurons (INs), although sparse in number, critically regulate timing of principle cell firing. In particular, parvalbumin expressing fast-spiking INs (PV-INs) regulate MSN firing via a feedforward mechanism. Action potential generation in both NAc MSNs and PV-INs is dependent upon excitatory drive from cortical, limbic and thalamic inputs. In order to understand how NAc PV-IN to MSN circuits function and this circuit's contribution to pathological conditions such as drug addiction, it is critical to understand the nature of excitatory and inhibitory control exerted onto MSNs. In this study, we utilized optogenetics to determine if NAc PV-INs are necessary and/or sufficient to induce associative learning. Specifically we investigate PFC to MSN and PV-IN to MSN synaptic and behavioral plasticity. We find that 1Hz and 30Hz stimulation of PFC terminals in the NAc induced long term depression (LTD) of optically evoked excitatory post synaptic currents. 1Hz and 30Hz stimulation of PV-INs caused LTD of optically evoked inhibitory post synaptic currents. In vivo, 1Hz stimulation of PV-INs caused place aversion, whereas 30Hz stimulation induced place preference. Investigation of the mechanisms underlying this plasticity will contribute to our understanding of how glutamatergic inputs and PV-INs shape NAc circuit function in both health and disease.

**Disclosures:** A. Taylor: None. K.M. Manz: None. J.C. Becker: None. B.A. Grueter: None.
**Poster**

324. Subcortical Circuitry: Reward Seeking and Reinforcement

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 324.14/W15

**Topic:** G.02. Motivation

**Support:** NIH R01 DA041808
University of Minnesota MnDRIVE Initiative

**Title:** Sex differences in optogenetic self-stimulation of prefrontal and hippocampal inputs to the nucleus accumbens shell

**Authors:** *E. B. LARSON, N. M. LOPRESTI, A. D. CHAPP, K. A. SILVIS, M. J. THOMAS, P. G. MERMELESTEIN; Neurosci., Univ. of Minnesota, Minneapolis, MN

**Abstract:** Sex differences in addictive behavior are well-documented in humans and animals, yet the neural circuitry mediating these differences remains unclear. Given that plasticity at excitatory inputs to the nucleus accumbens shell (NAcSh) has been implicated in addictive behavior in male rodents, we hypothesized that males and females may exhibit different sensitivities to reward-related excitatory transmission in this region. To investigate this, we utilized a novel context-based open-field spatial task to examine the sensitivity of drug-naïve male and female mice to self-administer optogenetic stimulation of glutamatergic inputs to the NAcSh. Male and female mice were injected with AAV2-CamKIIα-ChR2-eYFP into either the medial prefrontal cortex (mPFC) or ventral hippocampus (vHPC) to express the blue-light sensitive channelrhodopsin2 (ChR2) selectively in glutamatergic neurons. An optical fiber was implanted above mPFC or vHPC projection terminals in the NAcSh to selectively activate mPFC-NAcSh or vHPC-NAcSh circuits during behavioral testing. For this, mice were first habituated to the self-stimulation arena, which consisted of four spatially-restricted and contextually-distinct corner zones. Next, one of the corner zones was made active and the propensity to self-administer 10 Hz optogenetic stimulation was assessed (acquisition). The location of the active stimulation zone was then changed to further assess behavioral flexibility (reversal). We found that females were more likely to acquire mPFC-NAcSh self-stimulation behavior compared to males, while males were more sensitive to vHPC-NAcSh self-stimulation than females. For vHPC-NAcSh inputs, female also showed impaired reversal at higher frequencies (20 Hz), despite being able to acquire the task. Interestingly, although males and females exhibited different sensitivities to mPFC-NAcSh and vHPC-NAcSh stimulation, the reward-directed strategies exhibited across these two inputs was conserved. Together, these data suggest that sex differences in input-specific transmission to the NAcSh may be an important factor influencing reward-related behavior, and also implicates the mPFC-NAcSh and vHPC-
NAcSh circuits as potential sites of sex-dependent plasticity relevant for drug addiction and motivated behavior.


Poster

324. Subcortical Circuitry: Reward Seeking and Reinforcement

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: 324.15/W16

Topic: G.02. Motivation

Support: NIH grant DA042595
NIH grant DA043967

Title: Accumbal endocannabinoids attenuate impulsive choice

Authors: *J. F. CHEER1, J. M. WENZEL2, N. E. ZLEBNIK1;
2Anat. & Neurobio., 1Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Impulsive choice is characterized by preference for smaller immediate rewards over larger delayed rewards, and impulsive choice behavior is a determining factor in the development and persistence of drug abuse. However, the neural substrates of impulsive choice are still largely unknown. Emerging evidence from human and animal studies suggests frontal cortical regions exert influence over ventral striatal reward processing areas during decision-making in impulsive choice or delay-discounting tasks. Striatal endocannabinoids are powerful modulators of both cortical efferents and local circuitry, but their role in impulsive decision-making is yet to be examined. We used chemogenetic tools to selectively and reversibly inhibit corticostriatal projections and employed targeted intra-NAc pharmacology to interrogate the endocannabinoid system during the performance of a delay-discounting task. Inactivation of the PFC-NAc projection elicited a robust increase in impulsive choice, demonstrating a critical role for PFC afferents to the NAc during controlled choice behavior. Furthermore, blocking accumbal CB1 receptor signaling potentiated impulsive choice, whereas enhancing endocannabinoid signaling attenuated impulsive choice in a CB1 receptor-dependent manner. Together, these findings highlight new ways in which ventral striatal processing contributes to impulsive decision-making. Results such as these may have important implications for the pathophysiology and treatment of drug addiction and impulse control disorders such as attention deficit/hyperactivity disorder.

Title: Projections from oxytocin-releasing cells in paraventricular nucleus of hypothalamus to nucleus accumbens in male and female rats

Authors: *L. R. HAMMERSLAG¹, E. D. DENEHY¹, K. E. SAATMAN², M. T. BARDO¹;
¹Psychology, Univ. of Kentucky, Lexington, KY; ²Spinal Cord & Brain Injury Res. Cntr, Univ. Kentucky, Lexington, KY

Abstract: Purpose: Recent preclinical studies have highlighted the potential for the neuropeptide oxytocin (OT) to reduce drug seeking and drug-induced activation of the nucleus accumbens (NAc) through activation of OT receptors in NAc (Cox et al., 2017, Biol. Psychiatry, 81(11):949–958). Although magnocellular OT-releasing cells (magnOT), located in the paraventricular nucleus of the hypothalamus (PVN), are known to project to forebrain structures, it is unclear if there are direct PVN to NAc projections that may mediate these effects. It is also unclear if parvocellular cells (parvOT), which are thought to innervate primarily the spinal cord and brain stem, also project to NAc. In the present study, we used the retrograde tracer cholera toxin b (CTb) to identify and characterize projections from the PVN to the NAc in virgin male and female rats.

Method: Adult male (n=4) and female (n=4) Sprague-Dawley rats underwent surgery for infusion of CTb into NAc. One week later, they were perfused with 4% paraformaldehyde and brains were processed using fluorescent immunohistochemistry to identify CTb⁺ cells in NAc and PVN, as well as OT⁺ cells in the PVN. Images were captured at 20x and OT⁺ cells were classified as either magnOT (soma semiaxis > 13 µm) or parvOT (soma semiaxis < 10 µm), then further classified as CTb⁺ or CTb⁻. Whole slice images were captured to verify the accuracy of NAc CTb injections.

Results: We identified NAc projections among both the magnOT and parvOT OT-releasing cells in the PVN in both males and females; no sex differences were noted. These projections were more common among magnOT cells than among parvOT cells. There were also a large number of CTb⁺ cells in the PVN that were not OT immunopositive, indicating that other cells within the PVN also send projections to NAc.

Discussion: Our results demonstrate direct projections from OT-releasing cells in PVN to NAc in virgin male and female rats, consistent with the results of a previous study that had been
conducted in lactating females (Knobloch et al., 2012, Neuron, 73(3):553-566). Interestingly, we identified both magnOT and parvOT projections to NAc, adding to the evidence that parvOT cells, once thought to innervate primarily the spinal cord and brainstem, also project sparsely to forebrain. We are currently testing the hypothesis that OT may modulate drug seeking and drug-induced NAc through these monosynaptic connections from PVN.

**Disclosures:** L.R. Hammerslag: None. E.D. Denehy: None. K.E. Saatman: None. M.T. Bardo: None.

**Poster**

324. Subcortical Circuitry: Reward Seeking and Reinforcement

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 324.17/W18

**Topic:** G.02. Motivation

**Title:** Effect of an aversive stimulus on sexual motivation depends on the susceptibility to the induction of the motivated state

**Authors:** *C. SOTELO-TAPIA*¹, A. C. MEDINA², M. HERNÁNDEZ-GONZÁLEZ¹, R. M. HIDALGO-AGUIRRE¹, M. GUEVARA¹;
¹Inst. de Neurociencias, Univ. de Guadalajara, Guadalajara, Mexico; ²Neurobiologia Conductual y Cognitiva. Inst. de Neurobiologia-UNAM, Queretaro, Mexico

**Abstract:** Sexual motivation results from the adequate processing of sexually-relevant stimuli that trigger approach behaviors to a potential partner. The efficacy with which sexual stimuli induce this motivational state depends on several factors, including the individual’s physiological condition, which can be affected by an aversive stimulus such as an electrical shock applied to the foot. The changes produced by aversive stimuli can reduce or suppress behaviors, depending on their intensity and duration. Given that sexual motivation is a physiological state of activation that drives the individual to seek sexual interaction, and that it can be affected by aversive stimuli, the aim of this study was to determine the effect of electric foot shocks on sexual motivation in male rats. To this end, 33 male rats were used, divided into two groups, a control group (CG, n = 11) that did not receive shocks, and an experimental group (EG, n = 22) that received electric foot shocks *en route* to a compartment holding a receptive female. The parameters of sexual motivation (mount, intromission and ejaculation latencies) were recorded 48 hours before, immediately after, and then 48 hours after, receiving an electric shock. Only 11 male rats in EG copulated with the female 48 hours after receiving the shocks, and they took longer to enter the compartment with the female. Also, they had longer mount and intromission latencies immediately after receiving the aversive stimulus. The rats in EG that did not enter the compartment with the female rat after receiving the shocks had a high ejaculation latency 48 hours after receiving the aversive stimulus. These results show that the effect of an aversive stimulus
on sexual motivation depends on the time elapsed from the moment that the stimulus was received, and on the individual's susceptibility to the induction of sexual motivation. It is likely that this effect on sexual motivation depends on differences in the functionality of brain structures, and the levels of hormones related to sexual motivation and the processing of aversive stimuli.


Poster

324. Subcortical Circuitry: Reward Seeking and Reinforcement

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 324.18/W19

Topic: G.02. Motivation

Support: NIH DP2MH113095

Title: Evidence for distinct populations of D1 spiny projection neuron in NHP striatum


Abstract: The striatum is the major input nucleus of the basal ganglia and is critical for learning, cognition, movement, reward, and emotional responses. The striatum can be anatomically and functionally divided into three subregions: the caudate, putamen, and ventral striatum. The caudate forms circuits with association and oculomotor cortical regions. The putamen is interconnected with sensorimotor cortical regions. The ventral striatum, which includes the Nucleus Accumbens (NAcc), is integrated within limbic circuits and processes information about rewards and emotions. Multiple neurodegenerative and mental health disorders, such as Parkinson’s disease, Autism spectrum disorder, and addiction, are characterized by abnormal function in neural circuits that involve specific striatal subregions. To identify molecular access points to enable cell type and circuit specific investigation and therapeutic strategies, it is critical to understand how gene expression in different regions of the striatum regulates structure and function. Here, we set out to investigate the transcriptional architecture of the striatum in non-human primates (NHPs). We dissected caudate, putamen, and NAcc regions from 5mm thick coronal sections, immediately rostral to the anterior commissure, from two Rhesus monkeys. We isolated nuclei from the three striatal subregions and performed single nucleus RNASeq. Bimodality constrained hierarchical clustering of the resulting 80,000 RNASeq profiles identified D1 and D2 spiny projection neurons (SPNs), striatal interneurons, and marker genes
for each neuron type. Strikingly, the hierarchical clustering algorithm split D1 SPNs into two distinct cell-types (p < 0.05, Hartigan’s Dip test, Bonferroni corrected). The first type of D1 SPN was distributed across all three striatal subregions. However, the second type of D1 SPN was preferentially located in the NAcc. Further analysis revealed that gene expression in the NAcc-specific D1 SPNs was enriched for genes associated with dopamine signaling, learning, and higher cognitive functions. These result reveal genetic, and putative functional, heterogeneity in D1 SPNs in the NHP striatum, and they will provide a foundation for investigating cell type specific contributions to higher cognitive functions.


Poster

324. Subcortical Circuitry: Reward Seeking and Reinforcement

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 324.19/W20

Topic: G.02. Motivation

Support: R01-DA006214
K99DA045765
NMHRC CJ Martin 1072706
K12 GM093854

Title: Intermittent access to the opioid fentanyl induces amultifaceted addiction-like phenotype that is associated with an increased number of orexin/hypocretin neurons

Authors: *J. E. FRAGALE*1, M. H. JAMES1,2, R. GENERALI1, G. ASTON-JONES1; 1Rutgers Univ., Piscataway, NJ; 2Florey Inst. of Neurosci. and Mental Hlth., Parkville, Australia

Abstract: Clinical data indicate that human addicts, including opioid addicts, rarely maintain stable drug use patterns over extended periods. To better model abuse patterns in human addicts, the IntA self-administration paradigm (IntA; Zimmer et al., 2012) was established and has since emerged as a powerful preclinical model of drug addiction. IntA to cocaine results in a robust escalation of cocaine intake, increased motivation for cocaine in a behavioral economics (BE) procedure, increased compulsive responding, and greater cued and drug-primed reinstatement compared to traditional short (ShA) and long-access (LgA) models. Despite the utility of the IntA model, it has yet to be applied to the study of opioid addiction. Here, we extend our characterization of the IntA model to the widely abused opioid fentanyl. Male rats were either given ShA (1h, n=12), LgA (6h, n=8) or IntA (n=12) to fentanyl for 14 consecutive days. Like IntA to cocaine, we found that IntA to fentanyl causes escalation of fentanyl intake, increased
motivation for fentanyl on a BE procedure, persistent drug seeking during abstinence, and increased cue-induced reinstatement compared to rats given ShA or LgA to fentanyl. We have previously shown that IntA to cocaine is associated with a persistent increase in number and activity of lateral hypothalamic orexin neurons (James et al., 2018). To determine if IntA to opioids produces similar changes in orexin expression, rats previously given ShA (n=7) or IntA (n=6) to fentanyl were re-exposed to the drug-taking context (self-administration room) following 3mo of abstinence and sacrificed 90 later. Immunohistochemistry was performed to assess orexin and Fos (activity marker) expression. Rats given IntA to fentanyl exhibited increased orexin cell numbers and activity compared to rats given ShA to fentanyl. Unlike IntA to cocaine, these differences were apparent in both the lateral and medial hypothalamic orexin cell fields. Together, these results indicate that the IntA paradigm can serve as a strong preclinical model of opioid addiction and that IntA-induced behaviors may develop through persistent changes in orexin expression.


Poster

324. Subcortical Circuitry: Reward Seeking and Reinforcement

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program#/Poster #: 324.20/W21

Topic: G.02. Motivation

Support: 3R00AA023559-05S1

Title: Elucidating the role of a midline thalamus projection to nucleus accumbens in reward and aversion

Authors: *J. K. RIVERA IRIZARRY¹, K. E. PLEIL²;
¹Neurosci., ²Pharmacol. & Neurosci., Weill Cornell Med., New York, NY

Abstract: Substance use disorders are characterized by progressively uncontrollable drug use despite negative consequences. Repeated drug consumption may evolve into problematic use by precipitating sustained plasticity in glutamatergic synaptic transmission in regions including the nucleus accumbens shell (NacSh). Glutamatergic synaptic transmission in the NAcSh promotes reward-seeking behaviors including drug self-administration and receives dense glutamatergic input from the paraventricular thalamus (PVT), a midline thalamic region also involved in these behaviors and important for the integration of reward and aversion. Interestingly, the roles of the PVT and NAcSh in these behaviors may be topographically organized, and there is conflicting experimental evidence that the PVT-NAcSh projection promotes the rewarding or aversive components of drug-taking depending on the study. Here, we evaluated the hypothesis that
activity of the PVT to dorsomedial NAcSh (dmNAcSh) pathway is rewarding and sufficient to drive reward-seeking behaviors and mitigate behavioral responses to aversive stimuli using a pathway-specific chemogenetic approach. We found that mice expressing a Gq-DREADD specifically in this pathway preferentially self-administered CNO over water, and that systemic CNO activation of the DREADD resulted in increased sucrose consumption and preference, indicating that activation of this thalamo-limbic circuit is involved in promoting reward-seeking behavior. In addition, it delayed Pavlovian fear acquisition without altering pain perception in a sex-dependent manner, suggesting it may attenuate the aversive component of fear conditioning. Ongoing experiments are evaluating whether this is due to a reduction of aversion per se or imbalance between the activation of this and parallel PVT projections. In addition, while we show activation of this pathway is sufficient to drive reward, future studies will evaluate whether it is also necessary for reward-related behaviors.

Disclosures: J.K. Rivera Irizarry: None. K.E. Pleil: None.
the molecular identity of NAc neurons projecting to each subpopulation of the VP based on their Drd1a and Drd2 mRNA expression. In all, we provide comprehensive anatomical information regarding the VP which will allow for further interpretation of its function, including in the context of larger subcortical circuits.

Disclosures: S.C. Pate: None. D. Knowland: None. V. Lilascharoen: None. B. Lin: None.

Poster

324. Subcortical Circuitry: Reward Seeking and Reinforcement

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 324.22/W23

Topic: G.02. Motivation

Support: R00 MH109627

Title: Muscarinic M5 receptors modulate dopamine transmission and dopamine-dependent exploratory behavior in mice

Authors: J. A. RAZIDLO, *J. C. LEMOS;
Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Dopamine transmission in the nucleus accumbens (NAc) is a driving force for important behaviors involving motivation and reward learning. Dopamine dysregulation in the NAc is associated with a plethora of disorders that include Parkinson’s disease and depression. Thus, targeting biological substrates that modulate dopamine transmission may have important therapeutic potential. The muscarinic acetylcholine type 5 receptor (M5) is the only muscarinic subtype with detectable mRNA in the dopaminergic neurons of the midbrain. We replicated and extended that finding using RNAscope in situ hybridization and multiplexing technology. There is evidence that the M5 receptor can modulate dopamine transmission. However, since the discovery of the M5 receptor there has been a paucity of information on its function in relation to dopamine modulation in the NAc and dopamine dependent behavior. Here we demonstrate that non-selective muscarinic agonist oxotremorine (OXO-M, 10 µM) potentiates dopamine transmission in the NAc using fast scan cyclic voltammetry in an ex vivo slice preparation from wildtype control mice (M5 +/-). This occurs under conditions in which dopamine release resulting from dopamine fiber activation, as opposed cholinergic interneuron activation, is isolated. This effect is reversed with the non-selective antagonist scopolamine (1 µM). Importantly, OXO-M potentiation of dopamine transmission is absent in mice with global deletion of M5 receptors (M5 -/-), and a small but significant OXO-M-dependent inhibition is revealed. Interestingly, we did not detect any differences in OXO-M potentiation of dopamine peak transient between control and heterozygous mice (M5 +/-) suggesting that dopamine terminals contain spare M5 receptors. Next, we investigated the role of M5 receptors in
modulating exploratory and locomotor behavior using M5 +/+, +/-, -/- mice. In control animals, exposure to a novel environment transiently stimulates locomotor activity, stabilizing as the animal habituates. Our preliminary data suggests that mice with genetic deletion of M5 receptors show a blunted locomotor response in this initial novelty exposure but did not show a global locomotor impairment. These M5 -/- also show a significant reduction in center exploration, indicative of increased anxiety-like behavior. Interestingly, we detected no differences in exploratory behavior of opened and closed compartments of an elevated zero maze, indicating that deficits in exploratory behavior observed in M5-/- mice are context dependent. Furthermore, disruption of M5 signaling may lead to deficits in motivated behavior typical in mood disorders.

Disclosures: J.A. Razidlo: None. J.C. Lemos: None.

Poster

324. Subcortical Circuitry: Reward Seeking and Reinforcement

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 324.23/W24

Topic: G.02. Motivation

Support: WT108890MA

Title: Investigating reward motivation and sensitivity in healthy volunteers with high versus low state anhedonia

Authors: *C. L. SLANEY, M. R. MUNAFÒ, I. PENTON-VOAK, C. J. HOUGHTON, E. S. J. ROBINSON; Univ. of Bristol, Bristol, United Kingdom

Abstract: INTRODUCTION: Anhedonia is a common symptom of many psychiatric disorders including depression and schizophrenia. Despite this, currently available treatments do not adequately address this symptom. One hindrance in developing new effective therapies is that it is unclear whether anhedonia reflects deficits in motivation for reward and/or reward sensitivity. The aim of this study was to behaviourally examine reward motivation and reward sensitivity in healthy adults who have high vs low state anhedonia.

METHOD: Participants scoring high (25+) or low (below 18) on an online anhedonia questionnaire (Snaith Hamilton Pleasure Scale; SHAPS) were invited to take part in this study. In total, 101 participants took part (66 reliably met criterion at study visit). All participants completed a battery of tasks measuring reward motivation (Joystick Operated Runway Task; JORT and Effort Expenditure for Reward Task; EEfRT) and reward sensitivity (Sweet Taste Test; STT). Following this, they completed questionnaires that measured symptoms of anhedonia, apathy and depression. We predicted differences between groups (high vs low state anhedonia) on both reward motivation and reward sensitivity tasks (pre-registered;
RESULTS: Primary analyses were conducted on those who reliably met pre-defined criterion at study visit. Contrary to our predictions, there was no clear evidence of a difference between groups on reward motivation tasks (EEfRT and the JORT; \( p > .05 \)). As predicted, there was a difference between groups in the STT, the high anhedonia group had an overall reduced sensitivity to detect sucrose compared to the low anhedonia group (\( p < .05 \)). Exploratory correlational analyses including all participants revealed that poorer sucrose detection was also associated with other self-report measures (apathy and depression questionnaires). Additionally, performance on the EEfRT was related to anhedonia (measured using the Chapman Physical Anhedonia Scale; CPAS), replicating Treadway et al., 2009.

CONCLUSION: These findings suggest that anhedonia is related to changes in sensitivity to detect a reward (i.e. sucrose) and in effort-based decision making (using the CPAS). Importantly, changes in sensitivity to detect a reward do not appear to be anhedonia-specific.

brains were postfixed for 24h in paraformaldehyde before being saturated in cryoprotectant (20% sucrose in PB), then frozen to -80C. Sections of 40 μm thickness were taken from the dorsal lateral and dorsal medial striatum with a cryostat. Immunohistochemistry was performed using gold nanorods with covalently linked antibodies (rabbit anti-[rat D1], Abcam, 1:100). Primary antibodies were then reacted with a secondary amplification (goat anti-[rabbit Fab], Alexa Fluor® 488, Jackson ImmunoResearch, 1:100). X-ray microscopic imaging facilitated three-dimensional reconstruction of an approximately 500×500×40 μm region of striatum, revealing neuronal processes and dendritic spines with a 400 nm pixel size. This imaging process took less than 24h. These proof-of-concept data from rat striatum demonstrate the approach’s promise for connectomic imaging.


Poster

324. Subcortical Circuitry: Reward Seeking and Reinforcement

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 324.25/W26

Topic: G.02. Motivation

Support: 17ZDA323

Title: The neural mechanism of reward and punishment modulation on visual working memory

Authors: *Y. SUN1, X. CAI2, Y. KU1;
1East China Normal Univ., Shanghai, China; 2Neurosci., New York Univ. Shanghai, Shanghai, China

Abstract: Human do almost everything under the drive of motivation, but it still remains unclear how incentive information influences visual working memory (VWM) which is a fundamental component of cognition. Furthermore, little is known about how reward and punishment related neural circuits influence VWM related circuits. Here, we address this issue in a human functional magnetic resonance imaging (fMRI) study in which 20 subjects engaged in a free recall VWM task with a monetary incentive cue presented at the beginning of each trial. The subjects needed to remember four Gabor patches with different orientations, then recall one of those orientations after a short delay. Whether they got the monetary reward/avoid the punishment or not was determined by the response error and the specific criterion (which was calculated from the result of a pre-experiment). The cue could be low, medium or high monetary reward or punishment, and we manipulated the reward and punishment across blocks and the amount of money across trials. Behaviorally, there was no interaction but a main effect of the level of reward/punishment in response error, which indicated that the more the money, the better the performance. A significant difference between low and high punishment was found through paired sample T test.
Imaging data showed that BOLD signals in caudate and putamen tracked the level of incentive cues in both reward and punishment contexts. The frontal parietal network (superior parietal lobule, intraparietal sulcus, inferior frontal junction) showed enhanced activity at the high reward condition than low reward. However the de-activation of the default mode network(posterior cingulate cortex, precuneus, medial prefrontal cortex) signaled the level of punishment. In addition, the results of parametric modulation revealed that at the punishment context, dorsal striatum was negatively correlated with the response error whereas at the reward context the ventral striatum was negatively correlated with error. These findings suggest, although the behavioral performance was similar at reward and punishment condition, the neural mechanism seemed to be different. We additionally conducted the representational similarity analysis (RSA) to identify regions correlating with responses error at different condition. The results showed that the pattern of activation in the striatum could reflect the response error dissimilarity matrices as early as the cue stage. This study is critical to understand the similarity and difference of reward and punishment and their influence in VWM.

Disclosures: Y. Sun: None. X. Cai: None. Y. Ku: None.

Poster

324. Subcortical Circuitry: Reward Seeking and Reinforcement

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 324.26/W27

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Intramural Research Program

Title: Training squirrel monkeys on cognitive tasks for addiction studies

Authors: *D. EFFINGER, S. O. BROWN, R. A. MONTORO, H. P. JEDEMA, C. W. BRADBERRY;
NIDA Intramural Res. Program, NIH, Baltimore, MD

Abstract: Substance use disorder has become one of the largest and most rapidly growing health concerns in the United States. One of the hallmarks of substance use disorder is an inability to make logical decisions regarding drug use. Given the recent elevation in opiate use disorder, understanding the impact of abuse on neurological structure and cognitive function is of urgent importance. To explore these questions, we designed a longitudinal MR imaging and cognitive assessment study to look at the effects of prolonged remifentanil self-administration on brain structure, functional connectivity, and cognitive performance using non-human primates. Male squirrel monkeys (n=8) were trained to perform a discrimination/reversal task on a touchscreen wherein two abstract images representing a high and low milk reward were presented for the animal to choose between. Once the animal reached a criterion of 80% accuracy (choosing the
higher reward) on the stimulus discrimination over 15 consecutive trials, the contingencies were
switched, and the image previously associated with the higher reward would then deliver the
smaller reward. After reaching the same criterion performance on the reversal, two new stimuli
were presented. Within a set 150 trial session, the average number of trials it took to reach
criterion on the stimulus discrimination was 22.9 ± 1.6 trials [range: 17.2 ± .3 to 80.2± 6.8], with
an average number of 2.5 ± .2 discriminations completed within a 150-trial session. For the
reversal, the average number of trials it took to reach criterion was 30.9 ± 3.3 trials [range: 17 ±
9 to 73.2 ± 7.8], with an average number of 1.8 ± .3 reversals completed within a 150-trial
session. On a delayed match to sample task, we found inconsistent results, suggesting this task is
more difficult for the squirrel monkey to learn. With this baseline established, the monkeys have
received intravenous catheters and baseline structural and functional connectivity MR scans and
begun IV remifentanil self-administration in a food/drug concurrent choice paradigm. After a
period of prolonged exposure to remifentanil self-administration, cognitive measures on the
discrimination/reversal task, as well as a follow up structural and functional MRI scan will be
taken and compared to baseline.

Disclosures: D. Effinger: None. S.O. Brown: None. R.A. Montoro: None. H.P. Jedema:
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Poster

324. Subcortical Circuitry: Reward Seeking and Reinforcement

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 324.27/W28

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Intramural Research Program

Title: Squirrel monkey performance on opioid/food economic choice

Authors: *R. A. MONTORO, S. O. BROWN, D. P. EFFINGER, E. THORNDIKE, H. P.
JEDEMA, C. W. BRADBERRY;
NIDA Intramural Res. Program, NIH, Baltimore, MD

Abstract: We seek to understand opioid use disorder in the context of choice between drug and
non-drug reward, and to do so, are establishing a squirrel monkey (Saimiri sciureus) concurrent
choice paradigm using the short acting opioid remifentanil. Male squirrel monkeys, n = 4, were
trained to interact with touchscreens and familiarized with quantity-signifying stimuli for a
palatable non-drug reward (30% sweetened condensed milk). Animals were initially trained on a
milk/milk economic choice task which offered different quantities of milk, via two visual cues
on each trial. Animals proved to have the ability to differentiate the quantity-signifying stimuli
by choosing the larger of the two stimuli presented (80%, averaged over four sessions). When
presented with stimuli signifying one of four amounts (75 to 300 microliter/kg), animals reached that 80% criterion in 4 or fewer sessions consisting of 100 trials each. After catheterization, animals were familiarized with novel quantity-signifying stimuli for remifentanil (four amounts from 0.08 to 0.32 micrograms/kg). After several sessions in which animals responded for lone remifentanil-signifying cues, animals were presented with a drug/drug choice task, which, like the milk choice task, offered different quantities of drug simultaneously. To reach the same criterion (80% larger reward averaged over 4 sessions) required 7 ± 2.4 sessions consisting of 108 trials each. Animals are now being presented with a milk/drug choice task in which differing milk quantities are offered against differing drug quantities to study choice between drug and non-drug reward. We are currently exploring how economic choice between drug and non-drug reward can be manipulated with various pre-treatments including; milk satiation, substitution of drug by saline, and morphine pretreatment.


Poster

324. Subcortical Circuitry: Reward Seeking and Reinforcement

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 324.28/W29

Topic: G.02. Motivation

Support: ERC 682426
        KFI-2018-00097
        VKE-2018-00032
        NKP-2017-00001
        KTIA_NAP_12-2-2015-0006
        712821-NEURAM

Title: Cortex-wide activation of VIP interneurons by reward and punishment

Authors: *Z. SZADAI¹, H. PI², Q. CHEVY³, K. ÓCSAI⁴, G. KATONA⁴, B. CHIOVINI¹, L. POPARA⁴, A. KEPECS³, B. ROZSA⁵;
¹IEM-HAS, Budapest, Hungary; ²Neurosci., Brandeis Univ., Waltham, MA; ³Cold Spring Harbor Lab., Cold Spring Harbor, NY; ⁴PPCU, Budapest, Hungary; ⁵Inst.of Exptl. Med., Budapest, Hungary

Abstract: Reward- and punishment are the critical driving forces for behaviors to boost arousal, attract attention and drive learning. This may be important for cortical processing. Previously we found that the VIP+ cortical inhibitory neurons, which specialize in disinhibition, respond to reward and punishment in the auditory cortex (ACx). To test whether other VIP interneurons
across the brain do this, we used two-photon 3D acousto-optic microscopy and fiber photometry to monitor VIP neuronal activity across multiple cortical areas while mice performed an auditory decision task. We show that the majority of VIP interneurons recorded across the cortex were robustly activated by reward and punishment. VIP interneurons were also modulated by arousal, local cortical processes, as demonstrated by orientation- and direction-selective responses in the visual cortex. Our results reveal that VIP interneurons have multiple response modes, with both local and global contributions, and their global model signals reinforcement events, enabling the orchestration of cortex-wide learning mechanisms.

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**Poster**

325. Neural Mechanisms of Social Communication and Motivated Behaviors

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program#/Poster #:** 325.01/DP09/W30

**ControlExtraData.DynamicPosterDisplay:** Dynamic Poster

**Topic:** G.02. Motivation

**Support:** NIH Grant R01NS099288
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Helen Hay Whitney Postdoctoral Fellowship

**Title:** A midbrain-cortical dopamine circuit drives sexually-motivated singing

**Authors:** *M. BEN-TOV, F. DUARTE, R. D. MOONEY;
Duke Univ. Dept. of Neurobio., Durham, NC

**Abstract:** Male zebra finches learn to sing as juveniles and use their song in adulthood to court females. An adult male zebra finch sings in two distinct social contexts: an “undirected song” produced when in isolation from other birds, and a “directed song” that it sings to a nearby female. In fact, presenting a female to a previously isolated male finch motivates him to sing abundant directed songs to the female. The neural mechanisms through which social cues motivate and initiate song production are largely unknown. Song production is regulated by a specialized vocal motor network (the “song system”), which receives input from a variety of neuromodulatory regions that are highly conserved in all vertebrates, and which are likely to provide information to the song system about social context and reproductive state that could drive directed singing. To test this idea, we studied adult male zebra finches while they sang in the presence and absence of a female. We used sound recording and video tracking to monitor the bird’s singing and courtship behavior. Using pharmacological methods, we lesioned
dopamine (DA) terminals either in HVC, a premotor nucleus which lies in the apex of the song production circuity, or lesioned DA cell bodies in the midbrain part of the dopaminergic group A11, an area thought to be involved in social behavior and which is the major source of dopaminergic input to HVC. Either type of lesion abolished the ability of the males to produce female-directed songs, even though males in both experimental groups could still produce undirected songs. Furthermore, birds with DA lesions in HVC still showed courtship behaviors regardless of their inability to sing directed songs, including extensive production of introductory notes and orientation to the female. In contrast, birds with DA lesions in A11 failed to orient, call or sing to the female. Finally, we found that when we optogenetically activated A11 terminals in HVC while males were singing in the presence of a female they sang longer bouts and overall increased their singing rate. Together, these results support the idea that dopaminergic inputs from the A11 to HVC in male finches convey a social context-dependent signal important to female-directed singing.

Disclosures:  M. Ben-Tov: None. F. Duarte: None. R.D. Mooney: None.

Poster

325. Neural Mechanisms of Social Communication and Motivated Behaviors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 325.02/W31

Topic: G.02. Motivation

Support: DFG Grant SCHW 559/15-1

Title: Ultrasonic calling in response to playback of appetitive 50-kHz ultrasonic vocalizations in rats

Authors: *A. C. BERZ, T. M. KISKO, C. H. CHEN, M. WÖHR, R. K. W. SCHWARTING; Philipps-University Marburg, Marburg, Germany

Abstract: Rats emit ultrasonic vocalizations (USV) of different frequencies for communicational purposes. The so-called 50-kHz USV are thought to represent a positive emotional state, which is related to the meso-limbic reward system since both their production and perception, are associated with dopamine release in the nucleus accumbens. In contrast, 22-kHz USV might reflect a negative affective state and serve as alarm calls. To test the signal features of 50-kHz USV, our lab uses playback of these calls on a radial maze. When presenting 50-kHz USV, rats will approach the sound source. Besides this strong social approach, animals may exhibit a response on an ultrasonic level. Some of the emitted calls during and after presenting 50-kHz USV show low frequency features similar to 22-kHz USV, but are shorter and display a slightly higher frequency. These acoustic responses were found in males and females, but seem to depend on development, since they are typical for juvenile but not adult rats. Also during rough-
and-tumble play, juvenile rats may emit these low frequency calls while interacting with a partner. Results from pharmacological interventions with the D2 antagonist haloperidol indicate that the low frequency response calls are not dependent on dopamine D2 receptors. Considering different rat strains like Wistar and Sprague Dawley rats, our data imply that both strains show the phenomenon of low frequency response calls during or after 50-kHz USV presentation. With these studies, we are providing the first comprehensive characterization of the phenomenon of low frequency calls in response to 50-kHz USV playback and during juvenile social play. Besides distinguishing general features like frequency, amplitude and occurrence, we also compare different strains and pharmacological treatments. Our data indicate that these low frequency calls do not reflect aversive states since the animals do not show any avoidance behavior. Rather, it is tentative to assume that they serve as an important adaptive function of juvenile rats in anticipation of or during social interaction with conspecific animals, for example, to decrease the likelihood of aggression.


Poster

325. Neural Mechanisms of Social Communication and Motivated Behaviors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 325.03/W32

Topic: G.02. Motivation

Support: NIH Grant MH117778-01

Title: Identification of forebrain inputs to a midbrain vocalization circuit that promote and suppress the production of social vocalizations

Authors: *K. A. TSCHIDA*¹, V. C. MICHAEL¹, J. TAKATOH¹, S. ZHAO¹, B.-X. HAN¹, F. WANG², R. D. MOONEY¹;
¹Dept. of Neurobio., ²Duke Univ., Durham, NC

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. This work established the identity of the PAG neurons selectively required to gate the production of USVs (i.e., PAG-USV neurons), and an important remaining question is how PAG-USV neurons integrate upstream information to appropriately gate vocal output.

To identify neurons upstream of the PAG vocalization circuit, we performed trans-synaptic rabies tracing from PAG-USV neurons as well as nearby PAG inhibitory interneurons. We found that the PAG vocalization circuit receives inputs from a variety of cortical and subcortical regions implicated in social behavior, including neurons in the preoptic area (POA) of the hypothalamus and in the central amygdala (CeA). We used genetic tools to selectively activate subpopulations of POA and CeA neurons that project to the PAG, revealing that PAG-projecting POA neurons promote USV production, while activation of PAG-projecting CeA neurons halts ongoing vocalization. We then performed in situ hybridization to establish the neurotransmitter phenotypes of PAG-projecting neurons in the POA and CeA. Finally, we monitored the activity of the PAG vocalization circuit, to understand how activity in upstream neurons shapes the activity of PAG-USV neurons during natural social interactions. Taken together, these experiments further our understanding of how the midbrain vocalization circuit is embedded in and recruited by forebrain networks that control social behavior.


Poster

325. Neural Mechanisms of Social Communication and Motivated Behaviors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 325.04/W33

Topic: G.02. Motivation

Support: NIH 1R01MH117778
        NIH R01DC13826

Title: Descending control of vocalization in the mouse brain

Authors: *V. C. MICHAEL¹, K. A. TSCHIDA¹, J. TAKATOH¹, S. ZHAO¹, F. WANG², R. D. MOONEY³;
        ²Dept. of Neurobio., ¹Duke Univ., Durham, NC; ³Duke Univ. Dept. of Neurobio., Durham, NC

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 advanced genetic tools. The midbrain periaqueductal gray (PAG) is a critical node in the vocal motor circuit whose activity is obligatory for vocalizations. 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). In the current study we sought to dissect the larger vocal-control circuit in which these neurons reside. We used CANE-based trans-synaptic rabies tracing from PAG-USV neurons as well as from local PAG inhibitory neurons (PAG-GABA) to identify afferents to the PAG vocal control circuit. 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. We identified subpopulations of neurons in the preoptic area (POA) of the hypothalamus and the medial amygdala (MeA) that project to the PAG. We used genetic tools to show that the activation of GABAergic POA neurons that project to the PAG drives vocal production while the activation of GABAergic MeA neurons that project to PAG stops ongoing vocalizations. We further dissected the circuit mechanisms through which upstream inputs act on the PAG vocal control circuit by conducting \textit{in vitro} whole cell recordings of PAG-USV and PAG-GABA neurons while optogenetically activating terminals from the POA and MeA. Together these experiments demonstrate that inhibitory inputs from forebrain structures with known roles in social behavior are integrated by the PAG vocalization circuit to drive or suppress vocal behavior.

**Disclosures:** V.C. Michael: None. K.A. Tschida: None. J. Takatoh: None. S. Zhao: None. F. Wang: None. R.D. Mooney: None.

**Poster**

**325. Neural Mechanisms of Social Communication and Motivated Behaviors**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 325.05/W34

**Topic:** G.02. Motivation

**Support:** NIH Grant R01 DC00937-26

**Title:** Differing release patterns of dopamine and acetylcholine in the mouse basolateral amygdala in response to emotion-laden vocal sequences

**Authors:** *Z. GHASEMAHMAD*\textsuperscript{1}, D. PERUMAL\textsuperscript{2}, B. SHARMA\textsuperscript{2}, R. PANDITI\textsuperscript{2}, J. J. WENSTRUP\textsuperscript{1};

\textsuperscript{1}Dept. of Anat. and Neurobio., Northeast Ohio Med. Univ. (NEOMED), Rootstown, OH;

\textsuperscript{2}Northeast Ohio Med. Univ., Rootstown, OH
Abstract: Social vocalizations, reflecting the internal state of a sender, can change the internal state and behavior of listeners. These listener responses are mediated, in part by the basolateral amygdala (BLA), an emotional brain center shaping reactions to salient and valent stimuli and are thought to depend on inputs from neuromodulatory centers. Here, we examine how emotion-laden vocalizations evoke patterns of neurochemical release within the mouse BLA. A liquid chromatography/mass spectrometry (LC/MS) technique allowed simultaneous detection of non-electroactive chemicals (GABA and acetylcholine (ACh)) and catecholamines in the same samples. For playback of highly emotion-laden vocalizations of CBA/CaJ mice, we identified vocal sequences that were characteristic of restraint (aversive) and an intense stage of mating (appetitive). We presented 5 contextual exemplars from each context. Mice were tested in 3 groups: males-mating, males-restraint, and females-mating (w/estrous monitoring). These groups were chosen based on previous results showing sexually dimorphic reactions to mating vocalizations but similar behavioral responses to restraint calls. After experiencing mating and restraint situations in a counterbalanced order on consecutive days, mice were implanted with a microdialysis probe and CSF samples were collected from BLA before, during, and after vocalization playback in 10-min intervals. Probe location in or adjacent to BLA was histologically confirmed. Samples were analyzed using LC/MS and concentrations of neurochemicals were monitored and correlated with behavior. Here we report on the release of ACh and dopamine (DA). During 20-min playback of mating vocalizations, DA increased in both male and female mice and remained elevated after playback for 1 hour. However, ACh concentration decreased during playback and returned to baseline after the mating vocalizations stopped. In contrast, playback of restraint vocalizations decreased DA during and after playback of the vocalizations, while ACh showed a transient increase in concentration during playback and returned to baseline after playback stopped. Our findings suggest interplay between ACh and DA release in the BLA may contribute to distinct behavioral responses to appetitive and aversive vocal sequences.


Poster

325. Neural Mechanisms of Social Communication and Motivated Behaviors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 325.06/W35

Topic: G.02. Motivation

Support: Project 837-B5-124, University of Costa Rica
Project 723-B7-610, University of Costa Rica
Title: Muscarinic and glutamatergic regulation of self-grooming behavior and ultrasonic vocalizations in the context of open-field habituation in rats

Authors: *J. C. BRENES*¹, J. CHINCHILLA², R. LEANDRO², J. FORNAGUERA², M. ROJAS-CARVAJAL³;

Abstract: Glutamatergic and cholinergic signaling has been widely associated with spontaneous exploratory activity, non-associative learning, attention, and ultrasonic vocalizations. We tested whether (i.p.) administration of scopolamine (SCP; muscarinic antagonist) or MK-801 (MK; NMDA antagonist) was able to impair or delay open-field test (OF) habituation. In this context we analyzed different behaviors paying special attention to particular grooming subtypes, which have been previously linked to novelty habituation and emotional de-arousal. Both drugs were daily administered during four consecutive days 20-min before a 15-min OF session. On the fifth day, rats were given vehicle (saline, i.p) and tested as previously mentioned. On each testing day, ultrasonic vocalizations of 50-kHz and 22-kHz were analyzed. We found that neither SCP nor MK altered the behavioral kinetics of locomotion. However, SCP and MK strongly inhibited the emission of rearing behavior. Surprisingly, such an inhibition recovered in all groups on the last testing day without drugs. Moreover, MK but specially SCP strongly disrupts grooming syntaxes. Overall, MK and SCP decreased the time spent on grooming behavior, without affecting its frequency. In addition, both drugs increased the emission of short, head-directed sequences of grooming -the so-called cephalic grooming-, but dramatically abolished the most complex forms of this behavior, which have been associated with habituation learning and de-arousal. Interestingly, after drug clearance (day 5), neither MK, not SCP altered grooming emission. Further evidence about OF activity and the emission of ultrasonic vocalizations will be presented and discussed in the context of the glutamatergic and cholinergic mechanism mediating habituation, risk-assessment, and social communication in rats.


Poster

325. Neural Mechanisms of Social Communication and Motivated Behaviors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 325.07/W36

Topic: G.02. Motivation

Support: NIMH R01 MH119041
Title: Mu opioid receptors in the medial preoptic area govern social play behavior in adolescent male rats

Authors: *C. ZHAO*, L. CHANG, A. AUGER, L. RITERS;  
*Integrative Biol., Psychology, Univ. of Wisconsin-Madison, Madison, WI*

Abstract: Neural systems underlying important behaviors are usually highly conserved across species. Our research in songbirds has demonstrated a crucial role for opioids in the medial preoptic area (MPOA) in affiliative, non-sexual, social communication and reward. Unlike directed social behaviors (e.g., food-directed, mate-directed, drug-directed), affiliative social behaviors do not result in immediate, obvious extrinsic reward, suggesting that they may be intrinsically rewarded. One such affiliative behavior in mammals is social play. In rodent models, the MPOA is well-known for its role in directed social behaviors such as sexual and maternal reward; however, the role of MPOA in affiliative, intrinsically-rewarded social behaviors has not been well studied. Given that social play in mammals is a highly rewarding, motivated, affiliative behavior, and the MPOA is highly conserved (i.e., it is neuroanatomically, neurochemically and functionally similar) in birds and mammals, we predict that our studies of MPOA in songbirds are uncovering a central nucleus that is part of a core, conserved circuit across species in which opioids act to stimulate and maintain affiliative social behaviors. In this study, we applied our insights from songbirds to rats to determine whether opioids in the MPOA of adolescent rats govern social play behavior. We knocked down gene expression of mu opioid receptor (Oprm1) in the MPOA of Sprague-Dawley rats via adeno-associated viral (AAV) vectors and short hairpin RNA (shRNA)-mediated gene silencing. On postnatal day 21, AAV vectors expressing either rat Oprm1-shRNA (n=12) or scrambled shRNA (n=12) were injected bilaterally into the MPOA. Social play behavior was scored in group-housed animals undisturbed in the home cage on postnatal days 35-39. Animals were video-recorded for two 20-minute trials per day over 5 days for a total observation time of 200 minutes per animal. The frequency of each component of play behavior including pouncing, pinning, wrestling/boxing, biting and chasing was calculated over the entire observation time. We found that knockdown of Oprm1 in the MPOA reduced the number of total play bouts and the frequency of pouncing, a major index of social play behavior, with little effect on other components of play behavior in adolescent male rats. Taken together, these results demonstrate that knockdown of Oprm1 in the MPOA suppresses social play behaviors in adolescent male rats and support the hypothesis that the MPOA is a central nucleus that is part of a core, conserved circuit across vertebrates in which opioids act to govern affiliative, intrinsically-rewarded social behaviors.

Disclosures: C. Zhao: None. L. Chang: None. A. Auger: None. L. Ritters: None.
Title: Strain dependent effects of maternal behavior on hypothalamic oxytocin neurons

Authors: J. A. GENTES, *S. M. SIVIY;
Gettysburg Col., Gettysburg, PA

Abstract: Oxytocin (OT) modulates a variety of mammalian social behaviors, yet relatively little is known about the role of this neuropeptide in the control of playfulness. The F344 rat is consistently less playful than other strains of rat and we have been investigating whether strain differences in play may be associated with systematic differences in OT functioning. When comparing play between F344 and LEW rats we found that strain differences in play are fairly resistant to cross-fostering (Physiol Behav, 2017, 169, 147), suggesting that early postnatal influences have little impact on the levels of play in F344 rats. As part of that study and to determine whether early postnatal experiences may impact OT differentially in F344 and LEW rats, slices were processed immunohistochemically for OT-positive neurons in the paraventricular nucleus of the hypothalamus. Those rats reared by LEW dams were found to have significantly more OT-positive neurons than those reared by F344 dams, which engaged in less licking and grooming (LG) of their pups than LEW dams. This suggests that LG directed to pups is associated with increased hypothalamic OT. To further assess the relationship between licking and grooming received by pups and the number of OT-positive neurons, we did a correlation analysis between % time spent in LG by the dam and the number of OT-positive neurons for those pups that were sampled from each litter. The results are shown in the accompanying figure. A separate analysis was conducted for each strain and there was found to be a strong positive relationship between LG received and OT-positive neurons among LEW rats, with more LG received associated with more OT-positive neurons. On the other hand, there was no relationship between these two variables among F344 rats. This suggests that LEW rats use information provided by the mother (i.e., licking and grooming) and translate this into increased levels of hypothalamic oxytocin. This was not the case with F344 rats and suggests that rats of this strain may be impervious to the effects of early neonatal stimulation on OT structure and/or function.
Disclosures: J.A. Gentes: None. S.M. Siviy: None.

Poster

325. Neural Mechanisms of Social Communication and Motivated Behaviors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 325.09/W38

Topic: G.02. Motivation

Support: Comisión sectorial de investigación científica (CSIC, UdelaR)

PEDECIBA

Title: Nmda-lesions in the prefrontal cortex delay the onset of maternal behavior in adult female, but not infanticidal behavior in male mice (c57bl/6)

Authors: *M. ALSINA-LLANES, D. E. OLAZABAL;

Fisiología, Facultad de Medicina, Univ. de la República, Montevideo, Uruguay

Abstract: There is significant variability in the immediate behavioral response exhibited by inexperienced adult mice when they are exposed to pups for the first time. While most females can display parental behavior (PB) or sensitize rapidly, adult males are infanticidal or non-parental. The prefrontal cortex (PFC) participates in attentional selection, decision making, behavioral flexibility and planning that may be critical in the rapid decision to display PB or infanticidal behavioral (IB). We investigated if excitotoxic lesions in the PFC inhibited PB or IB in naïve adult female and male mice. Adult females and males received bilateral injections into the PFC of 0.2 μl of 25 mM phosphate-buffered saline solution (females: n=6; males: n=6) or 5
μg of N-methyl-D-aspartate (NMDA, females: n=6; males: n=7) dissolved in 0.2 μl of PBS. Two days later, animals were exposed to pups and then tested in an open field. All females with sham-lesions displayed full PB at the first encounter with pups. However, those with NMDA-lesions exhibited partial PB (50%) or were non-parental (50%). Four repeated exposures of 60-min to pups were needed to induce full PB in the NMDA-lesion female group. Sham-lesion males displayed non-PB (17%) or IB (83%), while all NMDA-lesion males were infanticidal. There was no difference in general locomotor and exploratory activity (i.e. peripheral crosses, rearings, immobility time). Nevertheless, both females and males with NMDA-lesions showed a reduction (p<0.05) in the number of central crosses and time that remained in the central area respectively, suggesting an increase in anxiety in lesioned groups. Our results suggest that the PFC is engaged in the rapid onset of PB in females, coordinating and planning its rapid execution, or reducing the anxiety to the first encounter with pups. However, IB may be a more stereotyped behavior with minor planning/coordination or increased by anxiety.


Poster

325. Neural Mechanisms of Social Communication and Motivated Behaviors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 325.10/W39

Topic: G.02. Motivation

Support: DA041482

Title: Temporal and spatial circuit activity dynamics in the interpeduncular nucleus differentially signal novelty and familiarity

Authors: *S. MOLAS, R. ZHAO-SHEA, P. KLENOWSKI, A. R. TAPPER; Dept. of Neurobiology, Brudnick Neuropsychiatric Res. Inst., Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract: Novel stimuli induce an increase in exploratory response that rapidly decreases as the stimulus becomes familiar upon repeated exposures. Novelty exploration and habituation with familiarity are fundamental for the adaptation to changes in the environment and prime goal-directed behaviors. The brain circuits that control behavioral responses to novelty and familiarity are still poorly understood. Our previous work demonstrated that the interpeduncular nucleus (IPN) of the midbrain represents a neuroanatomical substrate for familiarity signaling. In particular, using c-Fos expression as a read out of neuronal activation, we showed that as novel stimuli become familiar upon repeated exposures activity of IPN GABAergic neurons progressively increases. Here, we have deeply investigated IPN neuronal responses during interactions with novel and familiar stimuli by combining fiber photometry and genetically
encoded calcium indicators. We expressed CAG.Flex.GCaMP6m in IPN GABAergic neurons in the GAD2-Cre mouse line and recorded changes in Ca2+ signals as a proxy for neuronal activity in awake behaving mice during social and object novel preference tests. We have defined clusters of GABAergic neurons distributed throughout IPN sub-regions that exhibit differential responses to either novel or familiar stimuli, as well as clusters with no response. Importantly, changes in activity mainly occur within the second minute of the 5-minute behavioral testing. These results suggest IPN neurons signal novelty and familiarity through a highly dynamic temporal- and spatial-dependent manner.


Poster

325. Neural Mechanisms of Social Communication and Motivated Behaviors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 325.11/W40

Topic: G.02. Motivation

Support: NIH Grant R01 MH109450
NIH Grant R01 MH058616

Title: Transcriptomic regulations underlying pair bond maintenance in the socially monogamous prairie voles

Authors: *F. DUCLOT1,2, L. SAILER1,2, P. KOUTAKIS3, Y. LIU2,4, Z. WANG2,4, M. KABBAJ1,2;
1Biomed. Sci., 2Program in Neurosci., 3Nutrition, Food and Exercise Sci., 4Psychology, Florida State Univ., Tallahassee, FL

Abstract: Social affiliation is a core characteristic of human social behaviors and related impairments are a common feature in a multitude of neuropsychiatric disorders including schizophrenia and autism spectrum disorders. As a result, understanding the neurobiology of social attachment is of critical importance. In this context, the socially monogamous prairie vole (Microtus ochrogaster) provides an excellent opportunity to study the molecular mechanisms underlying the formation and maintenance of a pair bond. In prairie voles, prolonged cohabitation with an opposite-sex partner leads to an enduring social bond. Despite the variety of neurotransmitter systems involved in the initial formation of the bond, however, relatively little is known regarding the mechanisms associated with its maintenance. In this study, we thus aimed at identifying the pattern of gene expression related to pair-bond maintenance by analyzing the global transcriptional profiles in the nucleus accumbens (NAc) by RNA-sequencing. To this end, male and female adult prairie voles were cohabitated for 3 weeks with an opposite-sex partner—or a same-sex conspecific as a control—and tested for selective aggression to verify the
establishment of the pair bond. Notably, a third group was cohabitated for 24 hours in order to discriminate regulations specific to the maintenance phase of the bond. At baseline, sex differences in gene expression were widespread and involved many critical biological processes related to neuronal activity and plasticity. In both sexes, however, the early phase of the cohabitation (24 hours) triggered large transcriptional changes whereas few genes were differentially expressed in the late phase (3 weeks) when compared to sexually-naïve controls. Interestingly, the analysis of related biological pathways reveals a sex-specific profile of regulations. Indeed, while the biological pathways associated to the early and late phases were similar in females, but distinct in males. Although the related pathways underline a widespread alteration of several aspects of neurotransmission in both sexes, we observed notable sex-specific regulations. For instance, large regulations of genes related to mitochondrial activity were observed in females but not males, highlighting the mitochondria as a novel candidate for sex-specific regulations underlying pair-bonding in prairie voles. Overall, these data will provide a novel insight into the global transcriptomic profiles underlying the maintenance of a bond in the prairie vole NAc, and thus open the way for the identification of novel candidates mediating enduring social attachment.


Poster

325. Neural Mechanisms of Social Communication and Motivated Behaviors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 325.12/DP10/W41

ControlExtraData.DynamicPosterDisplay: Dynamic Poster

Topic: G.02. Motivation

Support:  NIDA K99 DA045662-01 (S.A.G.)
NARSAD 2018 #27082 (S.A.G)

Title: Automated analysis of prosocial and aggressive behaviours using computer vision and machine learning

Authors: *S. R. O. NILSSON, J. J. CHOONG, S. A. GOLDEN;
Biol. Structure, Univ. of Washington, Seattle, WA

Abstract: Background. Disrupted social behaviour is a fundamental shared symptom of many neuropsychiatric disorders, including drug addiction, depression and PTSD. However, freely behaving mice are seldom considered in the experimental design of preclinical models. This is predominately due to technical limitations preventing high-throughput, consistent, and unbiased
scoring of freely-moving complex social interactions.

**Method.** We developed predictive classifiers of social and aggressive behaviors during mouse dyadic encounters. Single C57BL/6J mice were placed into the home-cage of a CD-1 mouse and interactions were recorded in variable lighting conditions and different resolutions/frame-rates. We used DeepLabCut (Mathis et al., 2018, Nat Neurosci) to generate a model that tracks eight body-parts on each of the two mice. We detected and reduced tracking inaccuracies and calculated a battery of diverse features (>100) based on body-part movements, distances, angles, sizes, and their deviations across rolling windows. We used the features in sklearn-based machine learning algorithms against multiple socially-relevant targets (e.g., aggressive events, anogenital sniffing, tail rattling, pursuit, lateral threat display) and we visualized the tracking and the predictions with OpenCV.

**Results.** Model predictions were in excellent or good agreement with manual human frame-by-frame scoring. For example, random forest implementations based on re-sampled data predicted aggressive and tail rattling events with more than 95% accuracy. The model generalized well to new recording conditions.

**Conclusion.** The data support that complex social behaviors can be readily quantified in an unbiased, fast, and automated way in unmarked individual mice using DeepLabCut for feature detection and our python modules for machine learning.

**Disclosures:** S.R.O. Nilsson: None. J.J. Choong: None. S.A. Golden: None.

**Poster**

325. Neural Mechanisms of Social Communication and Motivated Behaviors

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

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**Topic:** G.02. Motivation

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NIH 1R01NS075421

**Title:** Oxytocin-dependent reopening of a social reward learning critical period with MDMA

**Authors:** *R. NARDOU*¹, E. M. LEWIS¹, R. ROTHHAAS¹, R. XU², A. YANG², E. S. BOYDEN², G. DÖLEN¹;
¹The Solomon H. Snyder Dept. of Neuroscience, Brain Sci. Inst., Johns Hopkins Univ., Baltimore, MD; ²MIT, Cambridge, MA
Abstract: A critical period is a developmental epoch during which the nervous system is expressly sensitive to specific environmental stimuli that are required for proper circuit organization and learning. Mechanistic characterization of critical periods has revealed an important role for exuberant brain plasticity during early development, and for constraints that are imposed on these mechanisms as the brain matures. In disease states, closure of critical periods limits the ability of the brain to adapt even when optimal conditions are restored. Thus, identification of manipulations that reopen critical periods has been a priority for translational neuroscience. Here we provide evidence that developmental regulation of oxytocin-mediated synaptic plasticity (long-term depression) in the nucleus accumbens establishes a critical period for social reward learning. Furthermore, we show that a single dose of (+/−)-3,4-methylenedioxyamphetamine (MDMA) reopens the critical period for social reward learning and leads to a metaplastic upregulation of oxytocin-dependent long-term depression. MDMA-induced reopening of this critical period requires activation of oxytocin receptors in the nucleus accumbens, and is recapitulated by stimulation of oxytocin terminals in the nucleus accumbens. These findings have important implications for understanding the pathogenesis of neurodevelopmental diseases that are characterized by social impairments and of disorders that respond to social influence or are the result of social injury.


Poster

325. Neural Mechanisms of Social Communication and Motivated Behaviors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 325.14/W43

Topic: G.02. Motivation

Support: NIH T32 NS058280
        NIH F31 MH117966
        ARCS Fellowship
        Marion Bowen Postdoctoral Grant
        Whitehall Foundation grant
        NARSAD Young Investigator grant
        Sloan Research Fellowship

Title: Correlated neural activity and encoding of behavior across brains of socially interacting individuals

Authors: *L. KINGSBURY, S. HUANG, J. WANG, K. GU, P. GOLSHANI, Y. E. WU, W. HONG;
        UCLA, Los Angeles, CA
Abstract: Social interactions involve some of the most complex decision-making tasks that animals must navigate to secure their survival and reproductive success, as individuals must integrate internal state variables with real-time decisions of others in a context-dependent manner. In interacting dyads, individuals thus become entrained as they attend to, predict, and react to each other’s decisions. To date, social neuroscience has mostly focused on behavior in individual animals to interrogate the neural computations underlying social decision-making. But a full understanding of the social brain, and how circuits are engaged during complex, dyadic interactions, requires a broader picture that reflects the dynamic nature of natural social engagement. An open question is whether and how emergent properties of neural systems, such as interbrain activity coupling, may arise across brains of interacting individuals to shape behavior.

Here, by simultaneously performing microendoscopic calcium imaging in pairs of socially interacting mice, we find that animals exhibit interbrain correlations of neural activity in the prefrontal cortex that are dependent on ongoing social interaction. Interbrain coupling arises from two neuronal populations that separately encode one’s own behaviors and those of the interacting partner. Strikingly, interbrain correlations predict future social interactions as well as dominance relationships between animals in a competitive context, suggesting a functional link between neural activity coupling and the evolution of real-time social engagements. They also reflect an asymmetry in encoding of partner behavior across dominants and subordinates, suggesting that brain coupling depends partially on attention of subordinates directed toward dominants. Together, these results demonstrate that interbrain synchrony exists in rodents, indicating generality and conservation of the phenomenon across diverse species beyond what has previously been observed in humans and non-human primates. By uncovering how interbrain synchronization arises from activity patterns at the single-cell level, this work also sets the groundwork for more incisive, circuit-level investigation into the emergent neural properties of multi-individual systems and their role in coordinating social interactions.


Poster

325. Neural Mechanisms of Social Communication and Motivated Behaviors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 325.15/W44

Topic: G.02. Motivation

Support: R00 DA037279

Title: Modulation of social behavior by mu opioid receptors in the brain reward network
Authors: *C. T. TODDES¹, L. ZUGSCHWERT², P. E. ROTHWELL³;
¹Univ. of Minneosta, Minneapolis, MN; ²Neurosci., Univ. of St.Thomas, Minneapolis, MN; ³Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: The rewarding aspects of social interaction require activation of mu opioid receptors within the brain reward network. However, it is unclear where in this network mu opioid receptor expression is essential for the regulation same-sex affiliative social interaction. The goal of this study was to determine how global and regional genetic knockout of the mu opioid receptor alters social behavior in male and female mice, using a variety of behavioral assays. Our behavioral battery includes the social conditioned place preference test, the dyadic social interaction test, and the reverse three-chamber social test. The reverse three-chamber test is a novel social assay that examines the social preference of a wildtype “judge” between a “typical” wildtype mouse and an “atypical” mu opioid receptor knockout mouse. These assays focus on different aspects of social behavior including social reward, motivation, and preference. Our data demonstrates that constitutive homozygous and heterozygous deletion of the mu opioid receptor impairs social behavior across tests. Additionally, preliminary data has shown a social impairment in mice following conditional knockout of the mu opioid receptor from cortico-limbic regions that converge upon the nucleus accumbens. These results highlight critical locations within the brain reward network where mu opioid receptor expression is necessary for normal social behavior.

Disclosures: C.T. Toddes: None. P.E. Rothwell: None.

Poster

325. Neural Mechanisms of Social Communication and Motivated Behaviors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program#/Poster #: 325.16/X1

Topic: G.02. Motivation

Support: Stiftung Freigeist fellowship, AZ88216

Title: Effect of lOFC lesion on social value unblocking of Pavlovian reinforcement learning in male rats

Authors: *S. VAN GURP¹, J. HOOG², T. KALENSCHER³, M. VAN WINGERDEN⁴;
¹Comparative Psychology, ²Heinrich Heine Univ., Duesseldorf, Germany; ³Univ. of Duesseldorf, Duesseldorf, Germany; ⁴Heinrich-Heine Univ. Düsseldorf, Düsseldorf, Germany

Abstract: Objectives: Reinforcement learning theory states that when stimuli are added to stimulus that fully predicts reward, learning about those additional stimuli will be blocked (Kamin, 1969). Learning about added stimuli can become unblocked by an increase in reward
value that is then associated to these additional stimuli (Holland, 1984). Here, we hypothesised that unblocking of learning about an added stimulus would occur when additional rewards are delivered to a social partner and that learning about added stimuli is blocked when a social partner is not rewarded. Furthermore, as Lateral Orbitofrontal Cortex (IOFC) neurons have been found to code upshifts in reward value during unblocking (Lopatina et al, 2015) we hypothesised that temporary inactivation of the IOFC would impair upshifts in social value.

**Methods:** Actor rats (N=36) and partner (N=36) learned to discriminate between a CS+ and CS-. Consequently, the rats went through compound conditioning consisting of 3 conditions: Both Reward/unblocked, both actor and partner rat were rewarded (Actor CS+, Partner CS-); Own Reward/blocked, only the actor was rewarded (Actor CS+, Partner CS-) and No Reward, both rats were not rewarded (Actor CS-, Partner CS-). Then the rats went through a probe session were the CS+, CS- and the unblocked (Partner CS+) and blocked cue (Partner CS-) were presented to the actor rat in extinction. In a control experiment, the exchange of visual and auditory information between actor and partner during the compound phase was impeded by an opaque wall. Finally, in a third experiment we temporarily inactivated the lateral OFC during the compound phase. **Results:** We found that when actor rats have fully learned a stimulus-reward association producing reward for themselves, delivering an additional reward delivery to a partner rat, in association with an additional a cue delivered in compound to the learned cue unblocked associative learning about this cue in the actor rats. In contrast, additional cues that did not predict additional reward remained blocked from acquiring associative value. In a control experiment where putative social cue exchange between the partnered rats was prevented, the normally unblocked cues now remained blocked as expected. Finally, preliminary data shows that inactivation of lateral OFC did not impair social unblocking. **Conclusions:** These results suggest that social value can drive reinforcement learning in rats, and that the transmission of social cues is necessary for this learning to occur. In addition, these results provide evidence that learning about changes in value derived from reward delivered to a social partner do not require the IOFC.

**Disclosures:** S. van Gurp: None. J. Hoog: None. T. Kalenscher: None. M. van Wingerden: None.

**Poster**

325. Neural Mechanisms of Social Communication and Motivated Behaviors

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 325.17/X2

**Topic:** G.02. Motivation

**Support:** R21MH111104

**Title:** Hypothalamic vasopressin regulates social behavior in female mice
**Authors:** *N. RIGNEY*¹, J. WHYLINGS¹, G. J. DE VRIES², A. PETRULIS²;  
²Neurosci. Inst., ¹Georgia State Univ., Atlanta, GA

**Abstract:** The neuropeptide arginine-vasopressin (AVP) has long been implicated in the regulation of social behavior and communication in diverse taxa, but the source of AVP release relevant for behavior has not been precisely determined. Potential sources include hypothalamic cell populations such as the paraventricular (PVN), supraoptic, and suprachiasmatic nuclei, as well as extrahypothalamic cell groups in the extended amygdala. To address if AVP cells in the PVN are important for mouse social communication in males and females, we deleted PVN-AVP cells using viral-mediated delivery of Cre-dependent caspase-9 suicide construct into the PVN of AVP-Cre positive mice (expressing Cre-recombinase under the control of the AVP promoter) or AVP-Cre negative littermate controls, and assessed their levels of social investigation. Preliminary results indicate that lesions of the PVN-AVP cell population in female mice increase social investigation of both opposite and same-sex individuals. These manipulations had little effect on non-social anxiety-like behaviors in the elevated-plus maze. Conversely, PVN-AVP cell deletion in male mice did not affect social investigation. These results suggest that AVP released from PVN normally inhibits social approach in female, but not male, mice. Parallel experiments will reveal if PVN-AVP influences other aspects of male and female social communication.

**Disclosures:** N. Rigney: None. J. Whylings: None. G.J. De Vries: None. A. Petrulis: None.

**Poster**

325. Neural Mechanisms of Social Communication and Motivated Behaviors

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 325.18/X3

**Topic:** G.02. Motivation

**Support:** CONACYT 253631  
Fronteras 374  
PAPIT In210215

**Title:** Manganese as contrast medium and its effects in the behavior of male rats for longitudinal studies

**Authors:** *J. A. AGUILAR MORENO, Jr.*¹, M. A. VEGA SOLACHE², E. CABRERA², R. G. PAREDES³;  
¹Plasticidad y Conducta sexual, Inst. De Neurobiología, Instituto de Neurobiologia, Mexico;  
²Instituto de Neurobiología, Querétaro, Mexico; ³Inst. De Neurobiología UNAM, Querétaro, QRO, Mexico
Abstract: In recent years, the use of manganese (Mn) as a contrast medium had increased considerably, due that Mn can be used as a tracer of neural activity in magnetic resonance (MR) studies, for its paramagnetic properties and accumulation in active neurons. Another important characteristic of Mn is that can have toxic effects, inducing motor impairment after the administration, leading alterations in the behavior of the animal. It has been reported the effects of Mn on the motor skill of rats, but it is unknown how it can impact motivated behaviors, such as sexual behavior and wheel activity of male rats. Then, we evaluated signal intensity changes in MR images of male rats with different levels of sexual experience. We used male Wistar rats, 300grs, without sexual experience. We use ovariectomized females as sexual stimulus. The males were randomly assigned to one of the following groups: Control (saline), and MnCl2 16mg/kg. Males were tested for sexual behavior (SB), where they freely control the sexual interaction for 30 minutes. Thereafter, they were exposed to a running wheel for 30min of free activity. Finally, they were evaluated in a Rotarod, in two different modes, regular speed and ramp (10rpm and 10-15rpm, respectively). Subjects were tested in the same behavioral sequence once a week for 10 weeks. MnCl2 was administered s.c. on sessions 1, 5 and 10. An additional group was injected with the same MnCl2 indicated dose and tested for sexual behavior in the same time as the other groups and scanned in weeks 1, 5 and 10. The results show that the performance in the experimental group was decreased in the activity wheel in sessions 1 and 5 (p<0.05) respect to control group, but it do not last in the following session, indicating a temporal but not permanent affectation in the behavior. The performance in Rotarod was not affected. SB parameters remain similar in both groups, having statistical differences only in the number of ejaculations in session 1, being lower in the group of SB+MRI (p<0.05). The statistical maps of MEMRI showed a significant brain activation in the amygdala, medial preoptic area in the experimental groups in session 5 and 10 (p<0.05). The results demonstrate that 16mg/kg of MnCl2 is an adequate dose to study SB with MEMRI without long lasting behavioral alterations.


Poster

325. Neural Mechanisms of Social Communication and Motivated Behaviors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 325.19/X4

Topic: G.02. Motivation

Support: NIMH R01 MH118237

Title: Brief social isolation increases social interaction and cortical drive of basolateral amygdala activity
Abstract: Adolescence is characterized by high social drive and ongoing brain maturation. The decline in social drive from adolescence to adulthood coincides with cortical development, which likely influences the age-dependent changes in the regulation of emotions. Increased activity in cortical regions, such as the anterior cingulate cortex (ACC), and the amygdala have been linked to social behaviors, and the projections from the ACC to the basolateral amygdala (BLA) are necessary for socially learned fear. Combined, this work suggests there may be existing developmental changes in the ACC-BLA pathway regulating social drive. Manipulations of social drive can be used to investigate the developmental shifts in social behaviors and uncover role of the ACC and amygdala. Brief social isolation facilitates social drive and promotes the value of social interactions. This can be used as an approach to investigate the influence of the developing ACC-BLA circuits to social behaviors. We found that brief social isolation (2hrs) increases the duration of social interaction, in adolescents and adults, and the overall duration of social interaction was substantially higher in adolescents. We next used anesthetized in vivo single unit recordings to determine the impact of brief isolation on BLA activity in adults. We found that brief isolation increased BLA neuronal activity. Further, ACC-evoked BLA activity was preferentially increased in groups exposed to brief isolation. These results suggest that increased social drive, through brief isolation, facilitates ACC drive of BLA activity in adults. These results begin to provide insight to the changes in cortico-amygdala circuitry from adolescence to adulthood that contribute to the refinement of social behaviors.

surrounding environment. Thus far, most studies have focused on better understanding how the brain regulates the expression of a single behavior or suite of behaviors associated with a single motivational state. However, in everyday life, we often find ourselves in situations of competing motivational states with multiple choices of how we can act. We aimed to develop a novel behavioral paradigm to test the competition between the motivation to seek social contact and the motivation to seek food, which can then be used to delineate the neural substrates that coordinate and support the expression of multiple motivated behaviors. Subjects were placed in a 3-chamber Plexiglas apparatus where a social stimulus (novel age- and sex-matched conspecific) and a food stimulus (standard lab chow) were corralled on opposite sides. To determine how the motivation to seek social contact and the motivation to seek food compete, we altered the motivational states of subjects by exposing them to acute social isolation and/or acute food deprivation. Preference was determined by the relative amount of time subjects spent investigating each stimulus. We first examined whether the competition between social contact-seeking and food-seeking is stable across the lifespan by comparing adolescent and adult male and female rats. Compared to adult rats, adolescent rats exhibited a greater hunger-mediated shift in stimulus preference; neither isolation nor sex affected behavior. Next, we examined whether this effect was conserved across rodent species, by repeating the experiment using adolescent male and female mice. Adolescent mice exhibited a similar hunger-mediated shift in stimulus preference compared to that observed in adolescent rats. However, while adolescent rats exhibited a strong preference for the social stimulus when sated which was attenuated by hunger, adolescent mice did not have a stimulus preference when sated and had a strong food preference when food deprived. Additionally, we found that total stimulus investigation (social + food) was greater in adolescent rats and mice than adult rats, consistent with prior reports that adolescence is characterized by increased motivation for rewarding stimuli. Given their higher investigatory drive and greater sensitivity to homeostatic manipulations of motivation, this behavioral paradigm appears to be ideally suited for future interrogations of the underlying neural systems (e.g., oxytocin, orexin, dopamine) in adolescent subjects.


Poster

325. Neural Mechanisms of Social Communication and Motivated Behaviors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 325.21/X6

Topic: G.02. Motivation

Support: NSF IOS 1735934 to AHV
**Title:** Sex differences in juvenile social recognition: Role of oxytocin in the bed nucleus of the stria terminalis

**Authors:** *K. E. YOEST, M. G. HENRY, R. BREDEWOLD, A. H. VEEHEMA;* Neurobio. of Social Behavior Laboratory, Dept. of Psychology & Neurosci. Program, Michigan State Univ., East Lansing, MI

**Abstract:** The ability to recognize and respond to previously encountered conspecifics is crucial for normal social interaction. The neuropeptide oxytocin (OT) and its corresponding receptor (OTR) have been identified as a brain system required for social behavior, and sex differences in the organization of the OT system have been linked to the sex-specific regulation of social recognition in adult rats. Sex differences in the OT system are already present early in development, but the functional implications remain unknown. Here, we sought to determine the role of OTR within the posterior region of the bed nucleus of the stria terminalis (BNSTp) in the regulation of social recognition in juvenile rats. We first determined whether juveniles show similar temporal patterns of social recognition to adults, by testing whether 5-week-old male and female rats were able to recognize a previously encountered same-sex conspecific 30, 60, or 120 minutes following initial investigation. Juvenile males showed social recognition 30 and 60 minutes following the initial encounter, but not after 120 minutes, similar to what has been previously seen in adults of both sexes. Juvenile females, however, did not show social recognition at any time point tested, indicating a developmental difference in social recognition ability in females but not in males. We are currently testing whether OT administered to the BNSTp is sufficient to induce social recognition in females and to prolong social recognition in males. In addition, we are characterizing whether OTR is expressed by neurons or glial cells within the BNSTp of juvenile male and female rats. Finally, we will determine potential sex differences in the extent to which OTR expressing cells within the BNSTp are activated in response to social investigation in juveniles. Together, these results will provide insight into how sex differences in the BNSTp-OT system are involved in the sex difference in social recognition in juvenile rats.

**Disclosures:** K.E. Yoest: None. M.G. Henry: None. R. Bredewold: None. A.H. Veenema: None.

**Poster**

325. Neural Mechanisms of Social Communication and Motivated Behaviors

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 325.22/X7

**Topic:** G.02. Motivation

**Support:** NIH R01MH102456
NSF IOS 1735934
Title: Role of vasopressin in the ventral pallidum in regulating social play behavior in juvenile male and female rats

Authors: *J. D. A. LEE, R. BREDEWOLD, A. H. VEE NEMA;
Dept. of Psychology, Michigan State Univ., East Lansing, MI

Abstract: Social play is predominantly displayed by juveniles of many mammalian species, including rats and humans. Engagement in social play helps develop social competence throughout life. Children diagnosed with autism spectrum disorder (ASD) show decrease involvement in social play. Moreover, ASD is more prevalent in males than females. Thus, there is a need to better understand the neural mechanisms underlying social play in both sexes. We recently showed that vasopressin acting in the lateral septum (LS) of juvenile rats regulates social play behavior in a sex-specific manner. Vasopressin projections to the LS originate in the bed nucleus of the stria terminalis (BNST) and medial amygdala (MeA). We further showed that the ventral pallidum (VP) also receives vasopressin projections from the BNST and MeA. Here, we hypothesized that, similar to the LS, vasopressin in the VP regulates social play in a sex-specific manner. We found that the VP and LS show a similar sex difference in vasopressin fiber density, with denser vasopressin fibers in juvenile males. In contrast, VP and LS show a brain region-specific sex difference in vasopressin 1a receptor (V1aR) binding density, with denser V1aR binding in the female LS and male VP. Using a specific V1aR antagonist, we are currently determining the effects of V1aR blockade in the VP on social play behavior in 5-week-old juvenile male and female rats. In addition, by combining retrograde tracing in the VP and LS with RNAscope for vasopressin and Fos mRNA expression, we will determine the extent to which vasopressin neurons in the BNST and MeA project to both the LS and the VP and are activated in response to social play. This study will provide insights into the brain regions recruited by vasopressin for the sex-specific regulation of social play behavior as well as the larger neural network modulated by the BNST/MeA-vasopressin system.

Disclosures: J.D.A. Lee: None. R. Bredewold: None. A.H. Veenema: None.

Poster

325. Neural Mechanisms of Social Communication and Motivated Behaviors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 325.23/X8

Topic: G.02. Motivation

Support: NIH R01MH102456
NSF IOS 1735934
NIH R01MH109471
Title: Regulation of social play behavior by oxytocin in the nucleus accumbens of juvenile male and female rats

Authors: *R. BREDEWOLD1, S. PROANO2, A. SCAZZERO1, J. MEITZEN2, A. H. VEENEMA1;

Abstract: Social play is a highly motivated and rewarding behavior that is predominantly expressed by juveniles. Exposure to social play in the juvenile period is important for the development of social competence in humans and rats. Social play deficits are seen in children with autism spectrum disorder (ASD) and these deficits may contribute to life-long impairments in social functioning. The neuropeptide oxytocin (OT) is currently being tested in clinical trials to help normalize social behavior in ASD patients. We aimed to better understand how OT modulates social play in male and female juvenile rats by focusing on its role in the nucleus accumbens (NAc), because this brain region is important for motivated and rewarding social behaviors. We found that injecting the specific OT receptor (OTR) antagonist des-Gly-NH2d(CH2)5[Tyr(Me2),Thr4]OVT into the NAc significantly decreased the duration of social play in both sexes, but required a higher dose of the OTR antagonist in males (100 ng/0.5 μl) than in females (10 ng/0.5 μl). This suggests that OT in the NAc facilitates social play in both sexes, but that females are more sensitive than males to disturbances in OTR activation. We further showed that OTR binding density in the NAc is similar in males and females, suggesting that factors other than the OTR contribute to the sex difference in behavioral sensitivity to the OTR antagonist. We are currently using microdialysis to determine the effects of the specific OTR antagonist des-Gly-NH2d(CH2)5[Tyr(Me2),Thr4]OVT (OVT) on changes in dopamine, serotonin, glutamate, and GABA release in the NAc while rats are exposed to social play. We also are employing whole-cell patch clamp to test how OVT modulates NAc neuron electrophysiology, and are specifically assessing intrinsic excitability and excitatory synapse properties. We also will be exploring potential sex differences in the type of NAc neurons expressing the OTR of juvenile rats. These experiments may provide insights into the mechanisms by which OT in the NAc facilitates social play in sex-specific ways, with applications for social play deficits and sex-biases seen in ASD.


Poster

325. Neural Mechanisms of Social Communication and Motivated Behaviors

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 325.24/X9
Abstract: Establishment of social hierarchy through intermale aggressive behavior helps to deflect excessive violence and injury, protects group’s valuable resources, and molds the societal structure. Successful acts of aggression may be rewarding, with a series of wins increasing aggressive motivation and propensity to engage in aggressive behavior, and a series of defeats having an opposite, aversive, effect. Social hierarchy is dynamic and may be altered after encounters between animals of a comparable rank. Several brain regions and circuits involved in aggressive behavior and social dominancy have been identified; however, the global maps of brain networks involved in establishing, maintaining, and reversing social status are not known. We used an animal model of intermale social conflict and applied global mapping of c-Fos expression to determine network activity across the brain regions and infer the circuits involved in aggression, defeat, and reversal of social status. Male mice were trained in a model of chronic social conflicts, and analyzed in several behavioral paradigms: (a) aggression (3, 10 or 20 days of consecutive agonistic interactions in pairs, producing winner and loser animals); (b) deprivation (20 days of agonistic interactions followed by 14 days of fight deprivation); (c) inversion (20 days of agonistic interactions followed by placing the animals in new pairs: winner vs. winner and loser vs. loser). Brains were processed using iDISCO protocol, imaged under the light-sheet microscope, postprocessed using ClearMap pipeline, and analyzed to reveal the interrelations between each of the behavioral states and to infer the connectivity patterns and related neuronal networks. We first analyzed the overall effect of the social status on the position of the activation patterns in a low-dimensional space using principal component analysis of the datasets and found that relative positions of particular patterns allow making conjectures and predictions on how the history of winning/losing defines the neural engram. We next used the data to identify groups of brain regions that show covarying activation upon experimental manipulations and therefore may be functionally related, with strong covariance explained by connectivity, and then asked whether such clustering can be related to the actual neuroanatomical mesoscopic connectivity. Finally, we identify functional subnetworks of coactivated brain regions by computing pairwise correlations between brain regions for animals with a defined social status and comparing these subnetworks to the known connectivity patterns to build mechanistic models of activation in specific subnetworks.

**Poster**

325. Neural Mechanisms of Social Communication and Motivated Behaviors

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program#/Poster #:** 325.25/X10

**Topic:** G.02. Motivation

**Support:** Intramural Research Program at the National Institutes of Health, National Eye Institute / EY000415

**Title:** Habenula and periaqueductal gray represent eye contact established by emotional history

**Authors:** *H. LEE, K. MAEDA, O. HIKOSAKA;
Lab. of Sensorimotor Res., Natl. Eye Inst., Bethesda, MD

**Abstract:** In the world, numerous animals have been living together, sharing many kinds of emotional history. For an appropriate social recognition beyond species, eye contact is rapid and useful social information seeking behavior. Herein we hypothesized that brain modulates different eye contact patterns, as a precursor of social interaction, based on emotional histories of relationships. For this hypothesis, we tested contextual Pavlovian-foraging task in monkeys by recording neuronal activity in lateral habenula (LHb), amygdala, and periaqueductal gray (PAG). In the task procedure, the monkey experienced three types (Rich, Poor, Dangerous) of visual scenes (i.e., variable faces and landscapes) which were probabilistically associated with distinct types of fractal objects: Big reward objects in Rich scene; Small reward objects in Poor scene; Big reward and Punishment objects in Dangerous scene. We found that eye is a highly salient feature on faces. Although eye contact is not necessary for acquiring reward and is not related to any reward value, eye contact was rapidly getting lower in the poor context than the rich or dangerous context. In neuronal recording, we found that many neurons in PAG and LHb changed their activity strongly in response to the visual scenes. There were different types of neurons in PAG. Some PAG neurons were strongly excited by the poor scenes than the rich or dangerous scenes. Other PAG neurons were excited tonically by the dangerous scenes than the rich or poor scenes. These data suggest that PAG contains different groups of neurons that encode different types of negative mood. On the other hand, many LHb neurons were excited phasically and tonically by the poor scenes than the rich or dangerous scenes. They were also sensitive to the fractal objects that appeared in each scene, depending on their values and dangers. Previously, we reported LHb and dopamine neurons can motivate reward anticipation (Bromberg-Martin ES, Matsumoto M, Hikosaka O., 2010) and amygdala can motivate excitement in dangerous context (Maeda K, Kunimatsu J, Hikosaka O., 2018). Present study suggests that PAG can relay these types of emotional information to control behaviors, including eye contact for social interaction.

**Disclosures:** H. Lee: None. K. Maeda: None. O. Hikosaka: None.
Title: Investigation on whether rule-violating behavior can be improved into rule-observing strategy

Authors: *J. BYUN\(^{1,2}\), A. ALKAHWAJI\(^{1,2}\), H.-S. SHIN\(^{1,2}\);
\(^{1}\)Ctr. for Cognition & Sociality, IBS, Daejeon, Korea, Republic of; \(^{2}\)Dept. of Basic Sci., Univ. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Following social rule is indeed orderly resolution of social conflict over limited resource. Thereby, most individuals take this as a strong strategy for the best choice. Meanwhile, how one could have decided to cling to a certain strategy is highly complicated. Here, we suggest a behavioral mechanism of strategy establishment in mice in social conflict. First, we trained pairs of mice to compete over wireless brain stimulation reward. Then, as the pairs developed mutual social rule by ‘reward zone allocation,’ we sorted out the pairs consist of rule-observing- and rule-violating mice. Those pairs went through further identical competition games. By the end, majority of rule-violating mice became rule-observing mice. We found the payoff equity and individual payoff were affected while the violating mice made attempts for following the mutual rule. We observed that mice could risk their own benefit by keeping the rule, enhancing payoff equity between their partners. These results support that altruistic decision can change socio-economic strategy in mice. To figure out what happens in their brain, a wireless EEG recording system is under development to record simultaneously in both of freely-moving mice.
Support: NINDS Grant NS091390
NIMH Grant MH112846
NARSAD YI Award 25066
MGH Fund for Medical Discovery
Howard Hughes Medical Institute

Title: Prefrontal mechanisms for tracking group behavior, reputation, and identity during three-agent interaction in macaques


Abstract: Primate group behavior poses the unique challenge of tracking others behavior, their reputation, and social identity. The ability to interact effectively within groups allows individuals to build affiliations and benefit from reciprocation with others. Furthermore, it can lead to dynamical interactions that can be affected by changes in reputation or distribution of wealth. The precise neuronal computations that underlie interactive group behavior, however, remain poorly understood. Here, we obtained multiple-neuronal recordings in the dorsal anterior cingulate cortex (dACC) and frontopolar cortex (FP) of individual rhesus macaques as they performed a structured reciprocity-based social task within a group. We devised a three-agent apparatus and social task in which three macaques interacted with each other over multiple rounds, and in which one individual could offer a food reward to one of the other two. Individuals could reciprocate past rewards that had been delivered to them by other group members in previous trials. Based on this design, we could dissociate computations associated with interactive behavior, social preference, and group dynamics. Within experimental sessions, we controlled for the animals’ position and gaze contact between actor and potential recipients. Across experimental sessions, we also controlled for group composition, social hierarchy, and the presence of conspecifics. Behaviorally, we find that the monkeys demonstrated a strategic preference for other individuals and that they favored rewarding those who reciprocated. The rate in which individuals reciprocated within a session and across sessions was reflected in different levels of reputation. Although the more dominant monkey obtained more rewards within a session, the group worked together to reduce inequality. At the neuronal-level, distinct subpopulations of dACC neurons tracked the identity of the current actor and reward recipient. In contrast, the activity of a subpopulation of FP neurons correlated with the current actor’s reputation for reciprocity. These findings reveal neurons in the primate prefrontal cortex that encode information about particular individuals, their behavior within social groups and their reciprocity. Together, they also lay the future groundwork for studying social behavior in humans and for testing neurobiology-guided clinical treatments.

Neurons in the primate amygdala simulate decision processes of social partners

Authors: *F. GRABENHORST¹, R. BAEZ-MENDOZA¹, W. GENEST¹, G. DECO², W. SCHULTZ¹;
¹Physiology, Develop. and Neurosci., Univ. of Cambridge, Cambridge, United Kingdom; ²ETIC, Univ. Pompeu Fabra, Barcelona, Spain

Abstract: Primates observe the choices of social partners to learn about the reward value of objects. Such values learned from observation not only inform own decision-making but may also provide a basis for predicting the decisions of others. Although the neuronal mechanisms underlying these social cognitive processes remain unclear, the amygdala may play an important role: the amygdala is a key component of ‘the social brain’ and its neurons participate in economic decisions (Grabenhorst et al., 2012, 2016). Here we show that monkeys’ amygdala neurons derive object values from social observation and use these values to simulate a partner monkey’s decision processes. While two monkeys alternately made reward-based choices, amygdala neurons encoded object-specific reward values learned from social observation. Dynamic activity patterns translated these values into representations of the recorded monkey’s own choices. Surprisingly, the same activity patterns unfolded spontaneously before the partner’s choices in separate neurons, as if these neurons simulated the partner’s decision-making. These neurons encoded signatures of a mutual-inhibitory decision computation, including value comparisons, value-to-choice conversions, and the difficulty of the partner’s decision, resulting in accurate predictions of partner’s choices. We refer to these neurons as ‘simulation neurons’ (Grabenhorst et al., 2019) because they dynamically encoded decision-making signatures during social observation, without decision requirements for the recorded monkey. Biophysically realistic modelling of amygdala circuits showed that simulation neurons emerge naturally from convergence between object-value neurons and self-other neurons. Our data and model suggest a
A neurobiological account of mental simulation as neural decision computation. The amygdala simulation neurons reported here could allow primates to reconstruct their social partners’ mental states and may constitute simple precursors for human mentalizing capacities, such as theory of mind. We speculate that dysfunction or absence of simulation neurons could impoverish social cognition, as is symptomatic in autism.

References


Poster
326. Emotion: Fear, Anxiety, and Pain I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 326.01/X14

Topic: G.03. Emotion

Support: NIMH Grant R01 MH112861
NIH Grant R21 ES027119
NWO and Alzheimer Nederland

Title: Chronic stress induces anxiety-associated negative valence behaviors by activating a corticotropin-releasing hormone-related molecular signature in oval bed nucleus of the stria terminalis

Authors: *P. HU1, I. MAITA1, C. KWOK1, E. GU1, M. GERGUES1, J. LIU3, M. PHAN1, Z. PANG3, D. F. SWAAB4, P. J. LUCASSEN5, T. A. ROEPKE2, B. A. SAMUELS1;
4Netherlands Inst. for Neurosci., Amsterdam, Netherlands; 5Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: The bed nucleus of stria terminalis (BNST) is a forebrain region highly responsive to stress that expresses corticotropin-releasing hormone (CRH) and is implicated in anxiety-associated negative valence behaviors. However, little is known about how chronic stress
modulates CRH signaling and neuronal activity in the BNST. We subjected C57BL6/J mice to 6-weeks of chronic variable mild stress (CVMS) and assessed effects on behavior, cellular neurophysiology and CRH-related molecular signaling in the BNST. CVMS elevated plasma corticosterone levels, induced anxiety-associated negative valence behaviors, diminished M-currents (a voltage-gated K+ current that stabilizes membrane potential and regulates neuronal excitability), and increased mEPSC amplitude in the oval nucleus of BNST (ovBNST). Additionally, the number of c-fos+, CRH+, and CRH activator pituitary adenylate cyclase-activating polypeptide (PACAP)+ cells were increased, whereas the number of CRH inhibitor striatal-enriched protein tyrosine phosphatase (STEP)+ cells were decreased in the ovBNST. CVMS also activated PKA in BNST and chronic infusion of the PKA antagonist H89 into ovBNST reversed all electrophysiological and behavioral effects of CVMS. Moreover, optogenetic activation of ovBNST mimicked the behavioral effects of CVMS. Together, these data demonstrate that ovBNST mediates the effects of chronic stress on behavior through alterations in cellular excitability and CRH signaling. These data highlight a novel role for PKA-dependent CRH signaling in the ovBNST, which has important implications for the understanding of the neural circuitry underlying stress-related anxiety disorders.


Poster

326. Emotion: Fear, Anxiety, and Pain I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 326.02/X15

Topic: G.03. Emotion

Support: R01MH108665
Phyllis & Jerome Lyle Rappaport Foundation
Eric Dorris Memorial Fellowship

Title: Dissecting the role of basolateral amygdala CRF neurons in learned and innate fear

Authors: *N. V. LUCHKINA, V. Y. BOLSHAKOV;
Psychiatry, McLean Hospital, Harvard Med. Sch., Belmont, MA

Abstract: Anxiety disorders are among the most commonly occurring mental illnesses, however, they remain difficult to address in clinical practice due to the lack of specificity in available treatments. Recent studies suggest a bidirectional role, i.e. anxiogenic or anxiolytic, for neuropeptide corticotropin releasing factor (CRF) in anxiety. This bimodality is strictly dependent on the participating cell types and neural networks involved and makes CRF an
attractive research topic and a potential therapeutic target. A complete characterization of distinct CRF subpopulations and understanding their roles, specifically within anxiety-related brain circuits, is of paramount importance for the development of treatments with desired anxiolytic effects. Towards this goal, we have identified a previously undescribed population of CRF-expressing neurons in the basolateral amygdala (CRF\textsuperscript{BLA}, constituting ~3\% of all BLA Nissl+ cells). CRF\textsuperscript{BLA} neurons demonstrated unique electrophysiological properties, distinguishing them from neighboring non-CRF\textsuperscript{BLA} cells, as identified with patch-clamp recordings in slices from Crh\textsuperscript{IRESCre::Ai14(tdTomato)} reporter mice. First, CRF\textsuperscript{BLA} neurons showed higher firing rates and input resistance. Second, CRF neurons did not display spike-frequency adaptation, in contrast to BLA pyramidal cells. Third, CRF\textsuperscript{BLA} cells were much more excitable, as indicated by a lower rheobase. Taken together, such properties suggest a possibility of more reliable activation of CRF\textsuperscript{BLA} cells by weaker synaptic inputs, which may result in an increased susceptibility for the induction of long-term plasticity. Furthermore, immunostainings revealed that a majority of CRF\textsuperscript{BLA} neurons are GAD67\+ (expressing GABA producing enzyme glutamate decarboxylase) and thus represent a subpopulation of GABAergic cells. Using ex vivo optogenetics, we determined that medial prefrontal cortex (mPFC) fibers form functional synapses on CRF\textsuperscript{BLA} neurons and, as expected, synaptic transmission at inputs to CRF\textsuperscript{BLA} cells is less efficient compared to BLA pyramidal cells. To analyze the functional role of CRF\textsuperscript{BLA} neurons in control of anxiety and fear extinction, we presently use DREADD-based methodologies to selectively manipulate (suppress or promote) the activity of CRF\textsuperscript{BLA} cells during fear extinction and assays of anxiety-related behaviors. Our initial results suggest that CRF\textsuperscript{BLA} cells may gate anxiety circuits in the BLA. In summary, these experiments can give mechanistic insights into the function of individual GABAergic neuronal subtypes in regulation of information flow in anxiety-driving microcircuits.

Disclosures: N.V. Luchkina: None. V.Y. Bolshakov: None.

Poster

326. Emotion: Fear, Anxiety, and Pain I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 326.03/X16

Topic: G.03. Emotion

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MOST 106-2410-H-010-002-MY2
MOST 107-2314-B-038-012
MOST 108-2636-H-038-001-
RD2017-005
TMU106-AE1-B32
Title: Serotonin transporter polymorphism (5-HTTLPR) biases neurophysiological response to threatening voices

Authors: *C. CHEN¹, R. M. MARTÍNEZ¹, C.-W. CHAN¹, T.-T. LIAO¹, Y. CHENG²; ¹Taipei Med. Univ., Taipei, Taiwan; ²Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: The 5-HTTLPR polymorphism has been regarded as a genetic contributor to anxiety-related traits and vulnerability to affective disorders. This polymorphism has been associated with the amygdala reactivity in response to threatening faces. However, the association between this polymorphism and the neural response to threatening voices remains unclear. This study genotyped 5-HTTLPR in ninety-five healthy Han Chinese participants, who varied in trait anxiety (STAI-T), as well as recorded their mismatch negativity (MMN), a component of the event-related potential elicited by the unexpected presence of angrily and fearfully spoken syllables embedded in a passive auditory oddball paradigm. Results showed that the short-allele (S) carriers of 5-HTTLPR appear to have significantly reduced fearful MMN than did the noncarriers. The fearful MMN amplitudes were negatively correlated with the STAI-T scores. Path analyses further indicated that 5-HTTLPR explained 4.9% of the variance in fearful MMN, which explained 6.5% of the variance in STAI-T. These findings provide preliminary evidence to corroborate the notion that 5-HTTLPR has a broad impact on social cognition. 5-HTTLPR could affect threatening voice processing already at the preattentive stage. Emotional MMN might shed some light on the emotional vigilance characteristic of trait anxiety.

Keywords: 5-HTTLPR; mismatch negativity (MMN); trait anxiety; emotion; voices


Poster

326. Emotion: Fear, Anxiety, and Pain I

Location: Hall A

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Topic: G.03. Emotion

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       NIH GM008804

Title: Increased evoked phasic dopamine release in the nucleus accumbens shell during sustained pain
Abstract: Dopamine signaling in the nucleus accumbens (NAc) regulates motivation and behavior through both tonic and phasic signaling. Tonic dopamine levels have been suggested to regulate the phasic dopamine signals in response to neuronal burst firing. We directly tested this hypothesis using fast scan adsorptive voltammetry (FSCAV) and fast scan cyclic voltammetry (FSCV) to measure tonic and phasic dopamine signaling, respectively, in the NAc shell in lightly anesthetized male, S.D. rats. Prior to dopamine measurements, the depth of anesthesia was standardized across animals by establishing a 6.3 +/- 0.2 second latency to a heat-induced tail-flick response. Application of capsaicin to the eye produced a significant increase in phasic dopamine signals as well as a hypodopaminergic state in the NAcSh that persisted for approximately two minutes; tonic dopamine levels were reduced by 22 +/- 8% in response to capsaicin. Following recovery of dopamine levels to pre-capsaicin baselines, application of capsaicin to the alternate eye again produced a hypodopaminergic state of similar magnitude. Electrical stimulation of the medial forebrain bundle one minute after the instillation of capsaicin on the eye, during the hypodopaminergic state, increased phasic dopamine release by 48 +/- 18%. These data show that phasic dopamine signals can be elicited by noxious stimuli, that ongoing pain produces a hypodopaminergic state, and directly demonstrate that phasic dopamine signals are increased when tonic dopamine levels are low. The data suggest that salient stimuli produce enhanced phasic dopamine signaling that may underlie behaviors such as impulsivity and impaired decisions that are observed in hypodopaminergic conditions including chronic pain.

Authors: *H. J. SZKUDLAREK, B. PEREIRA, M. DE FELICE, B. RASHEED, T. JUNG, S. N. WHITEHEAD, S. R. LAVIOLETTE;
Univ. of Western Ontario, London, ON, Canada

Abstract: Cannabis produces complex psychotropic effects across various brain circuits. Previous research in our laboratory has demonstrated that the two major phytocannabinoids in cannabis, delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) produce highly divergent effects on anxiety-related behaviours, cognition and emotional memory formation. In general, the anxiogenic effects of cannabis are linked to the actions of tetrahydrocannabinol (THC) whereas its anxiolytic effects have been associated with cannabidiol (CBD), the two main compounds produced by the plant. THC interacts with the endocannabinoid system via actions on CB1 receptors while CBD targets multiple receptors including the serotonergic 5-HT1a subtype. The basolateral amygdala (BLA) is a key brain region controlling affective and social behaviors and is functionally connected with other brain regions responsible for modulation of anxiety states like the prefrontal cortex (PFC) and ventral hippocampus (vHipp). In the present study we used behavioral, anatomical and electrophysiological approaches to study the effects of direct intra-BLA infusion of THC, CBD or its combination. We show that infusion of THC (100 ng) induces anxiolytic effects in the light-dark-box test while having no effects on anxiety in elevated plus maze and social motivation or social recognition. Similarly, CBD infusions (500 ng) had no effect on anxiety in the light-dark-box, elevated plus maze or on social behavior. Interestingly, co-infusion of THC (100ng) and CBD (500ng) induced strong anxiolytic effects expressed by longer durations spent in the open arm of the elevated plus maze and in the light compartment of the light-dark-box. Single unit and local field recordings in urethane anesthetized rats revealed that intra-BLA infusion of THC led to increased neuronal frequency, bursting rates and elevated low and high-gamma oscillations in the prefrontal cortex whereas vHipp activity was not affected. In contrast, intra-BLA infusion of CBD greatly reduced firing of vHIPP cells while having no effect in the PFC. Interestingly, THC+CBD induced no change in neuronal firing of PFC neurons while hippocampal cells were still inhibited, suggesting that CBD may counteract THC effects but not the opposite. Moreover, using retrograde tract-tracing with cholera toxin we show that PFC- and Hipp-projecting BLA neurons comprise two non-overlapping populations. These data suggest that intra-BLA THC and CBD target different neuronal BLA populations to affect anxiety behavior via dissociable pathways to the vHipp and PFC.


Poster

326. Emotion: Fear, Anxiety, and Pain I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 326.06/X19
Topic: G.03. Emotion

Support: VA 1I01BX003893-01A1
NIAAA T32 AA007577

Title: Fear conditioning regulates endocannabinoid degradation

Authors: *M. A. FAROOQ, J. LIU;

Abstract: Post-traumatic stress disorder, PTSD, is a debilitating psychiatric disorder that is characterized by a cluster of symptoms that include intrusive memories, avoidance, hyper arousal, and negative mood and feelings. Individuals with PTSD exhibit dysregulation of the fear system and enhanced stress response. Several animal models exist to study this extremely heterogeneous disorder. For instance, a fear conditioning paradigm is used to understand consolidation and extinction of fear memories, whereas predator odor exposure is used to model the psychological stress and emotional learning. The cerebellum, a brain structure traditionally known for motor coordination, is also implicated in emotional learning, and it is required for consolidation of associative fear memory. Endocannabinoid signaling regulates both memory processing and mood. Clinical studies have shown that individuals with PTSD exhibit reduced levels of circulating endocannabinoids. However, the mechanism underlying this decrease in endocannabinoids is unclear. Monoacylglycerol lipase, MAGL, is an enzyme that degrades 2-arachidonoyl glycerol, 2-AG, the major endocannabinoid in the cerebellum. We tested whether fear conditioning and predator odor stress upregulates MAGL activity, which leads to reduction in endocannabinoid tone. We used a fluorescence-based assay to assess MAGL activity. In a fear conditioning paradigm, mice were either subjected to a tone that is paired with a foot shock or an unpaired protocol as control. Twenty-four hours after fear conditioning training, we quantified MAGL enzymatic activity in the lobule V/VI of the cerebellar vermis, a region which is critical for fear memory consolidation and emotional learning. We found that fear conditioning increased MAGL activity by 70 ± 47% as compared to the unpaired control. This learning-induced increase in MAGL activity is predicted to accelerate 2-AG degradation and reduce endocannabinoid signaling. In contrast when mice were exposed to fox urine, MAGL activity remained unchanged as compared to the controls. Therefore an increase in MAGL activity may contribute to associative fear memory formation and the PTSD symptoms of intrusive memories and hyper arousal.

Disclosures: M.A. Farooq: None. J. Liu: None.

Poster

326. Emotion: Fear, Anxiety, and Pain I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 326.07/X20
Title: Role of PSD95 and nNOS interaction in gene regulation following fear conditioning and implications for molecular mechanisms underlying PTSD

Authors: *J. PATEL¹, A. I. MOLOSH⁴, E. T. DUSTRUDE², A. SHEKHAR³;
¹Dept. of Psychiatry, ³Indiana CTSI, ¹Indiana Univ. Sch. of Med., Indianapolis, IN; ⁴Psychiatry, IU Sch. of Medicine,, Indianapolis, IN

Abstract: Fear and anxiety are evolutionarily developed responses to perceived or anticipated threats. Normal learning can produce avoidance behavior that promotes survival, but excessive and persistent fear after trauma can lead to development of phobias and post-traumatic stress disorder (PTSD). Involvement of the amygdala in fear acquisition is very well described and requires activation of N-methyl-D-aspartic acid receptors (NMDARs). At a cellular level, NMDAR activation leads to production of nitric oxide (NO) by a process that is mediated by interaction between postsynaptic density protein 95 (PSD95) and nitric oxide synthase (nNOS). Our laboratory has previously shown that fear conditioning enhances PSD95-nNOS interaction and that the small-molecule ZL006 inhibits this interaction. Treatment with ZL006 attenuates rodent cued-fear consolidation, impairs long-term potentiation of neurons, and prevents fear-mediated shifts in glutamatergic receptor current densities in the basolateral amygdala (BLA). In addition, we have demonstrated that treatment with ZL006 avoids adverse effects on cognition that are observed following direct antagonism of NMDARs. To further elucidate mechanisms underlying the role of the PSD95-nNOS-NO pathway in conditioned fear, the current study utilized auditory cue paired-fear conditioning and RNA-sequencing of BLA tissues to examine fear conditioning-mediated gene changes. Expression of 403 genes was altered in the BLA following fear expression, and of these genes, 60 were restored by systemic treatment with ZL006. Network data analysis and gene ontology enrichment analysis of these genes with Ingenuity Pathway Analysis and DAVID software found that cell-cell interaction and cognition-related pathways were significantly altered. Our results reveal novel genetic targets that underlie plasticity of fear-memory circuitry via their contribution of NMDAR-mediated fear consolidation and can inform future strategies for targeting fear related disorders like PTSD.

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Poster

326. Emotion: Fear, Anxiety, and Pain I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 326.08/X21
Title: Adenosine A$_2$A receptors control the extinction of fear memories

Authors: S. L. REIS$^1$, V. LOURENÇO$^1$, R. A. CUNHA$^2$, P. M. CANAS$^1$, *A. P. SIMÕES$^1$;
$^1$Ctr. for Neurosci. and Cell Biol., Coimbra, Portugal; $^2$Ctr. for Neurosci. and Cell Biology; Fac. of Medicine-University of Coimbra, Coimbra, Portugal

Abstract: Adenosine A$_2$A receptors (A$_2$AR) control the acquisition of fear memories$^{1,2}$. Now, we studied the role of A$_2$AR in fear extinction. C57BL/6 mice were contextually fear conditioned (1$^{st}$ day: habituation followed by 3 footshocks) and then subjected to fear extinction (2$^{nd}$-4$^{th}$ day: re-exposure to the same context without footshock) and their freezing behavior was monitored. The role of A$_2$AR was investigated using a selective antagonist, SCH58261 (SCH) applied either intraperitoneally (ip, 0.1 mg/kg) or directly (50 nM) in cannulated basolateral amygdala (BLA) or ventral hippocampus (VH), 1h before each extinction session. Extinction memory was tested on the 5$^{th}$ day by re-exposing mice to the conditioning chamber. Electrophysiology was performed at two time periods - during extinction training and after the memory test - in transverse VH slices and horizontal BLA-containing slices from naïve (context exposed), fear conditioned (ACQ), fear conditioned + extinction (EXT) and fear conditioned + extinction in the presence of SCH (EXT+SCH) groups. Field excitatory postsynaptic potentials (fEPSP) were measured in VH CA3-CA1 synapses and population spikes (PS) were evaluated in BLA. Long-term potentiation (LTP) was triggered by high-frequency stimulation. Both ip and local blockade of A$_2$AR accelerated fear extinction: 23h after the first ip injection of SCH, mice already froze less than controls (EXT+SCH: 16.3±2.7% of freezing vs EXT: 29.4±4.1%). The same was observed when SCH was administered in VH (EXT+SCH: 14.5±3.9% vs EXT: 34.3±5.7%) and in BLA (EXT+SCH: 14.6±2.9% vs EXT: 26.2±2.9%). Moreover, SCH administration did not affect locomotion, anxiety or passive extinction of fear. During fear extinction, both ACQ and EXT had lower post-tetanic potentiation in BLA comparing to naïve and EXT+SCH (ACQ: 14.4±5.3% of potentiation and EXT: 19.1±4.5% vs naïve: 54.3±13.4% and EXT+SCH: 50.4±10.6%). In VH, LTP in ACQ was lower comparing to naïve and EXT+SCH (ACQ: 16.7±8.5% above baseline vs naïve: 48.8±9.5% and EXT+SCH: 48.5±3.3%). After the memory test, basal neurotransmission was increased in BLA of ACQ comparing to EXT and naïve but only the EXT+SCH group was equal to the naïve. In VH, LTP was now increased in ACQ compared to naïve and EXT+SCH (ACQ: 87.0±11.2% above baseline vs naïve: 49.2±9.3% and EXT+SCH: 51.4±9.6%). In conclusion, A$_2$AR blockade accelerates fear extinction by altering neuronal plasticity in BLA and in VH.$^1$- Simões et al., Neuropsychopharmacol. 2016, 41: 2862-2871; 2- Wei et al., Biol. Psychiatry. 2014, 75:855-63

Disclosures: S.L. Reis: None. V. Lourenço: None. R.A. Cunha: None. P.M. Canas: None. A.P. Simões: None.
Anxiolytic effect of ethanol on locomotor activity in rats

Early-life stress is associated with increased vulnerability to alcohol addiction. The exposure to reward uncertainty typical of training can reduce emotional self-medication (ESM) in rats with high levels of anxiety. We demonstrated, locomotion and anxiety behavior in Wistar (nonselected) male rats exposed to 32% to 4% sucrose downshift event in a consummatory successive negative contrast (cSNC) situation were given a two bottle, 2 h preference test immediately after consummatory training, and finally to the open field test (50 cm x 50 cm) to assess locomotor activity and anxiety behavior. One of the two bottles contained 2% ethanol or water different groups (the second bottle contained water for all groups). Because ethanol has anxiolytic properties in tasks involving reward loss, oral consumption after extinction sessions was interpreted as anti-anxiety or ESM. Three additional groups received the same postsession preference tests, but were always exposed to 4% sucrose during consummatory training. Rats showed the cSNC effect, suppressing consummatory behavior after the downshift relative to unshifted controls. This effect was accompanied by a selective increase of ethanol oral intake during the initial downshift sessions. Such increased fluid preference did not occur in animals with access to water or unshifted controls groups with postsession access to the anxiolytic during the postsession test. These results suggest that ESM may reverse symptoms of anxiety in animal model. The effect ESM increased locomotion activity in the open field relative to water controls. The origin of addictive behavior is often linked to the rewarding effects of drugs of abuse.

Disclosures: L. Manzo: None. A.C. Tafolla: None. A. Gordillo: None. C. Torres: None.
Poster

326. Emotion: Fear, Anxiety, and Pain I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

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ControlExtraData.DynamicPosterDisplay:
Dynamic Poster

Topic: G.03. Emotion

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NSF GRFP

Title: Neural control of organized escape from multimodal threats

Authors: W. WANG¹, P. J. SCHUETTE², B. TOBIAS¹, J. NAGAI⁴, F. M. REIS¹, A. L. SEVERINO⁵, M. CHAKERIAN¹, L. LIN¹, S. LEONARD¹, M. SEHGAL⁶, A. J. SILVA⁷, C. J. EVANS², C. M. CAHILL³, B. S. KHAKH⁴, *A. ADHIKARI¹;
¹Psychology, ³Psychiatry and Biobehavioral Sci., ²UCLA, Los Angeles, CA; ⁴Dept Physiol, Univ. of California Los Angeles Dept. of Physiol., Los Angeles, CA; ⁵Psychiatry and Biobehavioral Sci., ⁶Neurobio., Univ. of California Los Angeles, Los Angeles, CA; ⁷Dept Neurobiol, UCLA Med. Ctr., Los Angeles, CA

Abstract: Rapidly escaping from imminent danger is paramount for survival. However, the circuits controlling adaptive escape from threats can also provoke highly debilitating panic attacks in humans. Despite the biological and clinical relevance of panic-related escape, escape circuits are not well-understood. In particular, naturalistic organized escape that takes into account the geometry of the environment to promote flight through optimal escape routes has not been investigated. We optogenetically activated seven brainstem and hypothalamic nuclei that were shown or hypothesized to induce escape. Activation of six nuclei induced escape-related actions, such as jumping and running, but these actions were not coordinated correctly to allow escape from a geometrically complex environment. In contrast, activation of the hypothalamic dorsal premammillary nucleus (PMd) promoted versatile context-dependent flight, as mice jumped to escape only in the absence of a climbing route. In the presence of a climbing route, mice climbed instead of jumping to escape out of a complex environment. Chemogenetic inhibition and excitation of the PMd respectively impaired and increased organized escape from a wide variety of threats, including a live predator, carbon dioxide, a thermal threat and a shocking grid. Fiber photometry recordings show that the PMd is activated prior to escape,
regardless of the threat used or of the specific motor actions involved in flight. As the PMd is the strongest input to the panic-inducing dorsal periaqueductal gray (dPAG), we hypothesized that the PMd induces escape through the PMd-dPAG projection. Indeed, optogenetic inhibition of the PMd-dPAG projection decreased threat-induced organized escape. All results were replicated in 2-5 months old male and female mice (n=7-12 per group), the order of assays and drugs were counterbalanced. We identify the PMd as the first circuit shown to be sufficient and necessary for inducing organized context-dependent escape from numerous threats.


Poster

326. Emotion: Fear, Anxiety, and Pain I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 326.11/X24

Topic: G.03. Emotion

Support: NIH Grant F32-NS106720
NIH Grant R37-NS39395

Title: The medial cerebellar nucleus augments IPSC amplitude in the ventrolateral periaqueductal gray freezing circuit by activating local dopaminergic neurons

Authors: *C. E. VAAGA, I. M. RAMAN;
Neurobio., Northwestern Univ., Evanston, IL

Abstract: To avoid predation, mice engage in stereotyped behaviors such as innate freezing in response to threatening stimuli. Freezing requires activation of the ventrolateral periaqueductal gray (vlPAG), and stimulation of glutamatergic neurons in the vlPAG elicits freezing in naïve mice. Lesions of the cerebellar vermis also reduces freezing in rats, suggesting that the cerebellum may interact with freezing circuitry; however, the underlying synaptic mechanisms have not been investigated. Here, using anatomical and slice electrophysiological experiments in mice (male and female; ~p21-p90), we found that the medial cerebellar nucleus (mCbN) directly projects to the vlPAG where it modulates the synaptic efficacy of IPSCs in freezing-specific neurons. We recorded from putative freezing-specific glutamatergic neurons, which express the transcription factor Chx10. Chx10+ neurons project to the ventral gigantocellular nucleus of the reticular formation, which projects to the spinal cord to mediate freezing. Functionally, although the mCbN neurons were glutamatergic, optogenetic stimulation of ChR2-expressing mCbN neurons in slice experiments robustly increased electrically evoked IPSCs in Chx10+ neurons
(control: 180±42 pA; +mCbN optogenetic stimulation: 231±48. pA, n=7). We reasoned that the IPSC augmentation may result from cerebellar activation of local dopaminergic/noradrenergic neurons in the vlPAG. Consistent with this hypothesis, tyrosine hydroxylase (TH) positive neurons in the vlPAG received direct mCbN excitatory input (-31.9±13.1 pA, n=5 of 6 cells). Either bath applying dopamine, or optically stimulating TH-positive neurons mimicked mCbN-dependent IPSC augmentation. Finally, in wild-type mice, bath applying D1 and D2 receptor antagonists abolished mCbN-dependent IPSC augmentation (stim without blockers: 135.5±8.6% of control, stim with blockers: 101.9±1.0% of control, n=7, 4). These results suggest that the mCbN modulates IPSC amplitude in freezing-specific neurons, which are thought to be primarily driven through disinhibition following amygdala activation. Furthermore, given the high basal firing rates of mCbN cells, the data suggest that the dopaminergic tone in the vlPAG may be regulated by cerebellar activation.

Disclosures:  C.E. Vaaga: None. I.M. Raman: None.

Poster

326. Emotion: Fear, Anxiety, and Pain I

Location: Hall A

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Program #/Poster #: 326.12/X25

Topic: G.03. Emotion

Support: CIFAR Azrieli Global Scholar award
YNPRC - Office of Research Infrastructure Programs ODP51OD11132

Title: Dissecting projection-specific contributions of the zona incerta to fear

Authors: *A. VENKATARAMAN1, B. G. DIAS2,3;

Abstract: Fear expression towards threat-associated stimuli is an adaptive behavioral response. In contrast, generalization of fear responses toward non-threatening cues is maladaptive and is associated with psychopathology. Sensorimotor integration is critical to express appropriate and inhibit inappropriate fear responses towards fearful and neutral stimuli, respectively. The mammalian subthalamic region called the zona incerta (ZI), typically considered a sensorimotor relay, has been shown to play a crucial role in modulating fear responses. We have recently shown that the ZI can bidirectionally modulate fear generalization - decreasing ZI activity facilitated fear generalization while increasing ZI activity abolished fear generalization. Furthermore, we demonstrated that GABAergic cells in the ZI could modulate fear generalization. In this study, we were interested in understanding how projections of GABAergic cells in the ZI influence fear-related behavior. GABAergic cells in the ZI send strong projections
to the thalamic nucleus reuniens (RE) and midbrain periaqueductal gray (PAG); regions that play important roles in fear generalization, fear learning and fear expression. More specifically, the RE mediates specificity and long-term maintenance of fear memories, while the PAG generates and orchestrates fear and defensive responses. Using optogenetic manipulations, our preliminary results suggest that GABAergic cells in the ZI that project to the RE and the PAG differentially alter fear responses. Together, our results suggest a prominent role for GABAergic projections from ZI in calibrating fear responses.

Disclosures: A. Venkataraman: None. B.G. Dias: None.

Poster

326. Emotion: Fear, Anxiety, and Pain I

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Topic: G.03. Emotion

Support: NIH Grant T32MH015144-39
Leon Levy Foundation fellowship in neuroscience
Hope for Depression Research Foundation MPPN8883-1

Title: Optogenetic manipulation of ventral hippocampal parvalbumin interneurons during innate and learned fear

Authors: *W.-L. CHANG1, J. C. JIMENEZ2, E. Z. AKGUNGOR1, K. S. OTTE2, J. D. BARBOSA2, A. Z. HARRIS1, R. HEN1;

Abstract: Background: Lesion, pharmacological, and optogenetic studies in rodents have shown that the ventral hippocampus is important for the expression of anxiety-like behavior, particularly the output region CA1. The ventral CA1 (vCA1) exhibits increased neural oscillations in the theta frequency range (4-12 Hz) in anxiogenic environments, and this synchronous oscillation is coordinated by interneurons, such as parvalbumin (PV)-expressing interneurons, which target the soma and axons of excitatory cells. In this study, we determine the behavioral effects of acute manipulation of PV cell activity in the vCA1 using optogenetics during innate and learned anxiety-like behaviors. Methods: Male and female PV-Cre c57BL/6J mice received bilateral injections of adeno-associated virus (AAV) into the vCA1 and fiber optic implants. AAV containing Cre-dependent eNpHR3.0 was used for inhibitory experiments, while AAV with Cre-dependent hChR2(E123A) was used for excitatory experiments. Cre-dependent eYFP or mCherry AAV was used for controls. Behavioral tests were performed with 10 mW light delivery in timed blocks. Time blocks consisted of constant light for inhibitory experiments,
while 5 msec pulses at 20 Hz were used in excitatory experiments. Behaviors included the open field, elevated plus maze (EPM), elevated zero maze (EZM), and contextual fear conditioning (CFC). Results: Both excitation and inhibition of PV cells increased avoidance of innately anxiogenic environments. PV cell stimulation during the encoding/conditioning phase of CFC had no effect on freezing behaviors the next day. Experiments of PV cell inhibition during the retrieval/testing day of CFC are ongoing, but preliminary evidence suggests that PV inhibition decreases freezing behavior on Day 2 of CFC. Discussion: Our findings demonstrate that acute manipulation of PV interneurons in the vCA1 is sufficient to alter anxiety-related behavior. Unexpectedly, both stimulation and inhibition of PV cells increased avoidance of innately anxiogenic environments. It is possible that rhythmic stimulation of PV cells at 20Hz increased synchronous activity in the vCA1 to cause increased anxiety-like behavior. Inhibitory experiments were consistent with previous findings from vCA1 pyramidal cell manipulations. Inhibiting vCA1 pyramidal cell activity increases time spent in open arms of the EPM, while stimulation during CFC retrieval disrupts normal expression of conditioned fear. PV cell inhibition would be expected to disinhibit pyramidal cells, and therefore increase their activity. Our results from inhibitory experiments are consistent with that prediction.


Poster

326. Emotion: Fear, Anxiety, and Pain I

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Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 326.14/X27

Topic: G.03. Emotion

Support: National Natural Science Foundation of China (Grant 31661143038) Basic Research Project of Shanghai Science and Technology Commission (No. 16JC1400101 to L.L.)

Title: Parallel hippocampal-to-amygdala circuits distinct reactions from innate to learned threat

Authors: *Y. GU, M. WU, L. WANG, L. LIN; Inst. of Brain Functional Genomics, East China Normal Univ., Shanghai, China

Abstract: Innate behaviors are genetically determined and stereotyped. Whereas in the learned circuit, rapid learning of new CS-US association causes rapid overwriting of existing memories. How neural circuits processing innate fear instruct an adaptive learning process (for example, freezing in Pavlovian threat conditioning) in rodent? From a neuroscience perspective, this question might lead us to investigate the neural basis of learning and (inherently) memory. In this endeavor, we propose that distinct brain circuits guide different behaviors underlie
fundamental properties: feedforward, feedback and self-organizing networks. We focused on the hippocampal-amygdala circuits because (i) the extensive knowledge has confirmed their roles in innate and learned defensive behaviors; (ii) both brain regions employ a one-to-many wiring configurations to orchestrate multiple aspects of defensive behaviors; (iii) under certain circumstances, either activate or inhibit the same pathway can promote a broad spectrum of defensive behaviors.

We use different viral strategies for tracing retrograde and anterograde transsynaptic neural pathways within the dorsal hippocampal CA1 area and the basolateral/central amygdala in genetically defined populations of neurons in mice. Then we use chemogenetics and multi-channel recording techniques to manipulate and monitoring the circuits during defensive behaviors. In the mean while combining with CAMPARI to label the "snapshot firing neurons” for distinct behavioral readouts. The results potentially help us to better understand the interactions between hippocampus and amygdala circuits by segregated, integrated or antagonistic mechanisms in a nascent stage.


Poster

326. Emotion: Fear, Anxiety, and Pain I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 326.15/X28

Topic: G.03. Emotion

Support: NIH Grant R01MH119102

Title: Encoding fear of heights by single neurons in basolateral amygdala

Authors: *J. LIU¹, L. LIN², D. V. WANG³;

Abstract: Fear of heights, and related avoidance of heights, are essential for survival and everyday life. However, irrational or excessive fear of heights can lead to acrophobia, which may have a serious impact on people’s lives. Unfortunately, the neuronal activities and circuitry of fear of heights, acquired in the absence of any previous aversive experience (“visual cliff”, Gibson & Walk, 1960), have not yet been systematically studied. Converging evidence suggests that the amygdala is a key brain region involved in both learned and innate fear. By means of multi-channel tetrode recording and heart rate measurement in freely behaving mice upon high place exposure, we identified a distinct neuronal population in basolateral amygdala (BLA) that encodes the fear of heights. Unlike the prominent rapid habituation effects of amygdala neurons to conditioned fear, the high-place innate fear neurons presented greater resistance to habituation
by exhibiting sustained activation (up to 30 min). Transition between ground and a high platform, the high-place fear neurons exhibited an “all-or-none” responsive pattern, which is likely mediated by visual and/or vestibular inputs. Importantly, contrary to the responsive patterns of BLA anxiety-related neurons we reported before, the high-place fear neurons only respond to heights, but not anxiogenic conditions such as the open-field induced anxiety. To identify regulatory afferents that either activate or inhibit the firing of the BLA high-place fear neurons, we injected AAV viruses that encode Channelrhodopsin-2 (ChR2) into major BLA-projecting regions, including the prefrontal cortex (PFC) and lateral entorhinal cortex (LEnt), which have previously been implicated in fear processing. Then, we implanted an optrode (an optical fiber surrounded by eight tetrodes) into the BLA to examine how photoactivation of these afferents may potentially affect high place-fear neurons’ activities. Our results revealed that photostimulation of the PFC-to-BLA afferents activates a subpopulation of non-high-place fear BLA neurons. More interestingly, photostimulation of the LEnt-to-BLA afferents evokes differential effects on BLA neurons: a subpopulation of BLA neurons can be activated, while the majority of the BLA neurons, including the high-place innate fear neurons, are inhibited, indicating a LEnt-to-BLA neural circuit involved in regulating fear of heights. These findings provide insight into a better understanding of the neural circuits of fear of heights and may have clinical implications for treatment of excessive fear and anxiety disorders, such as acrophobia.


Poster

326. Emotion: Fear, Anxiety, and Pain I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 326.16/X29

Topic: G.03. Emotion

Title: Apical intercalated cell cluster: A novel sensory regulator in the amygdala

Authors: D. ASEDE, D. DODDAPANENI, A. CHAVEZ, J. OKOH, S. ALI, *M. BOLTON; Max Planck Florida Inst., Jupiter, FL

Abstract: It is increasingly appreciated that distinct classes of GABAergic neurons regulate different aspects of information processing in the amygdala. One unique class is the amygdala intercalated cells (ITCs) which are masses of GABAergic neurons organized in clusters around the basolateral amygdala (BLA). Recent evidence indicates that different fear states activate distinct ITC clusters, highlighting the distinct nature and function of each ITC cluster in fear-related behaviors. Although the role of a few of the ITC clusters has been studied, the functional role of apical ITCs (apITCs) is unknown. Here, using optogenetics, we show that apITCs receive direct sensory input from thalamic and cortical areas. Upon fear conditioning and memory retrieval, thalamic inputs undergo pre and postsynaptic plastic changes, indicative of their
involvement in fear behaviors. 3D reconstruction of biocytin-filled aplITCs revealed that they arborize extensively within the aplITC cluster itself and also innervate the dorsal striatum and LA. We further showed that aplITCs provide sensory feedforward inhibition to LA principal cells, a putative mechanism for controlling plasticity during fear learning.


Poster

326. Emotion: Fear, Anxiety, and Pain I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 326.17/X30

Topic: G.03. Emotion

Support: NIH Grant 1R21MH109722-01A1
NIH Grant 1R21MH113103-01A1

Title: Reshaping fronto-amygdalar circuitry through electrically forced hebbian plasticity

Authors: *F. J. DA SILVA, M. J. SCHATZA, E. B. BLACKWOOD, M.-C. LO, A. S. WIDGE; Psychiatry, Univ. of Minnesota, Minneapolis, MN

Abstract: Fronto-amygdalar circuits (IL-BLA in rats, and prefrontal cortex-Amygdala in humans) are implicated in fear learning and fear extinction. Altering the functional connectivity of these circuits may facilitate the extinction of learned fear responses, which may reduce the debilitating effects of fear-related diseases such as post-traumatic-stress-disorder (PTSD). At the neuronal level, when one neuron regularly contributes to the firing of another, the connection between them is strengthened (Hebb’s rule). Similarly, at the regional neuronal network level, paired pulse electrical stimulation (the activation of many neurons in one area followed by similarly broad activation of neurons in another) also leads to temporarily increased connectivity between the regions involved in the paired stimuli. However, paired electrical stimulation likely activates both inhibitory and excitatory sub-populations. It is not clear whether such broad alteration of connections will translate to lasting changes in functional connectivity and behavior. Rather than modulating the activity in both regions, a more efficient approach to Hebbian plasticity might be to stimulate a downstream region only after the activity in another has naturally gone above some threshold. This more closely mimics natural activity patterns, and thus might produce more lasting effects. We are currently implementing that protocol in the IL-BLA circuits, inducing Hebbian-like connectivity enhancements by stimulating one BLA region in response to increases in IL activity. We will compare multiple variations on this stimulation, including the definition of an IL activity increase (ensemble spiking rate vs. power in the high gamma local field potential), timing of BLA stimulation relative to IL activity, and amplitude of
the BLA stimulation. We will also explore single pulse BLA stimulation versus stimulation that mirrors the activity recorded in the originating area, to determine which combination of parameters results in the greatest and longest-lasting changes in connectivity. This poster will present the initial results from those experiments, demonstrating that this activity-dependent stimulation facilitates IL-BLA synapses and that facilitation requires precise stimulation timing. Future work will use these protocols to modulate fear extinction in rats, as a step towards translating the technique into humans.


**Poster**

**326. Emotion: Fear, Anxiety, and Pain I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 326.18/X31

**Topic:** G.03. Emotion

**Support:** CAPES
PNPD/CAPES
CNPQ (309201/2015-2)

**Title:** Environmental enrichment reverses anxiogenic-like behavior induced by cohabitation with mice submitted to chronic neuropathic pain

**Authors:** *I. M. CARMONA* ¹, ², P. E. CARNEIRO DE OLIVEIRA ¹, A. CANTO-DE-SOUZA ¹, ², ³;

¹Dept Psychology-Psycobiology group/UFSCar, São Carlos, SP, Brazil; ²Joint Grad. Program in Physiological Sci. UFSCar/UNESP, São Carlos, SP, Brazil; ³Grad. Program in Psychology/UFSCar, São Carlos, SP, Brazil

**Abstract: AIM:** Animal models are fundamental tools for understanding the neurobiological bases implicated in anxiety modulation. Our group recently demonstrated that cohabiting with a conspecific submitted to chronic pain produces enhancement anxiety-like behaviors in observer mouse. Although their anxiety treatment is usually pharmacological, alternative interventions such as environmental enrichment may prevent or reverse psychological disorders. Therefore, the present study aimed to evaluate the effects of environmental enrichment in anxiety-like behavior tested on the elevated plus-maze (EPM) in mice that cohabiting with a cagemate in neuropathic pain. **METHODS AND RESULTS:** Male Swiss mice (n=10-14/group) were submitted to 28 days protocol. On the 21st after birth (weaning) mice were housed in pairs for 14 days to familiarity establishment. On the 14th day, the animals were divided into four groups: cagemate nerve constriction exposed to standard environmental (CNC+SE), in which one animal
from each pair was subjected to sciatic nerve constriction and cagemate nerve constriction exposed to EE (CNC+EE); cagemate sham exposed to standard environmental (CS+SE), one animal from each pair was subjected to the same surgery but without constriction and cagemate sham exposed to EE (CS+EE). On the 28th day all groups were exposed to elevated plus-maze. Two-way ANOVA followed by post-hoc Duncan test revealed that cohabitation with mice in sciatic nerve constriction condition induces anxiogenic-like behavior and enriched environment reverted this effect in percentage of open arm entries (CS/SE: 34.6±5.6; CNC/SE: 6.1±1.9*; CS/EE: 16.0±5.4#; CNC/EE: 29.1±5.1*#) and percentage of open arm time (CS/SE: 14.9±3.1; CNC/SE: 1.6±0.7*; CS/EE: 6.7±2.9#; CNC/EE: 13.1±2.8#) and closed arm entries (CS/SE: 6.9±0.7; CNC/SE: 9.1±0.6; CS/EE: 8.9±1.0#; CNC/EE: 12.2±1.4*#) of the EPM. Data are presented as mean ± SEM. *P<0.05 versus respective CS group. #P<0.05 versus respective SE group.**CONCLUSIONS:** These findings suggest that EE reverses anxiogenic-like behavior induced by cohabitation with mice submitted to chronic sciatic nerve constriction.

**Disclosures:** I.M. Carmona: None. P.E. Carneiro de Oliveira: None. A. Canto-de-Souza: None.

**Poster**

**326. Emotion: Fear, Anxiety, and Pain I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 326.19/X32

**Topic:** G.03. Emotion

**Support:** FAPESP Grant 2017/25409-0
CNPq Grant 306556/2015-4
FAPESP Grant 2016/24568-4

**Title:** Inhibition of the left medial prefrontal cortex (mPFC) prolongs the anxiogenic effect induced by social defeat in mice: Modulation by NMDA receptors in the right hemisphere

**Authors:** *R. L. NUNES-DE-SOUZA*¹, N. SANTOS-COSTA², D. C. MASCARENHAS², G. VICTORIANO²;
¹Pharmacol., Univ. Estadual Paulista, UNESP, Araraquara, Brazil; ²Pharmacol., Univ. Estadual Paulista - UNESP, Araraquara, Brazil

**Abstract:** Injections of Cobalt chloride (CoCl₂, a nonselective synaptic inhibitor) and NOC-9 [a nitric oxide (NO) donor], respectively, into the left and right medial prefrontal cortex (L- and RmPFC) provoke anxiety in mice. Also, LmPFC inhibition immediately followed by a single social defeat stress (SDS) lead to anxiogenesis in mice exposed to the elevated plus maze (EPM) 24 hours later. Given that glutamate NMDA (N-methyl-D-aspartate) receptors are densely present in the mPFC, we investigated (i) the time course of the anxiogenesis induced by the
LmPFC inhibition + SDS and (ii) the effects of intra-RmPFC injection of AP-7 (a NMDA receptor antagonist) on this long-lasting anxiety. Male Swiss mice received intra-LmPFC injection of CoCl₂ (1mM) and 10 min later were subjected to a single SDS episode and then (i) exposed to the EPM 2, 5, or 10 days later or (ii) 2 days later, received intra-RmPFC injection of AP-7 (0.05 nmol) and were exposed to the EPM. EPM analysis comprised the spatiotemporal measures [percentage of entries and time in open arms (%OE; %OT) and frequency of closed arms entries (CE)]. Results showed that the combination LmPFC inhibition + SDS reduced the %OE and %OT 2, 5 or 10 days later. In addition, the expected anxiogenic effects induced by the LmPFC + SDS were absent in animals that received intra-RmPFC injection of AP-7 two days later. Importantly, drug injections outside the dorsal region of the mPFC (i.e. prelimbic and cingulate cortex, area 1) did not produce significant behavioral effects on anxiety. These results suggest that the integrity of the LmPFC is important for mice to properly cope with SDS, and that NMDA receptor blockade in the RmPFC facilitates the resilience phenomenon to the anxiogenic effects induced by the SDS in male mice.


Poster

326. Emotion: Fear, Anxiety, and Pain I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 326.20/X33

Topic: G.03. Emotion

Support: UFSCar
CNPQ (309201/2015-2)
FAPESP (2015/00006-4)

Title: Dissimilar activation pattern of the anterior cingulate cortex, insula and amygdaloid complex in the modulation of pain empathy in mice

Authors: *A. CANTO-DE-SOUZA¹²³, D. BAPTISTA-DE-SOUZA¹, R. NUNES-DE-SOUZA³⁴;
¹Psychobiology Group, Dept of Psychology, UFSCar, São Carlos, SP, Brazil; ²Grad. Program in Psychology/UFSCar, São Carlos, SP, Brazil; ³Joint Grad. Program in Physiological Sci. UFSCar/UNESP, São Carlos, SP, Brazil; ⁴Pharmacol, FCF/Unesp, Araraquara, SP, Brazil

Abstract: The pain experience not only includes nociceptive and nocifensive but also emotional-affective and cognitive components. It has been shown that see a conspecific with pain can increase or decrease the sensation of pain in the observer mouse. Recently, our research group has shown that living with a conspecific in chronic pain increase the nociceptive responses in
animals subjected to the writhing test. In addition, chemical inhibition of amygdala and insula, respectively, enhances and attenuates the hyperalgesia. **Aim:** Here we investigated the activation pattern of the anterior cingulate, insular cortices and amygdaloid complex (e.g., central, lateral and basal nuclei) in mice submitted to sciatic nerve constriction and the cagemates living with a conspecific in chronic pain. **Methods:** Male Swiss mice (n=6-7/group) were housed in pairs for 28 consecutive days. On day 14th, pairs of mice were grouped as follow: cagemate nerve constriction [CNC; i.e. one animal from each pair was subjected to sciatic nerve constriction (SNC) surgery] or cagemate sham (CS; i.e. one animal from each pair was subjected to (SS) sham surgery). After that, each pair was returned to its homecage to live together for further 14 days. On testing day (day 28th), all mice were euthanatized and their brains removed for immunohistochemistry assay. **Results:** Two-way ANOVA [Factor 1: condition (SNC or SS), Factor 2: hemisphere (left or right)] revealed decreasing of the activation in the anterior cingulate cortex (F(1,20)=18.52; P < 0.05) of the SNC group compared to the CS, similarity observed in mice submitted to constriction (F(1,20)=12.86; P < 0.05) compared to the sham group. In contrast, SNC group showed an increase of FosB bilaterally in the insular cortex (F(1,20)=28.46; P < 0.05) as observed in animals on neuropathic pain condition (F(1,20)=24.24; P < 0.05). On the same way, for mice submitted to sciatic nerve, we observed the increase of activation in the central (F(1,20)=5.14; P < 0.05) and lateral (F(1,20)=11.87; P < 0.05) nuclei of the right amygdala. However for cagemates we observed the decrease of activation of the central nucleus in the left amygdala (F(1,20)=5.36; P < 0.05). **Conclusion:** These results demonstrate that living with a mouse subjected to SNC induces hyperalgesia and alters the activation pattern of the anterior cingulate, insular cortices and amygdaloid complex. Taken together, the present results suggest the involvement of brain areas that are known to play a role in emotional states, such as anterior cingulate cortex, insula and amygdaloid complex in the modulation of pain empathy in mice.

**Disclosures:** A. Canto-de-Souza: None. D. Baptista-de-Souza: None. R. Nunes-de-Souza: None.

**Poster**

326. Emotion: Fear, Anxiety, and Pain I

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 326.21/X34

**Topic:** G.03. Emotion

**Support:** UFSCar
CAPES (88887187389/2018-00)
CNPQ (309201/2015-2)
Title: Chemical inactivation of the anterior cingulate cortex produces an anxiolytic-like effect in mice exposed to the model of empathy for pain

Authors: *G. B. CEZAR1,2, L. M. SILVEIRA1,2, L. A. ROSA1,2, I. M. CARMONA1,3, A. CANTO-DE-SOUZA1,2,3,
1Dept Psychology-Psycobiology Group/UFSCar, São Carlos, SP, Brazil; 2Grad. Program in Psychology/UFSCar, São Carlos, SP, Brazil; 3Joint Grad. Program in Physiological Sci. UFSCar/UNESP, São Carlos, SP, Brazil

Abstract: AIM: Empathy is expressed by the ability to recognize and respond to the emotional signals of others. This capacity is crucial for social interaction and has valuable adaptive behavior that provides essential skills for the survival of species. Recently, our group have demonstrated that mice cohabiting with a conspecific submitted to chronic neuropathic pain show anxiogenic-like behaviors in the elevated plus-maze (EPM). Evidence has emphasized the role of anterior cingulate cortex (ACC) in modulation of anxiety, pain and emotional contagion. This study investigated the effects intra-ACC injection of cobalt chloride (CoCl2, a reversibly non-selective synaptic blocker), on the modulation of anxiety induced by cohabiting with a cagemate in neuropathic pain in mice exposed to the EPM. METHODS AND RESULTS: Male Swiss mice (n=11-12/group) were submitted to a 28 day protocol. On the 21st after birth (weaning) mice were housed in pairs for 14 days to establish familiarity. On the 14th day, the animals were divided into two groups: cagemate sciatic nerve constriction (CNC), in which one animal of each pair was subjected to sciatic nerve constriction and cagemate sham (CS), in which one animal from each pair was subjected to the same surgery without nerve constriction. After surgery, the mice returned to the box with their respective conspecific (cagemate). On the 23rd day, cagemates were submitted to stereotactic surgery for implantation of guide cannulas directed to the ACC. On the 28th day, the CNC and CS groups received bilateral injections of saline or CoCl2 intra-CCA, and the cagemates were submitted to EPM for five minutes. Two-way ANOVA revealed that cohabitation with mice CNC induces anxiogenic-like behavior and demonstrated that temporary chemical inactivation of the ACC increased percentage of open arm entries (CS/Saline: 48.8±4.9; CNC/Saline: 26.7±3.4*; CS/CoCl2: 53.9±7.3; CNC/CoCl2: 55.4±6.4#) and percentage of open arm time (CS/Saline: 41.5±7.0; CNC/Saline: 20.1±5.2*; CS/CoCl2: 47.8±7.5; CNC/CoCl2: 48.3±7.8#), without altering closed arm entries (CS/Saline: 8.7±0.8; CNC/Saline: 8.4±1.1; CS/CoCl2: 9.9±1.2; CNC/CoCl2: 6.7±1.1) of the EPM. Data are presented as mean ± SEM. *P<0.05 versus respective group saline. #P<0.05 versus respective group CS/saline or CNC/saline. CONCLUSIONS: These data suggest that inactivation of the ACC provokes an anxiolytic-like effect in the EPM in mice. Furthermore, it suggests that ACC is essential for exhibition of anxiety-like behaviors induced by emotional contagion, evaluated through cohabitation with conspecific submitted to chronic neuropathic pain.

**Title:** The inactivation of the anterior cingulate cortex provokes an anxiolytic-like effect and impairs the evocation of the aversive memory in mice exposed to a model of posttraumatic stress disorder

**Authors:** *L. A. ROSA*¹,², L. M. SILVEIRA¹,², G. B. CEZAR¹,², A. CANTO-DE-SOUZA¹,²,³; ¹Dept Psychology-Psychobiology Group/Universidade Federal de São Carlos-UFSCar, São Carlos, SP, Brazil; ²Grad. Program in Psychology/UFSCar, São Carlos, SP, Brazil; ³Joint Grad. Program in Physiological Sci. UFSCar/UNESP, São Carlos, SP, Brazil

**Abstract:** AIM: Two of the most important aspects of the Posttraumatic Stress Disorder (PTSD) are the stability of a traumatic (aversive) long-term memory, and hypervigilance, which may be associated with higher levels of anxiety. And since the dorsal anterior cingulate cortex has been associated with context representation, and aversive encoding and anticipation, we carried out a study to evaluate the effects of microinjections of cobalt chloride (CoCl₂, a reversible non-selective synaptic blocker) intra-anterior cingulate cortex (ACC/Cg2) of male Swiss male mice on anxiety-like behavior and memory by using an animal model of PTSD that employed reexposures to situational reminders (SR). METHODS AND RESULTS: The experimental setup consisted of exposing male Swiss mice (n= 16/group) to an inescapable footshock (0.5 mA/10 s) into the dark side of a light-dark box (LDB), followed by three weekly reexposures to the light side of the box for 2 minutes (SR). Forty-eight hours after the last session, all animals were stereotaxically implanted with guide cannulas bilaterally aimed to the ACC/Cg2. On the twenty-eighth day, 10 minutes after bilateral microinjections of vehicle or CoCl₂ (1 mM/0.1 µl) intra-ACC/Cg2, all animals were tested in the elevated-plus maze (EPM) for 5 minutes. On the thirty-fourth day, following the same microinjection procedure, the same mice were submitted to explore both sides of the LDB for 5 minutes. Our results demonstrated that temporary chemical inactivation of the ACC/Cg2 in mice increased percentage of open arm entries (t₃₀ = -5.31, p < 0.05) and percentage of open arm time (t₃₀ = -5.53, p < 0.05), while decreased closed arm entries (t₃₀ = 6.20, p < 0.05), percentage of protected head-dipping (t₃₀ = 2.69, p <0.05), and percentage of protected stretched attend posture (SAP) (t₃₀ = 4.81, p < 0.05). In relation to LDB, the results demonstrated that inactivation of the ACC/Cg2 decreased latency to enter into the dark side of
the box ($t_{30} = 4.99, p < 0.05$), and increased entries ($t_{30} = -2.71, p < 0.05$) and time spent ($t_{30} = -6.55, p < 0.05$) in the dark side of the LDB. **CONCLUSIONS:** Together, these data suggest that temporary inactivation of the ACC/Cg2 provokes an anxiolytic-like effect in the EPM, and impairs the evocation of the aversive memory in the LDB in mice.

**Disclosures:** L.A. Rosa: None. L.M. Silveira: None. G.B. Cezar: None. A. Canto-de-Souza: None.

**Poster**

**326. Emotion: Fear, Anxiety, and Pain I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 326.23/X36

**Topic:** G.03. Emotion

**Support:** FAPESP 2016/24568-4
FAPESP 2017/25409-0

**Title:** N-methyl-d-aspartate (NMDA) receptor activation in the left prelimbic (but not infralimbic) cortex restores social interaction in chronically defeated mice

**Authors:** *N. SANTOS-COSTA*¹, M. MATTHIESEN², R. L. NUNES-DE-SOUZA³;
⁰Uni. Estadual Paulista - UNESP, Araraquara, Brazil; ²Unesp, Araraquara, Brazil; ³Univ. Estadual Paulista, UNESP, Araraquara, Brazil

**Abstract:** The chronic social defeat stress (CSDS) in mice allows the identification of two subgroups of animals: susceptible [low social interaction (SI)] and resilient (normal SI). A recent optogenetic study showed that while the activation of the LmPFC in susceptible mice restores sociability to control levels, its inhibition leads to social avoidance in resilient mice. Thus, the LmPFC seems to regulate social behavior independent of the activity of the RmPFC, and confers resilience. Besides, recent findings of ours have shown that NMDA activation of the Left (L) mPFC provokes anxiolytic-like effects of mice exposed to the elevated plus maze. Here, we investigated whether the social avoidance induced by CSDS is reversed by NMDA activation in the L mPFC of mice exposed to the SI test. Methods: Male Swiss mice were submitted to SDS for 10 consecutive days (n=7 no-stressed;10 defeated). 24h later, they were individually exposed to the SI test to record the social interaction profile. The SI ratio were calculated as follows: time in the interaction zone with the aggressor / time in the interaction zone without the aggressor. After that, mice were classified as susceptible (SI ratio < 1 ) or resilient (SI ratio = or > 1). The day after, mice received saline or NMDA (0.04 nmol/0.2 µL) in the LmPFC and were reexposed to the SI test. Results: On the 1st exposure to SI test, no-stressed subjects had SI ratio equal ou higher than 1 (average=1.2; SEM=0.3), what were maintained in the reexposure (average =1.4; SEM=0.3). Ninety percent of defeated mice were classified as susceptible (SI average: 0.3;
SEM=0.09) and then were separated to receive sal (n=4) or NMDA (n=5). On the retest, saline-injected group maintained its SI ratio average (0.3; SEM=0.2). Interestingly, mice who received NMDA within the L prelimbic cortex (n=3) increased SI to resilient levels (SI ratio average: 1.8; SEM=0.1). However, NMDA microinjection into infralimbic subregion of mPFC (n=2) did not change mice phenotype. These results are suggestive that i) CSDS protocol is able to induce high percentage of susceptible mice and ii) the social avoidance induced by CSDS seems to be reversed by activation of Glu-NMDA receptors located in the left prelimbic (but not infralimbic) cortex of mice.

Disclosures: N. Santos-Costa: None. M. Matthiesen: None. R.L. Nunes-de-Souza: None.

Poster

326. Emotion: Fear, Anxiety, and Pain I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 326.24/X37

Topic: G.03. Emotion

Support: UFSCar
        CNPQ (153163/2016-0; 309201/2015-2)
        FAPESP (2018/047750; 2015/00006-4)

Title: Empathy for pain: Interplay between antagonist 5-HT₃ and benzodiazepine/GABAᵦ receptors in amygdala modulates nociceptive response in cagemate that cohabited with a mice undergoing neuropathic pain

Authors: *L. R. R. TAVARES¹,², D. BAPTISTA-DE-SOUZA¹, A. CANTO-DE-SOUZA¹,²,³; ¹Dept Psychology-Psycobiology Group/UFSCar, São Carlos, SP, Brazil; ²Joint Grad. Program in Physiological Sci. UFSCar/UNESP, São Carlos, SP, Brazil; ³Grad. Program in Psychology/UFSCar, São Carlos, SP, Brazil

Abstract: AIM: Serotonergic and GABAergic receptors within amygdala are involved in pain and emotional responses, suggesting that this structure acts in the modulation of empathy for chronic pain models in mice. The study investigated the role of 5-HT₃ and benzodiazepine/GABAᵦ receptors (GABAᵦR) on social modulation of pain induced by cohabiting with a mouse submitted to sciatic nerve constriction. METHODS AND RESULTS: Male Swiss mice (n=6-12), were housed in pairs for a period of 28 days. On the 14th day, one animal from each pair underwent constriction surgery of the sciatic nerve or not, divided into two groups: cagemate nerve constriction (CNC), in which one animal of each pair was subjected to sciatic nerve constriction; cagemate sham (CS), in which one animal from each pair was subjected to the same surgery but without constriction. On the 24th day, the CNC or CS animal were subjected to a stereotaxic surgery. On the 28th day each cagemates received injections of
vehicle or ondansetron (OD, 5-HT3 antagonist, 0.3, 1.0 or 30 nmol/0.1µl) intra-amygdala (Experiment 1), saline or midazolam (MDZ, benzodiazepine/GABAA receptor agonist, 3.0 or 30 nmol/0.1µl) intra-amygdala (Experiment 2) and pre-treatment with vehicle or OD (0.3 nmol/0.1µl) follow by saline or MDZ (3.0 nmol/0.1µl) intra-amygdala (Experiment 3). After intra-amygdala injections, mice received acetic acid (0.6%, 0.1 ml/10g i.p.) injection and were submitted to the writhing test. For experiment 1, two-way ANOVA revealed significant effects for treatment \[F(3,71)=19.85; p<0.05\] and interaction between treatment and cohabitation \[F(3,71)=8.16; p<0.05\]. In experiment 2, two-way ANOVA show statistically significant effects for treatment \[F(2,44)=11.66; p<0.05\] and interaction between treatment and cohabitation \[F(2,44)=3.64; p<0.05\]. In experiment 3, three-way ANOVA show statistically significant effects for cohabitation \[F(1,57)=5.11; p<0.05\], pre-treatment \[F(1,57)=5.10; p<0.05\], treatment \[F(1,57)=13.02; p<0.05\], and interaction effects for cohabitation x pre-treatment x treatment \[F(1,57)= 4.91; p<0.05\]. Post hoc test revealed that intra-amygdala OD and MDZ attenuated hypernociception induced by living together with a cagemate in CNC groups. In experiment 3, the attenuation of the hyperalgesia found by MDZ in experiment 2 was impaired by OD. CONCLUSIONS: Our results suggest that the amygdaloid complex modulates the hypernociception induced by cohabitation with a pair in chronic pain through the interplay of 5-HT3 and benzodiazepine/GABAA. This evidences confirming the significative role on serotonin and GABA neurotransmitters on affective-emotional components of pain-related empathy.

Disclosures: L.R.R. Tavares: None. D. Baptista-de-Souza: None. A. Canto-de-Souza: None.

Poster

326. Emotion: Fear, Anxiety, and Pain I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 326.25/X38

Topic: G.03. Emotion

Support: COBRE II GM103642
RCMI Pilot Project MD007600
PRCTRC U54MD007587
UPR MSC Chancellor's Office
UPR School of Medicine Deanship
NSF CREST PRCEN
NARSAD

Title: The effect of glyphosate-based herbicide on locomotion and anxiety-like behavior in rats

Authors: *M. CÁCERES-CHACÓN¹, O. MARTÍNEZ-GUZMÁN¹, M. RIVERA-LÓPEZ¹, D. M. OJEDA-MARTÍNEZ², R. Y. RAMOS-SÁNCHEZ², C. J. REYES-SEPÚLVEDA³, P. ALVELO-FERNÁNDEZ³, D. SIERRA-MERCADO¹,
Abstract: Glyphosate-based herbicides (GBH) are the most commonly used herbicides in agriculture. Glyphosate was initially considered safe for mammals because it acts by inhibiting a metabolic route not present in mammals. However, the use of glyphosate has been correlated with an increased diagnosis of neurological diseases such as movement and anxiety disorders. Similarly, recent studies in rodents have shown that GBH causes decreased locomotion and increased anxiety-like behavior through various methods of exposure including intranasal application, oral gavage and intra-peritoneal injections. Additionally, GBH exposure in rats decreased dopamine levels in the striatum. These studies, however, utilized a dose far larger than that to which animals and humans are likely exposed. Also, the experimental methods often used to treat rodents with GBH are unrealistic (i.e. injections and gavage). Therefore, we aimed to evaluate the effect of prolonged oral consumption of drinking water containing low dose GBH on locomotion and anxiety-like behavior. To achieve this, we gave rats access ad libitum to drinking water containing GBH at a concentration of 0.7mg/l, which corresponds to maximum contaminant level allowed by the environmental protection agency, whereas control rats received filtered drinking water. We assessed locomotion and anxiety-like behavior after two weeks of exposure using the open field test. No difference was seen in total distance traveled (control: 28.65, GBH: 31.63; p=0.55), amount of time spent in the center (control: 32.05, GBH: 15.30; p = 0.39), or the amount of entries into the center (control: 14.00, GBH: 9.50; p=0.54). Anxiety-like behavior was reassessed in the elevated plus maze where no difference was seen in the ratio of time spent in the open arm (control: 0.36, GBH: 0.19; p=0.21), ratio of entries into the open arm (control: 0.31 GBH: 0.21; p=0.48), or anxiety index (control: 0.66, GBH: 0.80; p=0.28). In conclusion, GBH did not affect locomotion or anxiety-like behavior through drinking water at a concentration of 0.7mg/l, after two weeks. Future directions include prolonging the exposure time, assessing other emotional memories and performing immunohistochemistry on brain regions involved in locomotion and anxiety-like behaviors.

Title: Effects of atrazine on locomotion and anxiety-like behavior


1Univ. of Puerto Rico Rio Piedras Campus, San Juan, PR; 2Dept. of Anat. & Neurobio., Univ. Puerto Rico Sch. of Med., San Juan, PR; 3Univ. of Puerto Rico Bayamón, Bayamón, PR

Abstract: Atrazine (ATR) is a herbicide widely used in agriculture across the globe. Given its excessive use, it has been detected in water systems and has reached drinking water. Intake of ATR has been shown to influence locomotion and anxiety-like behaviors in rodents. For example, recent studies in rodents have shown that ATR consumption can lead to increased locomotion. Therefore, we aimed to evaluate the effect of prolonged oral consumption of drinking water containing the maximum contaminant level of ATR on locomotion and anxiety-like behavior. To achieve this, we gave rats access ad libitum drinking water containing ATR at a concentration of 0.03mg/l, while control rats received filtered drinking water. We assessed locomotion after two weeks of exposure using the open field test. No difference was observed in total distance traveled (Control: 28.65, ATR: 29.98; p=0.76). No difference was seen in the amount of time spent in the center (Control: 32.05, ATR: 7.93; p=0.18. Anxiety-like behavior was assessed using the elevated plus maze, where experimental rats showed 0.66 and 0.72 for controls p=0.62 anxiety-like behavior. Future directions include increased exposure time to drinking water containing atrazine, as well as assessing brain regions important for locomotion and anxiety using immunohistochemistry. These results may help elucidate if prolonged consumption of oral atrazine affects normal behavior.


Poster

327. Consequences of Alcohol and Drug Exposure During Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 327.01/X40

Topic: G.08. Drugs of Abuse and Addiction
Title: Perinatal exposure to drugs of abuse causes permanent aberrations in somatosensory circuits

Authors: *J. B. ALIPIO*¹, Y. LI¹, C. W. HAGA², R. BALAJI², M. E. FOX², D. EL-METWALLY¹, M. K. LOBO², M. PICCIOTTO³, A. KELLER¹; ²Anat. & Neurobio., ¹Univ. of Maryland Sch. of Med., Baltimore, MD; ³Dept Pyschiat, Yale Univ. Sch. Med., New Haven, CT

Abstract: Perinatal exposure to drugs of abuse during pregnancy and while breastfeeding may cause lasting changes in offspring. Perinatal exposure to nicotine and opioids is associated with—along with other devastating outcomes—permanent changes in sensory processing, suggesting that functional impairments in somatosensory circuits may underlie these neurodevelopmental consequences. We hypothesize that perinatal fentanyl or nicotine exposure permanently alters synaptic transmission in the thalamocortical (TC) and corticothalamic (CT) projections that regulate somatosensory function. Preliminary findings suggest that perinatal fentanyl exposure permanently suppresses excitatory synaptic transmission in both the primary somatosensory cortex (S1) and the ventroposteromedial (VPM) nucleus of the thalamus. This includes a decrease in the probability of evoked glutamate release, a decrease in the frequency and amplitude of miniature excitatory postsynaptic currents (mEPSCs), and a decrease in the ratio of NMDA/AMPA responses. In contrast, perinatal exposure to nicotine caused opposite changes in the TC/CT circuit - an increase in the probability of evoked glutamate release, an enhancement in the frequency and amplitude of mEPSCs, and a decrease in NMDA/AMPA response ratios. Ongoing studies are examining how perinatal drug exposure influences S1 neural activity in vivo, using electrocorticogram (ECoG) recordings of neural oscillations in awake behaving mice. These studies will contribute to our understanding of the enduring consequences associated with perinatal exposure to drugs of abuse, impact on infant neurodevelopment, and potentially inform improved treatment options.


Poster

327. Consequences of Alcohol and Drug Exposure During Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 327.02/X41

Topic: G.08. Drugs of Abuse and Addiction
Title: Buprenorphine exposure during gestation results in dose-dependent consequences for the dam and her litter in a translational model of opioid-maintenance therapy

Authors: *C. M. WALLIN, S. E. BOWEN, S. BRUMMELTE; Psychology, Wayne State Univ., Detroit, MI

Abstract: Significance: The opioid crisis has led to increases in pregnant opioid-dependent women treated with opioid-maintenance therapy (buprenorphine, BUP). However, not much is known about the consequences of gestational BUP exposure on pregnancy outcomes or fetal development. Previous work has not accounted for the critical fact that women are likely already using opioids at the time of conception and continue to use throughout the postpartum. Unsurprisingly, the initiation of drug use in a new user has considerable physiological effects as compared to a regular user, which necessitates consideration. Method: Our translational animal model aimed to resemble human BUP treatment therapy by starting vehicle or BUP exposure subcutaneously (s.c.) in adult female Sprague-Dawley rats (N=30) at least 7 days before conception and continuing exposure throughout the postpartum period. We evaluated effects of therapeutic (low-dose, 0.3 mg/kg) and overexposure (high-dose, 1 mg/kg) BUP exposure against saline-control (1 mg/mL). Females were randomly assigned to exposure groups (n=10/group) and were bred in house with drug-naïve adult male Sprague-Dawley rats. At parturition, successful litters (N=26) were culled to 5 males/5 females per litter. One male and one female from each litter were randomly assigned to various behavioral tests during either the neonatal period (precipitated withdrawal, postnatal day (PN) 2) or during adolescence (anxiety-like behavior, stress-sensitivity, nociceptive response). Pregnancy outcomes, maternal care behavior, neonatal opioid withdrawal syndrome (NOWS), as well as offspring development were evaluated. Results: Overexposure (high-dose) BUP exposure resulted in increased pup mortality, increased symptoms of NOWS, and decreased maternal care behavior. In fact, most high-dose BUP pups did not survive past PN 2. Therapeutic (low-dose) BUP exposure delayed offspring development, decreased body weight, length, and body temperature, as well as increased tolerance to morphine’s analgesic effect (p’s < .05). Conclusion: Overall our results demonstrate that the therapeutic (low-dose) level of BUP was relatively safe though it did have subtle effects on exposed offspring. However, the overexposure (high-dose) level of BUP interfered with maternal caregiving behavior and thus offspring survival and increased NOWS. More research is needed to validate the translational implication of these findings for human opioid-dependent mothers maintained on buprenorphine-maintenance therapy or other opioids.

Disclosures: C.M. Wallin: None. S.E. Bowen: None. S. Brummelte: None.
**Poster**

**327. Consequences of Alcohol and Drug Exposure During Development**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 327.03/X42

**Topic:** G.08. Drugs of Abuse and Addiction

**Title:** Effects of prenatal cocaine exposure in the neurodevelopment of DAT-Cnr2 and Cx3cr1-Cnr2 conditional knockout mice

**Authors:** *A. CANSECO-ALBA*¹, M. A. HAMMOUDA², Q.-R. LIU⁴, E. S. ONAIVI³;
¹CINVESTAV-IPN, Mexico City, Mexico; ²William Paterson Univ., Carlstadt, NJ; ³William Paterson Univ., Wayne, NJ; ⁴LCI/NIA/NIH, Baltimore, MD

**Abstract:** The endocannabinoid system (eCB), including endocannabinoids and their receptors (CB1Rs and CB2Rs) has been detected from the earliest stages of embryonic development and throughout pre- and postnatal development. Consequently, the eCB may play an important role in neurodevelopment. The presence of CB2 receptors in microglia cells and neurons of discrete parts of the brain has been demonstrated. Therefore, the functional relevance of the CB2 receptors in the CNS is emerging. Through the use of new genetic strategies like the generation of conditional knockout mice, new possibilities are open, in studying the role of the components of the ECS system and in particular, CB2 receptors. The objective of this study was to evaluate the postnatal behavioral development of mice which do not express cannabinoid CB2 receptors in dopaminergic neurons and microglia (DAT-Cnr2 and CX3cr1-Cnr2 cKO mice), and to evaluate the effect of perinatal exposure to cocaine in these animals on the neurodevelopmental features of the conditional knockout mice in comparison with the wild type (WT) mice.

Ultrasonic vocalizations (USVs), physical development, and reflex activities of the pups from postnatal day 1 to the weaning day were analyzed. A separate group of pregnant cKO females received subcutaneous cocaine injections of 20 mg/kg from gestational days 7 through 17. We report that cocaine treatment of pregnant dams decreased their weight gain during pregnancy in all genotypes. CX3cr1-Cnr2 cKO mice treated with cocaine show a significant decrease in the number of pups, and this group presented the lowest survival rate. DAT-Cnr2 cKO pups started crawling and walking sooner than the WT mice, but this feature was lost in the group treated with cocaine. We concluded that the deletion of CB2Rs in dopamine neurons and microglia revealed a neuro-immuno- modulatory effects of CB2Rs in the psychostimulant effects of cocaine.

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**Disclosures:** A. Canseco-Alba: None. M.A. Hammouda: None. Q. Liu: None. E.S. Onaivi: None.
Abstract: Background and aim: Current knowledge concerning cognitive performance in children born to mothers in opioid maintenance treatment (OMT) is limited and conflicting. In humans OMT is associated with multiple confounding factors making it difficult to draw conclusions about prenatal opioid exposure and its subsequent effects. Hence, the aim of this study was to examine the possible effects on learning and memory in young adult rats prenatally exposed to methadone or buprenorphine.

Methods: Female rats were implanted with 28-day osmotic minipumps delivering methadone (10 mg/kg/day), buprenorphine (1 mg/kg/day) or vehicle 5 days prior to mating. To examine possible effects on learning and memory, the offspring were included in three different behavioral tests. At 7 weeks of age, the offspring underwent the simultaneous brightness discrimination (SBD) test, which reflects non-spatial reference memory. At 8 weeks of age, the rats were included in a novel object recognition test. Finally, the Morris water maze (MWM) test was used to investigate spatial learning and memory in 10 weeks old offspring.

Results: Young adult rats prenatally exposed to methadone or buprenorphine exhibited impaired non-spatial reference memory and recognition memory, shown by reduced performance in the SBD and novel object recognition tests. In the MWM, all animals displayed normal learning abilities, however, methadone exposed offspring displayed impaired spatial memory in the retention task performed 8 days after the learning sessions.

Conclusions: Our study shows that prenatal exposure to methadone or buprenorphine causes impaired cognitive performance in young adult rats. This is of major concern given the escalating increase in opioid use and OMT among fertile women.

Disclosures:  M. Kongstorp: None. I. Bogen: None. T. Stiris: None. J.M. Andersen: None.
Poster

327. Consequences of Alcohol and Drug Exposure During Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 327.05/X44

Topic: G.08. Drugs of Abuse and Addiction

Support: START UP FUNDS TO DR. YELAMANCHILI
START UP FUNDS TO DR. PENDYALA

Title: Transgenerational epigenetic inheritance associated with prescription opioids abuse: How far has the apple fallen from the tree?

Authors: K. ODEGAARD¹, V. SCHAAL², D. MOORE³, A. CLARK⁴, G. PENDYALA², *S. V. YELAMANCHILI¹;
¹Pharmacol. and Exptl. Neurosci., ²Dept. of Anesthesiol., ³Univ. of Nebraska Med. Ctr., Omaha, NE; ⁴Creighton Univ., Omaha, NE

Abstract: Recent statistics from the Centers for Disease Control and Prevention (CDC) show that every day, more than 115 Americans die from overdosing on opioids including heroin as well as prescription opioid pain relievers. Adding another layer of complexity is the increased risk of dependency on prescription opioids such as oxycodone (oxy) during pregnancy and postpartum. Although limited studies have linked maladaptive behaviors and cognitive deficits in exposed offspring, a significant knowledge gap remains in understanding how short- and long-term oxy exposure impacts the epigenetic architecture in the exposed offspring and whether these changes persist in the subsequent generations. We, accordingly have developed an animal model using Sprague Dawley rats to mimic oxy exposure in utero and in post-natal pups to examine long-term consequences of its abuse on the exposed offspring (F1) as well as on subsequent generations (F2) in the absence of drug exposure. Using RNA-Seq on RNA isolated from the nucleus accumbens (NAc), a region of the brain involved in reward and sensitive to drug abuse, we identified key changes in genes associated with synaptic signaling and neuron differentiation in both the F1 and F2 generations of each group. Western blot experiments done on synaptosomes isolated from the prefrontal cortex of both F1 and F2 P14 pups show differences in the expression of synaptic genes, such as EAAT2, synaptophysin, and PSD95, among the control, in utero, and postnatal groups. We also identified phenotypic differences (head size, body weight, and body length) among the groups in both generations. To further elucidate how these changes impact glutamatergic and dopaminergic systems, we conducted behavioral tests to assess repetitive behaviors and social alterations among the experimental groups and between the two generations. Sex differences were also considered during the behavior tasks. Given the increased use of opiates (both medical and non-medical),
understanding the persistent developmental effects of these drugs will delineate potential risks associated with opiate use beyond the direct effects on the user.


Poster

327. Consequences of Alcohol and Drug Exposure During Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 327.06/X45

Topic: G.08. Drugs of Abuse and Addiction

Title: Assessing physiological and behavioral changes in rodents following in utero opioid exposure

Authors: *S. STEVENS1,2, S. MOHAN1;
1Manchester Col. of Pharm., Fort Wayne, IN; 2Dept. of Pharmaceut. Sci. and Res., Marshall Univ. Sch. of Pharm., Huntington, WV

Abstract: As opioid use among pregnant woman increases, the number of infants born with neonatal abstinence syndrome (NAS) continues to rise. Although the short term withdrawal symptoms are well characterized, the neuropathology behind opioid-mediated NAS and the long term effects on behavior and memory are unclear and warrants further research. Current preclinical models of NAS are limited by short gestational periods, large litter sizes and primary organogenesis occurring after birth. Using a novel mouse model with an extended gestational period, we aim to study the short and long term effects of in utero morphine exposure by assessing withdrawal behavior, memory and investigating key cellular mediators underlying neuropathology of NAS. To model maternal opioid use, dams were treated daily with saline or morphine 10 and 30 mg/kg S.C. beginning on G18 until day of birth; this resulted in a cumulative exposure for 19-21 days. Physiological (body weight, body temperature) and withdrawal behaviors (jumps, wet dog shakes and ultrasonic vocalizations) for each pup were recorded daily for the first eight days. Differences in memory were measured using Y-maze and novel object recognition tests starting at one month of age. Preliminary data from 3 month-old mice born exposed to morphine were found to exhibit increased physiological changes and withdrawal behavior commonly associated with opioid withdrawal. Additionally, decreased Y-maze performance was found in morphine exposed mice compared to saline treated mice. These data suggests that our method of in utero morphine exposure does result in postnatal withdrawal behavior. A decrease in Y-maze performance suggests deficits in learning and memory in adult mice that were prenatally exposed to morphine. Further studies are currently in progress to determine any underlying cellular changes responsible for these differences. These
results coupled with the lengthened gestational period, small litter size and increased prenatal organogenesis make this mouse model of in utero opioid exposure an improved translational model of NAS compared to current animal models. We are hopeful this novel mouse model will further our understanding of the consequences of in utero opioid exposure on neurodevelopment and behavior.

Disclosures: S. Stevens: None. S. Mohan: None.

327. Consequences of Alcohol and Drug Exposure During Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 327.07/DP14/X46

ControlExtraData.DynamicPosterDisplay: Dynamic Poster

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

Support: San Antonio Life Science Foundation

Title: Using human brain organoids to model prenatal buprenorphine exposure

Authors: *J. J. DONEGAN¹, H. ELAM¹, V. NIETO-ESTEVEZ², C. MCMAHON², J. HSIEH², D. LODGE¹;
¹Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX; ²Biol. and Brain Hlth. Consortium, Univ. of Texas at San Antonio, San Antonio, TX

Abstract: The misuse of opioids has reached epidemic proportions over the last decade, with over 1.5 million people in the U.S. suffering from substance use disorders related to prescription opioid pain relievers. This increase in opioid misuse affects all demographics of society, including women of child-bearing age, which has led to a rise in opioid use during pregnancy. Infants exposed to opioids in utero show an increased risk for neurological and behavioral deficits, including neonatal abstinence syndrome, a cluster of withdrawal symptoms that include tremors, diarrhea, fever, irritability, and seizures. Currently, opioid use disorder in pregnant women is treated with long-acting opioid agonists, including buprenorphine. Although buprenorphine is widely considered safe for the developing fetus, few long-term studies have been conducted. The goal of the current experiments was to examine the consequences of buprenorphine on the developing human brain. While prenatal opioid exposure can be modeled in rodents, species differences limit our ability to recapitulate all aspects of human nervous system development. Therefore, we used human induced pluripotent stem cells to grow 3D brain cell cultures that model the cellular diversity, connectivity, and activity of the developing human brain. Specifically, we used patterning factors to generate dorsal forebrain progenitors (cortical organoids) or ventral forebrain progenitors (subcortical spheroids). RNA Sequencing and
immunohistochemistry were used to confirm cortical or subcortical specificity. To then measure the effect of buprenorphine exposure on neuronal network properties, cortical or subcortical spheroids were treated with buprenorphine (2 ng/ml) at various time points during development and multielectrode arrays were used to record neuronal activity. Immunohistochemistry was used to measure markers of cortical development. Finally, to model the developmental process by which inhibitory interneurons migrate tangentially into the cerebral cortex, cortical and subcortical spheroids were fused in the presence of buprenorphine. Interneuron progenitors were labelled with a GFP tag and migration was monitored using a confocal microscope. Together, our results will provide new information about the developmental consequences of buprenorphine exposure during development and may ultimately lead to new therapeutic strategies to treat infants exposed to opioids in utero.


Poster

327. Consequences of Alcohol and Drug Exposure During Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 327.08/Y1

Topic: G.08. Drugs of Abuse and Addiction

Title: Characterizing the impact of prenatal drug exposure on emotion processing: Findings from the ABCD study

Authors: *A. WAGNER1, A. RAMAKRISHNAN2, I. IVANOVA, M. A. PARVAZ3; 1Psychiatry, Icahn Sch. of Med. At Mount Sinai, New York, NY; 2Biomed. Informatics, Rutgers Sch. of Hlth. Professions, Newark, NJ; 3Psychiatry, Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Background: Prenatal drug exposure has shown to alter the trajectory of neurodevelopment and put an individual at risk for impairments long before functional disorders arise. Adolescence is a period where atypical development becomes apparent in behavior and brain function that can lead to psychiatric illnesses including mood and anxiety disorders, and substance abuse. However, the current literature on precise neural underpinnings of prenatal drug exposure and their manifestations on behavior is sparse, ambiguous, and primarily based on small cohort studies. Therefore, here we leverage data from the Adolescent Brain Cognitive Development (ABCD) study to determine the effects of prenatal drug exposure on working memory and emotional arousal and the underlying brain activity. Methods: From 8,829 children with fMRI data in the ABCD cohort, we defined groups by extent of prenatal drug exposure based on mothers’ self-report of drug use during pregnancy: continued exposure (CDE; continued use after pregnancy confirmation), limited exposure (LDE; discontinued use after
pregnancy confirmation), and no exposure (NDE). The N-back fMRI task with emotionally-salient stimuli was used to assess emotion processing during a task which demanded working memory. The primary outcomes were task performance, and brain activity of pre-selected (based on a prior systematic review) regions of interest (inferior frontal gyrus, dorsolateral prefrontal cortex, ventromedial prefrontal cortex, and amygdala). Results: After adjusting for the mother’s age at birth, task performance showed main effects of Memory [F(1,8578)=225.7, p<0.001], Emotion [F(2,8577)=4.9, p=0.007], Stimulus [F(2,8577)=699.7, p<0.001], and Exposure [F(2,8578)=5.008, p=0.007] as well as Stimulus*Exposure [F(4,17156)=4.6, p=0.001] and Memory*Stimulus*Exposure [F(4,17156)=3.2, p=0.001] interactions. These differences in task performance as well as in regional brain activations are being further explored and will be included in the final presentation. Conclusion: Although previous studies show that prenatal drug exposure is associated with differences in brain function, those findings should be replicated in a larger study. The ABCD study presents an opportunity to answer important questions about the effects of prenatal drug exposure on development.


Poster

327. Consequences of Alcohol and Drug Exposure During Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 327.09/Y2

Topic: G.08. Drugs of Abuse and Addiction

Title: Alcohol and tobacco use during pregnancy: A Native American perspective

Authors: *R. C. ROSENTHAL¹, K. E. CONNER², K. J. HUFFMAN¹;
¹Psychology, ²Interdepartmental Neurosci. Program, Univ. of California, Riverside, Riverside, CA

Abstract: Tobacco and alcohol abuse are prevalent among Native Americans. Thus, Native American offspring are at risk for toxic prenatal exposures. Of specific concern are prenatal nicotine exposure (PNE) and prenatal alcohol, or ethanol exposure (PrEE), as these maternal consumptions may induce developmental brain damage leading to cognitive-behavioral deficits (Abbott et al., 2018). A primary goal of this study is to elucidate preconceived ideologies and the significance they play in children’s health, in a specific population where tobacco and alcohol abuse is prominent. For example, the U.S. Department of Health and Human Services reports high incidence rates for Fetal Alcohol Syndrome (FAS) in Alaska (1.5 per 1,000 live births) and, within Native Americans and Alaskan Natives specifically, the rate rises to 5.6. Also, considering the prevalence of teenage pregnancy in the Native American population, it is important to gain insight on individuals’ beliefs about what is acceptable, customary and safe to
consume during pregnancy. This project examines Native Americans’ perceptions of the use of tobacco (ceremonial and recreational smoking), alcohol, cannabis, certain medications, as well as nutritional choices and sweat lodge practices during pregnancy. Using an online survey, we investigate beliefs about consumption safety during pregnancy in young Native Americans, age 18 to 25, throughout the United States. Our results suggest tribal differences in beliefs about tobacco use during pregnancy. Specifically, some tribes view ceremonial tobacco practices as safe, whereas most believe recreational tobacco is unsafe to use during pregnancy. Additionally, survey data suggest individual differences in beliefs about the safety of alcohol consumption during pregnancy; some subjects perceive wine and beer as safe, while others view all alcohol consumption as unsafe. Regional tribal differences were observed concerning the perceived safety of salmon consumption during pregnancy. Salmon contains polychlorinated biphenyls (PCBs) which are known developmental teratogens. Our results indicate that Alaskan Natives consider salmon safe but red meat unsafe to consume during pregnancy, whereas southwestern tribes believe the reverse. It is important to identify populations that consume or use substances that may cause developmental harm. Nicotine, alcohol and PCBs are examples of teratogens that are known to impact brain development and have lasting behavioral effects on offspring. Pregnancy is a sensitive period, understanding how Native Americans view maternal practices during pregnancy will elucidate gaps in awareness and guide educational outreach.

Disclosures:  R.C. Rosenthal: None.  K.E. Conner: None.  K.J. Huffman: None.

Poster

327. Consequences of Alcohol and Drug Exposure During Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 327.10/Y3

Topic: H.01. Animal Cognition and Behavior

Title: Modulation of polysialated neural cell adhesion molecules (PSA-NCAM) ameliorates learning and memory deficits associated with prenatal cannabinoid exposure

Authors: *P. D. PINKY*, 1 J. BLOEMER, 1 Y. DU, 1 S. E. SETTI, 1 R. T. HESLIN, 1 W. D. SMITH, 1 A. DITYATEV, 2 M. N. REED, 1 V. SUPPIRAMANIAM;  
1Drug Discovery and Develop., Auburn Univ., Auburn, AL; 2German Ctr. for Neurodegenerative Dis., Magdeburg, Germany

Abstract: Cannabis is currently the most commonly used illicit drug in the United States, and becoming more popular in pregnant women based on its anti-emetic properties in alleviation of morning sickness. The active ingredient of cannabis is delta-9-tetrahydrocannabinol (THC) which crosses blood placental barrier to reach the fetus causing enduring alterations in synaptic circuitry responsible for cognition and behavior. The aim of our study is to investigate the mechanisms of learning and memory deficits in prenatal cannabinoid exposure (PCE) in
adolescent offspring. We hypothesize that PCE induced reduction in neural cell adhesion molecule (NCAM) and its active form polysialated NCAM (PSA-NCAM) are responsible for glutamatergic neurotransmission mediated deficits in learning and memory. We have performed a series of behavioral, electrophysiological and immunochemical studies to identify the mechanisms of memory deficits in PCE. An osmotic pump filled with either vehicle or the cannabinoid receptor full agonist WIN55,212-2 (2 mg/kg body weight/day) was implanted subcutaneously on Gestational Day-3 to deliver the drug at a constant rate until the pups were born. Open field test was performed to determine the effects of PCE on locomotor activity, and found no difference in general motor activity. Contextual fear conditioning revealed a significantly reduced freezing behavior in the PCE group compared to the vehicle exposed group. The hippocampus based spatial memory test, Morris water maze, revealed a preference for the platform quadrant in control animals, while the PCE animals did not show any preference for a particular quadrant. Electrophysiological experiments were performed on acute hippocampal slices to measure synaptic plasticity. Impairment in both long term potentiation (LTP) and long term depression (LTD) were observed. These impairments were mirrored by alterations in expression of glutamate receptor subtype N-methyl-D-aspartate receptors (NMDAR). An increase in cannabinoid receptor type 1 (CB1) expression along with reduced PSA-NCAM expression have been observed in western blot analysis. The LTP in the PCE group was improved when E.coli derived colominic acid sodium salt was applied on acutely isolated hippocampus slices. Based on the previous studies along with our data, it can be postulated that the observed hippocampus based behavioral deficits could be due to altered NMDAR mediated glutamatergic neurotransmission associated with reduction in PSA-NCAM expression. Present study provides evidence that modulation of NMDA receptors as possible therapeutic option to improve PCE mediated memory deficits.

Disclosures:  P.D. Pinky: None.  J. Bloemer: None.  Y. Du: None.  S.E. Setti: None.  R.T. Heslin: None.  W.D. Smith: None.  A. Dityatev: None.  M.N. Reed: Other; Center for Neuroscience Initiative, Auburn University, Auburn, AL.  V. Suppiramaniam: Other; Center for Neuroscience Initiative, Auburn University, Auburn, AL.

Poster

327. Consequences of Alcohol and Drug Exposure During Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 327.11/Y4

Topic: G.08. Drugs of Abuse and Addiction

Support:  PHS NIH F30 AA025534  
PHS NIH RO1 AA023410  
PHS NIH R21 AA024036
Title: Prenatal ethanol exposure and enduring effects on pyramidal neuron function in the somatosensory cortex

Authors: *L. C. DELATOUR, P. W. YEH, H. H. YEH; Mol. and Systems Biol., The Geisel Sch. of Med. At Dartmouth, Hanover, NH

Abstract: Fetal alcohol spectrum disorders (FASD) represent the broad range of cognitive and behavioral deficits associated with prenatal exposure to ethanol. Previous work demonstrated the effects of in utero exposure to binge-type ethanol on the radial migration of primordial pyramidal neurons and the form and function of pyramidal neurons in the somatosensory cortex of early postnatal mice. Specifically, changes in spontaneous synaptic activity in layer V/VI pyramidal neurons resulted in a shift in the excitatory/inhibitory balance in postnatal day 9-11 mice. However, the mechanisms underlying these changes and whether they would persist are unknown. We therefore asked how prenatal ethanol exposure affects synaptic strength and pyramidal neuron morphology in the somatosensory cortex in the long-term.

Pregnant mice were exposed to either a liquid food diet containing ethanol (5% w/w) or an isocaloric control from embryonic day 13.5 through 16.5 and both male and female offspring were analyzed. Whole-cell patch clamp electrophysiology revealed an increase in the frequency of spontaneous and miniature synaptic activity in layer V/VI pyramidal neurons in the somatosensory cortex of postnatal day 28-32 mice. Furthermore, optogenetic experiments conducted in Emx1\textsuperscript{IRESCre}\textsubscript{Ai32} and Nkx2.1\textsubscript{Cre-Ai32} mice to investigate paired-pulse-evoked EPSC and IPSC responses, respectively, revealed a change in the release probability of neurotransmitter following prenatal exposure to ethanol. These changes in function were found in the absence of any changes in the dendritic morphology of layer V/VI pyramidal neurons in the control and ethanol-exposed cohorts. These data suggest an effect of ethanol on the presynaptic terminal, resulting in a shift in the excitatory/inhibitory balance. However, a prominent presynaptic mechanism does not preclude an effect of prenatal ethanol exposure on the postsynaptic terminal. In fact, we found a significant change in the density and morphology of spines along the apical dendrites in layer V/VI pyramidal neurons following prenatal ethanol exposure. However, a potential postsynaptic mechanism needs to be addressed systematically. Our findings indicate that prenatal ethanol exposure exerts long-term effects on synaptic strength with a prominent effect on the presynaptic terminal. Furthermore, we demonstrate that morphology does not necessarily predict function, especially during development. These data strongly support an enduring effect of prenatal ethanol exposure on the function of pyramidal neurons in the somatosensory cortex and contribute to our understanding of the underlying etiology of FASD.

Disclosures: L.C. Delatour: None. H.H. Yeh: None. P.W. Yeh: None.
Poster

327. Consequences of Alcohol and Drug Exposure During Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 327.12/Y5

Topic: G.08. Drugs of Abuse and Addiction

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European Union’s Horizon 2020 research and innovation program 2014-2020 under Grant Agreement No 634143
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Plan Nacional sobre Drogas (#2014/020) and Generalitat de Catalunya (2014SGR34)

Title: Alcohol exposure during prenatal and lactational periods enhances reinforcing effects of alcohol, without presenting changes in the mRNA expression of mu and kappa receptors

Authors: *F.-J. FERNANDEZ-GOMEZ*1,2, S. MONTAGUD-ROMERO3, C. NUÑEZ1,2, M.-V. MILANES1,2, O. VALVERDE3,4;

Abstract: Maternal binge alcohol drinking during the developmental brain stage can produce deleterious effects on the fetus. In the utero, alcohol exposure may lead to a wide range of morphological and behavioral long-lasting consequences known as fetal alcohol spectrum disorders, which the most severe type is the fetal alcohol syndrome. We aimed to assess the effect of prenatal and lactational alcohol exposure (PLAE) on the reinforcing effects of alcohol during the adulthood. Furthermore, we assessed the possible neurobiological mechanisms underlying the addictive effects of alcohol, such as the opioid system in brain areas involved in drug intake. Pregnant C57BL/6 female mice were exposed to binge alcohol drinking throughout gestation up to weaning. Then, male offspring performed the two bottle choice paradigm induced by alcohol, at early adulthood. After that, brain samples were analyzed by qPCR to quantify the mu and kappa opioid receptors. Our findings demonstrate that PLAE produces an increase of alcohol intake in the two bottles choice procedure. The qPCR analysis of the prefrontal cortex, striatum and amygdala did not display changes in the expression of the evaluated receptors. The current study highlights that PLAE animals increased alcohol consumption due to a lower sensitivity to the pleasant effects of alcohol. The pattern of alcohol consumption used in our study may not be enough intense or prolonged to cause changes in the receptors. For future
research, it may be interesting to analyze the opioid peptides as they have been involved in alcohol reward.

**Disclosures:**  
F. Fernandez-Gomez: None.  
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C. Nuñez: None.  
M. Milanes: None.  
O. Valverde: None.

**Poster**

**327. Consequences of Alcohol and Drug Exposure During Development**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 327.13/Y6

**Topic:** G.08. Drugs of Abuse and Addiction

**Support:**  
PHS NIH R01 AA023410  
PHS NIH R21 AA024036

**Title:** Prenatal ethanol exposure affects the development of GABAergic interneurons in the striatum

**Authors:** *A. R. TOUSLEY, H. YEH, P. YEH;*  
Mol. and Systems Biol., Geisel Sch. of Med. at Dartmouth, Hanover, NH

**Abstract:** Fetal Alcohol Spectrum Disorder (FASD) is a neurodevelopmental disorder that presents with varying degrees of cognitive and behavioral dysfunction including hyperactivity and behavioral inflexibility. Imaging studies have found striatal dysmorphology and changes to corticostriate connectivity to be associated with FASD diagnosis and symptom severity. However, less is known about how FASD-related striatal changes develop. Previous work in the lab has shown that prenatal alcohol exposure (PAE) can increase both the number of GABAergic interneurons in the developing cortex and neurogenesis in the medial ganglionic eminence (MGE), where cortical interneurons are born. Here we asked if PAE affects the migration and function of striatal interneurons, which are also born in and migrate from the MGE into the striatum during the same time period.

We chose to assess the density of striatal interneurons on embryonic day 16.5 (E16.5) in Nkx2.1Cre/Tdtomato mice exposed to 5% binge ethanol during the peak of tangential migration (E13.5-16.5). We found that E16.5 animals exposed to ethanol have an altered distribution of GABAergic interneurons compared to control animals, with the density of interneurons in the dorsal striatum increased and that of the ventral striatum unchanged. This observation suggests an accelerated migration of GABAergic interneurons into the dorsal striatum.

Ongoing studies will clarify how this embryonic change in interneuron distribution influences striatal GABAergic circuit formation post-natally. We are assessing the onset, frequency and amplitude of spontaneous GABAergic and glutamatergic activity in Nkx2.1+ interneurons in the developing striatum during the first two postnatal weeks. Preliminary observations point to an
earlier onset of inhibitory and excitatory synaptic activity in the striatum of neonatal mice. Our results indicate that altered interneuron development in the striatum could contribute to PAE-related pathology. Future experiments will allow us to continue to explore the potential role of striatal interneuronopathy and altered inhibitory circuit formation on the development of behavioral and cognitive changes related to PAE.

Disclosures: A.R. Tousley: None. H. Yeh: None. P. Yeh: None.

Poster

327. Consequences of Alcohol and Drug Exposure During Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 327.14/Y7

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA041529

Title: Long-term consequences of perinatal opioid exposure

Authors: *H. J. HARDER, L. HANUS, C. SEARLES, W. KENKEL, A. Z. MURPHY; Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: The incidence rate of infants born exposed to opioids during gestation has increased over 400% in the last decade. However, current preclinical models of perinatal opioid exposure (POE) lack clinical relevance and translatability such that these models typically utilize a truncated schedule of opioid exposure restricted to the second embryonic week in the rodent (roughly equivalent to the second trimester in humans). In contrast, women with an opioid use disorder do not restrict their use to pregnancy, but rather, are more likely to initiate drug use in adolescence, become pregnant, and continue opioid use throughout pregnancy. As such, the ability to translate the results from preclinical models are limited. We have developed a novel, more clinically-relevant model of gestational opioid exposure that allows for the assessment of potential long-term consequences on the offspring. In this model, female Sprague Dawley rats are implanted with slow release morphine pellets (75 mg; NIDA) beginning in early adulthood (P40), continuing through gestation, and ending following weaning. Morphine pellets are replaced every two weeks allowing for regular exposure to morphine at clinically relevant plasma levels. Pups are indirectly exposed to morphine via the dam for the entire gestational period, including lactation. Following parturition, pups are tested on a battery of behavioral assays known to be influenced by opioids and/or early life experience. These assays include juvenile play, response to acute and chronic anxiety and stress, nociceptive sensitivity and morphine efficacy, and response to an immune challenge. Parallel changes in the neurocircuitry underlying opioid signaling will further delineate the impact of perinatal opioid exposure. Generation of a complete phenotype (behavioral, anatomical and molecular) of domains
impacted by a highly translatable model of perinatal opioid exposure will facilitate the
development of new targeted pharmacotherapies for the amelioration of the long-term
consequences, which are largely unknown in the human population.

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**H.J. Harder:** None.  
**L. Hanus:** None.  
**C. Searles:** None.  
**W. Kenkel:** None.  
**A.Z. Murphy:** None.

**Poster**

**327. Consequences of Alcohol and Drug Exposure During Development**

**Location:** Hall A  
**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM  
**Program #/Poster #:** 327.15/Y8  
**Topic:** G.08. Drugs of Abuse and Addiction  
**Support:**  
R21 DA036667 (GAB)  
James Battaglia Endowed Chair (GAB)

**Title:** Late maturing influence of immune activation on morphine withdrawal and tolerance in the infant rat

**Authors:** S. Wang¹, *B. S. East, Jr², G. A. Barr¹;  
¹Children’s Hosp. of Philadelphia, Philadelphia, NY; ²Nathan Kline Inst., Orangeburg, NY

**Abstract:** Between 50-80% of infants in the neonatal intensive care unit are dependent on opiates by the end of their medical treatment. Many of these infants have on-going infections. Best current medical practice is to provide declining amounts of opiates to taper the infant patient off the drugs. As such, this prolongs hospitalization and constitutes a significant burden on the health care system, and an emotional burden on the family whose child remains in the hospital, and has unknown consequences for subsequent development of the newborn. We know little about opiate dependence in the neonate, except that it differs in important mechanisms from dependence in the adult. We also know that there is interplay between opioids and the immune system. Whether that immune-chronic opioid interplay is present or not in the infant has not been studied and thus is not known, despite how common infection is in these babies. We tested the effects of activating the immune system with LPS during chronic morphine treatment in the first or third week of life in the infant rat and tested for precipitated withdrawal or analgesic tolerance, mRNA and protein for immune-related markers. Around weaning (PN21) LPS worsened affective (ultrasonic vocalizations) aspects of withdrawal but not the physical (behavioral signs) aspects. The same immune activating treatment in 7 day old (PN07) infant rats had small protective effect on USV and on behavioral signs, reducing both. Fos-positive cells were increased in the PAG and NAcc, in withdrawal at PN21 only. There were no changes induced by LPS on morphine induced tolerance at either age. These behavioral changes were accompanied by age-dependent differences in the expression of immune-related markers in the
spinal cord and brain. Morphine was immunosuppressant for expression of cytokines at both ages in the spinal cord but only in the older animals in the periaqueductal gray. There were no differences between ages in the number of microglia or astrocytes as a result of LPS treatment. Thus, the effects of activating the immune system during development are late developing in altering precipitated withdrawal and in some but not all aspects of immune function.

Disclosures: S. Wang: None. B.S. East: None. G.A. Barr: None.

Poster

327. Consequences of Alcohol and Drug Exposure During Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 327.16/Y9

Topic: G.08. Drugs of Abuse and Addiction

Title: Effects of early fentanyl exposure on morphine-induced antinociception

Authors: *G. I. PARK, J. A. TAYLOR, J. W. RIOS, J. A. BUNCH, C. A. CRAWFORD; California State Univ. San Bernardino, San Bernardino, CA

Abstract: Opioid use has reached epidemic levels in the United States. There has been a 200% increase in opioid overdose (poisoning) deaths since 2000, and 61% of all drug overdose deaths now involve opioids. An unfortunate consequence of this crisis has been the increase in opioid use in women of childbearing age. Because of the large number of children potentially affected, it is important to understand the long-term consequences of early opioid exposure. While there is an older literature examining the effects of morphine and heroin, there are relatively few studies assessing at the long-term effects of high potency synthetic opioid compounds like fentanyl. Thus, in the present study we examined whether repeated fentanyl during the early neonate period alters later opioid-mediated behaviors. To examine this issue, we assessed the antinociceptive effects of morphine using the tail flick and hot plate tasks. Male and female rats (n = 7-8) were exposed to fentanyl (0, 1, 10, 33 or 100 µg/kg, sc) for 6 consecutive days starting on PD 4. On PD 60, rats were habituated to a tail flick apparatus and a room temperature hot plate. On the following day, tail-flick and paw-lick latencies were measured three times in sequence, with a 10 min interval between trials. After these baseline trials, rats were injected with morphine (0, 1.25, 2.5, 5, or 10 mg/kg, sc) and returned to their home cage for 20 min. Rats were subsequently tested three times on the tail flick and hot plate tasks, with a 10 min interval between each test. As expected, morphine caused a dose-dependent increase in tail flick and paw lick latencies. This morphine-induced effect varied according to pretreatment condition, sex, and analgesic task. Specifically, fentanyl (100 µg/kg) pretreatment reduced the effect of morphine (10 mg/kg) on the hotplate task, while potentiating the antinociception effects of morphine (1.25 mg/kg) on the tail flick task. Interestingly, the effects of fentanyl were only significant in female
rats. The present data suggest that early exposure to fentanyl may have long-term consequences on the functioning of the opioid receptor system of adult female rats.


Poster

327. Consequences of Alcohol and Drug Exposure During Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 327.17/Y10

Topic: G.08. Drugs of Abuse and Addiction

Support: CNPQ
FAPERJ

Title: Effects of caffeine and/or ethanol exposure during development on anxiety-like behavior and learning and memory of adolescent mice

Authors: *A. N. FREITAS, A. C. C. CARVALHO-SILVA, P. C. LISBOA, A. C. MANHÃES, Y. ABREU-VILLAÇA, C. C. FILGUEIRAS;
Univ. do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

Abstract: In spite of epidemiological evidence of the concomitant use of caffeine and ethanol by pregnant women, the consequences of this combined exposure during development have received little attention in experimental studies. In this work we investigated the effects of co-exposure to caffeine and ethanol during development on the anxiety-like behavior, learning and memory, and cortical levels of oxidative stress of adolescent mice. Adult female Swiss mice received one of two concentrations of caffeine (0.1g/L; n=14 or 0.3g/L; n=12) in the drinking water during 7 days before mating. Exposure persisted until their pups were 21 days-old. Control mice (n=10) had ad libitum access to tap water. Every other day, from PN2 to PN8, animals in each litter were injected (i.p) with one of the following solutions: 0.25 µl/g ethanol (ETOH25), 0.5 µl/g ethanol (ETOH50) or saline solution (SAL). From PN30 to PN35 mice were submitted to behavioral tests. Anxiety-like behavior was assessed in elevated plus maze test (EPM) and learning and memory in passive avoidance paradigm (PA). On PN9, cortical levels of oxidative stress were evaluated by the measurement of thiobarbituric acid reactive species (TBARS), antioxidant capacity (DPPH) and protein carbonylation. In EPM, ethanol promoted a dose-dependent decrease in anxiety-like behavior, mice from ETOH50 group spent less time and had fewer entries in open arms than those mice from ETOH25 and SAL groups. Interestingly, in the group of mice exposed to 0.3 g/L of caffeine, anxiety-like behaviors were not affected by ethanol exposure. While caffeine did not affect behavior of mice in PA, learning and memory deficits were found only in males exposed to ethanol. In spite of ethanol did not change oxidative stress...
levels, caffeine had a marked antioxidant effect in all oxidative stress assays. Our data corroborate other studies that show that early exposure to ethanol promotes a number of neurobehavioral disorders. Furthermore, we suggest that caffeine exposure during development has a protective effect on deleterious effects of ethanol.


Poster

327. Consequences of Alcohol and Drug Exposure During Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 327.18/Y11

Topic: G.08. Drugs of Abuse and Addiction

Title: Effects of adolescent sucrose consumption on resistance to punished cocaine in adult male and female rats

Authors: R. PARMAR1, M. M. COBB3, R. ARANGO4, J. PARENT4, M. CRAWFORD4, C. RAMIREZ4, I. C. SUMAYA2, *A. M. GANCARZ-KAUSCH5; 1Psychology, 2Psychology - 24 DDH, California State Univ. Bakersfield, Bakersfield, CA; 3Psychology, UCLA, Los Angeles, CA; 5Psychology, 4California State University, Bakersfield, Bakersfield, CA

Abstract: Environmental challenges during this crucial time of neural plasticity could potentially result in dire neurodevelopment and behavior consequences in adulthood. It is known that exposure to drugs of abuse during adolescence increases future susceptibility to drug addiction in adulthood, yet little is known about exposure to other natural stimuli that may also alter functioning of the mesolimbic dopamine circuitry and their effects on drug abuse in adulthood. Given that natural and drug reinforcers activate the same neural circuitry (Wise & Rompe, 1989), this pathway may also be responsible for such increases in reactivity to natural reinforcers (sucrose) during the adolescent period. Further research is needed to understand exposure to naturally rewarding stimuli during the adolescent period that may alter functioning and behavior. Here, we seek to understand how exposure to sucrose could alter behavior in adulthood. In order to test this, male and female adolescent rats (PND 28-42) were exposed to either 10% noncontingent sucrose or water delivery in fourteen 30 min sessions in operant settings. In adulthood, rats were fitted with chronic indwelling jugular catheters and allowed to recover (PND 51-53). Rats were then tested for cocaine self-administration (PND 54-60). During this phase, rats emitted an active response to receive 1.0 mg/kg/infusion of cocaine according to a Fixed Ratio 1 (FR1) schedule of reinforcement. The schedule was increased to FR5 across the 7 days of testing. Following this phase, rats were then tested for resistance to punished cocaine self-administration (PND 61-65), which included a cocktail of cocaine and histamine. Histamine
was used to produce an aversive, delocalized itching sensation throughout the body that has previously been shown to act as an aversive stimulus. We hypothesized that rats exposed to non-contingent sucrose solution during the adolescent period would self-administer significantly greater number of infusions of cocaine/histamine compared to rats exposed to water. Sucrose treated adolescent male and female rats self-administered significantly more infusions of the cocaine/histamine cocktail compared to water exposed controls suggesting sucrose exposure increased resistance to punished cocaine in adulthood.

Disclosures:  

Poster

327. Consequences of Alcohol and Drug Exposure During Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 327.19/Y12

Topic: G.08. Drugs of Abuse and Addiction

Support:   CIHR MOP-123378
            NSERC

Title: Adolescent nicotine exposure induces long-term depressive and anxiety-like effects through hyper-phosphorylation of the ERK 1-2 and Akt-GSK-3 pathways and neuronal oscillatory dysregulation in the nucleus accumbens

Authors: *S. R. LAVIOLETTE1, R. M. HUDSON2, M. GREEN4, T. D. JUNG5, J. RENARD6, C. E. JOBSON3, W. J. RUSHLOW7;  

Abstract: Nicotine dependence typically develops during adolescence and is associated with a variety of neuropsychiatric co-morbidities, including mood and anxiety disorders. Neurodevelopmental nicotine exposure has been reported to influence the later development of affective and anxiety-related behaviours in both clinical and pre-clinical studies. The mammalian nucleus accumbens shell (NASh) is critically involved in regulating emotional processing and both molecular and neuronal disturbances in this structure are associated with mood and anxiety-related pathologies. Specifically, aberrant γ-band oscillation patterns, disturbances in extracellular-signal related kinase 1-2 (ERK 1-2), protein kinase B (Akt)-glycogen-synthase-kinase-3 (GSK-3) signaling pathways and dopamine (DA) D1/D2 receptor expression patterns have been linked to both the effects of chronic nicotine exposure as well as mood/anxiety-related
phenotypes. In the present study, we used a rodent model of adolescent neurodevelopmental nicotine exposure to examine the expression of several molecular biomarkers associated with mood/anxiety-related phenotypes. We report that nicotine exposure during adolescence (but not adulthood) induces profound upregulation of the ERK 1-2 and Akt-GSK-3 signaling pathways directly within the NASh that persists into adulthood. These adaptations were accompanied by profound decreases in theta, alpha, beta and gamma-band oscillatory states, hyperactive medium spiny neuron (MSN) activity with depressed bursting rates and anxiety and depressive-like behavioural abnormalities. Pharmacologically targeting these molecular and neuronal adaptations directly in the NASh revealed that the Akt-GSK-3 pathway, high-γ-band oscillatory signatures and spontaneous bursting rates in the NASh are involved selectively in mediating adolescent nicotine-induced depressive and anxiety-like neuropathological trajectories.


Poster

327. Consequences of Alcohol and Drug Exposure During Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 327.20/Y13

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA R00 Grant DA035251
NIDA P50 Grant DA044118

Title: Dose- and sex-dependent effects of acute delta9-tetrahydrocannabinol in adolescent rats

Authors: *C. M. RUIZ, J. R. CEVALLOS, E. CASTILLO, E. HARDER, D. N. JUSTESON, J. S. MONTESINOS, S. V. MAHLER; Neurobio. and Behavior, UC Irvine, Irvine, CA

Abstract: Cannabis legalization is rapidly spreading throughout North America and the world, which is expected to increase the availability of the drug to all age groups, including adolescents whose brains are in a critical neurodevelopmental period. Numerous studies in rodents have shown persistent, perhaps permanent effects of adolescent exposure to cannabinoid drugs including delta(9)-tetrahydrocannabinol (THC), but little attention has been given to the translational relevance of the dosing delivered in these studies. Indeed, most studies employ moderate to large doses that typically cause anxiogenic effects rather than rewarding ones. Here we test the effects of acutely delivered low or high doses of THC (0, 0.5, 5mg/kg, i.p.) on behaviors in male and female adolescent rats. Male and female long-evans rats were acquired at postnatal day (PD) 22 or bred in-house and all testing was conducted from PD27 to PD50, during adolescence. First, the effects of acute THC on elevated plus maze (EPM) and open field
anxiety-related behaviors was tested. Time spent on the closed and open arms of the apparatus was measured in the 5min test. Subsequently, effects of THC on time spent in the center or periphery of a novel open field was measured for 5min, then locomotion in this environment was monitored for an additional hour. Next, the rewarding effects of THC were assayed in a three-chamber conditioned place preference task, where vehicle (5% Tween80 in saline) was paired four times with one chamber and THC paired with a distinct chamber over 8 alternating, daily training sessions. Finally, the effects of acute THC on neural activity was measured by analyzing c-Fos immunoreactivity after acute vehicle or THC injections. Behavioral and neural activity effects of THC in adolescents were dose- and sex-dependent with both anxiogenic and rewarding effects observed and recruitment of several limbic reward- and stress-related regions. Ongoing work will determine mechanisms of these sex- and age-dependent THC effects, in pursuit of a rat adolescent cannabinoid drug dosing protocol that matches key aspects of human teenage cannabis use.


Poster

327. Consequences of Alcohol and Drug Exposure During Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 327.21/Y14

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA042595  
NIH Grant DA043967

Title: Cannabinoid exposure in adolescence dysregulates genes that orchestrate dopamine development and cocaine-motivated behavior

Authors: *N. E. ZLENNIK¹, S. CUESTA⁴, J. M. WENZEL², G. A. HERNANDEZ⁵, D. NOUEL⁴, S. KUMMER⁷, L.-Y. ZHANG⁸, C. FLORES⁶, J. F. CHEER³;  
¹Univ. of Maryland Sch. of Med., Baltimore, MN; ²Anat. & Neurobio., ³Anat. and Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD; ⁴Psychiatry, ⁵Dept of Psych, ⁶McGill Univ., Verdun, QC, Canada; ⁷Univ. of Pompeu Fabra, Barcelona, Spain; ⁸Peking Univ., Beijing City, China

Abstract: Cannabis is the most commonly abused illicit drug among adolescents, and excessive use in this population is associated with the development of psychiatric conditions, including drug addiction. Adolescence is a critical period for the refinement and organization of neuronal connectivity, especially within the mesocorticolimbic dopamine circuitry. In particular, dysregulation of the guidance cue receptor, Dcc, in ventral tegmental area (VTA) dopamine
neurons disrupts spatiotemporal targeting of dopamine axons to the nucleus accumbens (NAc) and the medial prefrontal cortex (mPFC). We have previously demonstrated that exposure to amphetamine in early adolescence (PND21-32) disrupts the development of dopamine circuitry development, leading to alterations in cognitive processing and drug seeking in adulthood. Here, we examine whether exposure to the synthetic cannabinoid-1/2 receptor agonist WIN-55,212-2 (WIN) in early adolescence regulates Dcc mRNA expression in the VTA and induces alterations in drug-motivated behaviors and in dopamine function in adulthood. Preliminary findings demonstrate that adolescent exposure to WIN downregulates the Dcc receptor in the VTA and its ligand, Netrin-1, in the NAc and mPFC, suggesting disruption of pre- and postsynaptic components of mesocorticolimbic dopamine circuitry. Additionally, WIN-treated mice display aberrant responding for cocaine as well as potentiated cocaine-mediated anxiety. Ongoing experiments will elucidate functional changes in cocaine-evoked phasic dopamine release in the NAc and mPFC. Overall, these findings support that repeated exposure to a cannabinoid-1/2 receptor agonist in adolescence impacts mesocorticolimbic dopamine system maturation and may have important implications for dopamine-mediated learning and psychostimulant-motivated behavior later in life.


**Poster**

327. Consequences of Alcohol and Drug Exposure During Development

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 327.22/Y15

**Topic:** G.08. Drugs of Abuse and Addiction

**Title:** Adolescent but not adult exposure to nicotine produces long-term effects on anxiety measured in light-enhanced startle

**Authors:** *R. C. BARNET, D. KOSEREISOGLU;* Col. William & Mary, Williamsburg, VA

**Abstract:** Chronic adolescent nicotine exposure may cause long-term changes in the brain pathways that mediate anxiety. The Bed Nucleus of the Stria Terminalis has been linked to anxiety as expressed in one animal model known as the Light-Enhanced Startle Effect (LES; Walker & Davis, 1997). Experiment 1 examined whether adolescent nicotine exposure in rats would cause long-lasting changes in anxiety that persist into adulthood. Adolescent Sprague-Dawley rats were exposed to either nicotine (.15 mg/kg, .40 mg/kg) or saline from postnatal days 28-42. On approximately postnatal day 67 the effect of earlier adolescent nicotine exposure on anxiety was assessed in the LES paradigm. Adult males but not females exposed to nicotine as
adolescents showed significant increases in anxiety measured in LES compared to saline controls. In Experiment 2, the same nicotine preexposure administered during adulthood (beginning on postnatal day 65) rather than adolescence failed to produce nicotine-induced alterations in anxiety. These findings suggest nicotine exposure during adolescence can produce changes in the neural pathways that mediate anxiety and that these changes persist into adulthood. Given anxiety has been implicated with the progression of drug taking in addiction, and relapse, the present findings further extend use of light-enhanced startle as a useful model for examining adolescent liability factors in addiction.

Disclosures: R.C. Barnet: None. D. Kosereisoglu: None.

Poster

327. Consequences of Alcohol and Drug Exposure During Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 327.23/Y16

Topic: G.08. Drugs of Abuse and Addiction

Support: Fundacion Universitaria Konrad Lorenz
 Universidad Nacional de Colombia

Title: Differences of nicotine-induced locomotor sensitization in adolescent and adult Wistar rats

Authors: *L. A. ORTEGA¹, M. LAMPREA², C. NOVOA², J. SOLANO²;
¹Fundacion Universitaria Konrad Lorenz, Bogota, Colombia; ²Univ. Nacional de Colombia, Bogota, Colombia

Abstract: Locomotor sensitization is an increased behavioral response to nicotine administration after repetitive drug exposure. Although such reported effects tend to be reliable, little is known about the behavioral mechanisms underlying nicotine-induced locomotor sensitization. In the present study we evaluated the effects of acute nicotine administration on locomotor performance, for adolescent and adult rats, in the context of previous experience with chronic nicotine. Four locomotor activity tests (5 minutes each) were performed in an open field. Behavioral analyses were centered on free exploration, using a virtual division of the peripheral and center areas of the open field. During day 1, the experimental procedure started with a baseline activity test. Immediately after, subjects were randomly assigned to either Nicotine or Vehicle groups, and nicotine (0.14 mg/kg; reported as free base; same dose for all acute and chronic nicotine administrations) or control saline were administered via SC injections. Ten minutes after the injection, animals underwent an acute challenge activity test. From days 2 to 20, animals were assigned to groups receiving one daily injection of chronic nicotine or saline. On day 21, rats underwent a long-term re-exposure activity test. Immediately after, they received an injection of nicotine or saline. Ten minutes after the injection, animals were assessed on a...
chronic challenge activity test. Results suggest that chronic nicotine induced locomotor sensitization among adolescent and adult rats. Interestingly, open field area preference was differentially modulated by nicotine among adolescents and adults. These results suggest the importance of (1) developmental age on the interpretation of locomotor sensitization, and (2) the orientation of the rat to potentially dangerous contexts as a mechanism underlying locomotor sensitization.


Poster

327. Consequences of Alcohol and Drug Exposure During Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 327.24/Y17

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant GM083883

Title: MK801-induced locomotor activity in preweanling and adolescent male and female rats: Role of the dopamine and serotonin systems

Authors: M. G. APODACA, G. I. PARK, N. R. MONTEJANO, A. TERAN, T. J. BAUM, *S. A. MCDOUGALL;
Psychology, California State Univ., San Bernardino, CA

Abstract: Ketamine is a dissociative anesthetic that causes hyperactivity in both rats and mice; however, the strength of this effect varies greatly depending on the age and sex of the animal being tested. MK-801 shares some of the same pharmacological properties as ketamine (i.e., both are NMDA receptor channel blockers) and, like ketamine, MK-801 stimulates locomotor activity in rats. With both drugs, there is evidence that the increased locomotor activity is ultimately mediated by direct or indirect activation of monoamine (MA) systems. Whether the neural mechanisms mediating MK801-induced locomotor activity differ according to age and sex has not been fully determined. The purpose of this study was to examine if MA depletion, dopamine (DA) depletion, and/or serotonin (5-HT) depletion attenuates MK801-induced locomotor activity, and whether this effect differs among male and female preweanling and adolescent rats. Rats were treated with vehicle, reserpine (a MA depleting agent; 1 or 5 mg/kg), α-methyl-DL-tyrosine methyl ester HCl (AMPT; a DA synthesis inhibitor; 2 × 200 mg/kg), or 4-chloro-DL-phenylalanine methyl ester HCl (PCPA; a 5-HT synthesis inhibitor; 3 × 200 mg/kg) prior to an acute injection of saline or MK801 (0.3 mg/kg, ip). Locomotion was then measured for 2 h. MK801 stimulated substantially more locomotor activity in preweanling rats than adolescent rats. Among adolescent rats, females exhibited significantly more locomotor activity than males. Both AMPT and PCPA decreased the MK801-induced locomotion of preweanling rats and
female adolescent rats, but these compounds did not depress the basal or MK801-induced locomotor activity of male adolescent rats. Reserpine (5 mg/kg), on the other hand, reduced the MK801-induced locomotion of rats regardless of age or sex. Preweanling rats were more sensitive to the effects of reserpine than adolescent rats, since the lower dose of reserpine (1 mg/kg) only attenuated the MK801-induced locomotor activity of the younger age group. When considered together, these results suggest that the neural mechanisms underlying MK801-induced locomotor activity vary according to both age and sex. In general, preweanling rats respond like female rats, as both groups are sensitive to the locomotor depressing effects of DA and 5-HT synthesis inhibitors. In contrast, the same synthesis inhibitors did not affect the MK801-induced locomotion of male adolescent rats. Consistent with results from past ketamine studies, it appears that the locomotor activating effects of MK801 weaken in male rats (but not female rats) as they mature, and some of these effects may be due to age- and sex-dependent differences in MA system functioning.

**Disclosures:** M.G. Apodaca: None. G.I. Park: None. N.R. Montejano: None. A. Teran: None. T.J. Baum: None. S.A. McDougall: None.

**Poster**

**327. Consequences of Alcohol and Drug Exposure During Development**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 327.25/Y18

**Topic:** G.08. Drugs of Abuse and Addiction

**Support:** NIH Grant GM083883

**Title:** Ketamine-, cocaine-, and amphetamine-induced locomotor activity in preweanling and adolescent male and female rats: Role of the serotonin system

**Authors:** *J. W. RIOS, J. A. TAYLOR, M. G. APODACA, J. A. M. ROBINSON, S. A. MCDougALL;* Psychology, California State Univ., San Bernardino, CA

**Abstract:** High doses of ketamine (an NMDA receptor channel blocker) cause sedation, analgesia, and anesthesia in both rats and mice. At subanesthetic doses, ketamine causes prolonged periods of hyperactivity. One of the most interesting aspects of this ketamine-induced effect is that it varies greatly according to both age and sex. Specifically, male rats show a progressive decline in ketamine-induced locomotor activity with increasing age, while female rats show a strong locomotor response across all age groups. The neural mechanisms responsible for ketamine’s locomotor activating effects are uncertain, but there is substantial evidence that ketamine directly or indirectly modulates monoaminergic system functioning. Indeed, we previously showed that dopamine depletion causes a substantial reduction in ketamine-induced
locomotor activity. This finding does not rule out the possible involvement of other monoamine systems, since serotonin (5-HT) receptor antagonists reduce the locomotor activating effects of MK801 (an NMDA receptor antagonist). The purpose of this study was to determine whether 5-HT depletion attenuates ketamine- and psychostimulant-induced locomotor activity, and whether this effect differs among preweanling and adolescent male and female rats. Rats were treated with vehicle or the 5-HT synthesis inhibitor 4-chloro-DL-phenylalanine methyl ester HCl (PCPA, 3 × 200 mg/kg) for three days prior to the administration of an acute injection of saline, ketamine (20 or 40 mg/kg), amphetamine (2 mg/kg), or cocaine (15 mg/kg). Locomotor activity was measured for 2 h. In all age groups and both sexes, 5-HT depletion caused a modest decline (25%) in the locomotion of rats treated with 40 mg/kg ketamine. Only in female adolescent rats, did 5-HT depletion reduce the locomotor activating effects of 20 mg/kg ketamine. 5-HT depletion did not affect amphetamine-induced locomotion at any age. Interestingly, PCPA dramatically reduced the locomotor activating effects of cocaine in both preweanling and female adolescent rats, but had minimal effect on the cocaine-induced locomotor activity of male adolescent rats. In sum, (a) both age and sex are critical biological constraints that influence whether 5-HT depletion attenuates ketamine- and cocaine-induced locomotor activity; (b) ketamine caused different patterns of locomotion than amphetamine or cocaine, suggesting that ketamine did not function in the same manner as these prototypical psychostimulant drugs; and (c) when considered together with past results, ketamine’s locomotor activating properties are primarily dependent on dopamine neurotransmission, but some 5-HT involvement is evident.

**Disclosures:** J.W. Rios: None. J.A. Taylor: None. M.G. Apodaca: None. J.A.M. Robinson: None. S.A. McDougall: None.

**Poster**

327. Consequences of Alcohol and Drug Exposure During Development

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 327.26/Y19

**Topic:** G.08. Drugs of Abuse and Addiction

**Support:** 1R01AA024798

**Title:** Pre-conception vs post-conception exposure to ethanol: Similar effects on the development of hypothalamic hypocretin/orexin neurons and behavior in larval zebrafish

**Authors:** *A. D. COLLIER, S. MIN, S. CAMPBELL, M. ROBERTS, K. CAMIDGE, S. F. LEIBOWITZ; The Rockefeller Univ., New York, NY

**Abstract:** There are clinical and pre-clinical studies showing that exposure of the embryo to ethanol markedly affects neuronal development and stimulates alcohol drinking and related
behaviors. In rodents and zebrafish, our studies show that embryonic exposure to low-dose ethanol, in addition increasing voluntary ethanol intake during adolescence, increases the density of hypothalamic hypocretin/orexin (Hcrt) neurons, a neuropeptide known to regulate reward-related behaviors. The question addressed here in zebrafish is whether maternal ethanol intake before conception affects neuronal and behavioral development, phenomena suggested by clinical reports but seldom investigated, and how these effects compare to those of embryonic ethanol exposure. The goal of the present study was to characterize and compare how pre- and post-conception ethanol exposure affects early development of the Hcrt system and behavior, including voluntary ethanol-gelatin consumption and locomotor activity in larval zebrafish. We used both a pre-conception model, involving 14-day maternal ethanol-gelatin (10%) consumption, and a post-conception model, involving embryonic ethanol (0.5% v/v) exposure in the water from 22-24 hours post-fertilization (hpf). We measured the number, distribution and anatomical location of Hcrt neurons, using live imaging and Imaris software in Hcrt:EGFP transgenic zebrafish during early development. We found that pre-conception maternal ethanol intake and post-conception embryonic ethanol exposure have similar effects on the Hcrt neurons and behavior. They both increased the number of Hcrt neurons and caused these neurons to become more widely dispersed, an effect that was persistent throughout early larval development. Also, these effects on Hcrt neurons in both models were found to be asymmetric, occurring predominantly on the left side of the brain, and they both involved a greater dispersion and altered anatomical location, with some Hcrt neurons detected outside their normal position in the anterior hypothalamus, again primarily on the left side of the brain. In addition, both models of ethanol exposure stimulated voluntary ethanol consumption in 12 dpf zebrafish, measured using a new feeding paradigm, which was positively correlated with Hcrt neuron number, and they also caused an increase in locomotor activity. These findings demonstrate that both pre-conception and post-conception ethanol exposure markedly and similarly alter Hcrt development and behavior, producing unnatural asymmetries and altered anatomical locations that may contribute to the increased ethanol consumption and other behavioral disturbances in offspring.


Poster

327. Consequences of Alcohol and Drug Exposure During Development

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 327.27/Y20

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA020022
**Title:** The effect of adolescent intermittent binge ethanol and ethanol challenge on Arc expression in young adult rat brain

**Authors:** *W. LIU*¹, F. T. CREWS²;

²Pharmacol & Psychiat, ¹Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

**Abstract:** Underage drinking is the most common form of substance abuse in adolescent humans, it significantly increases the risk of developing an alcohol abuse problem. In the current study, adolescent intermittent binge ethanol was modeled using adolescent male Wistar rats treated with intermittent binge ethanol (AIE exposure: 5 g/kg/day, i.g., 2 days on-2 days off, P25-P54) and assessed in early adulthood (P80). Arc (the activity-regulated cytoskeletal-associated protein) is a member of the immediate-early gene family that acts as an important regulator of synaptic plasticity. To investigate the effect of AIE exposure on Arc expression in young adult rats (P80), four study groups were assigned. At weaning on P21, male offspring were weight matched and pair-housed into two groups, water control and AIE group. At P80 following 26 days without ethanol, both control and AIE groups were separately divided into two groups challenged with water or ethanol (4 g/kg, i.g., groups: control, AIE, control-challenge and AIE-challenge), and then sacrificed 2 hours later. Arc+IR expression in multiple brain regions of the young adult rat was determined using immunohistochemistry (IHC). Our studies found that Arc+IR baseline expression at P80 was remarkably increased following AIE exposure in prelimbic cortex (92%, p<0.05), infralimbic (91%, p<0.05), insula (93%, p<0.05), anterior cingulate 1 and 2 (124% and 123%, p<0.01), lateral septal nucleus (369%, p<0.05) as well as the nucleus accumbens shell (99%, p<0.05) and core (96%, p<0.01). These persistent increases in Arc+IR expression suggest AIE exposure may cause persistent hyper-excitability. Acute ethanol (4 g/kg) challenge of controls significantly increased Arc+IR expression in most brain regions of the interest ranging from 2-5 fold (including frontal, entorhinal, ectorhinal, and perirhinal cortex; nucleus accumbens; granular cell layer, amygdala subregions and lateral septal nucleus) except dorsomedial hypothalamic nucleus at P80. Further, AIE exposure rat ethanol-challenge Arc+IR expression was reduced in the shell of nucleus accumbens (29%, p<0.05), lateral septal nucleus (44%, p<0.05), basolateral (63%, p<0.001) and lateral (38%, p<0.01) amygdaloid nucleus compared to control ethanol challenge Arc expression in those brain regions. These findings indicate that adolescent intermittent binge ethanol has a long lasting impacts on Arc expression induced neuronal plasticity that varies in different subregions of young adult rat brain (Supported by the NADIA from NIAAA).

**Disclosures:** W. Liu: None. F.T. Crews: None.

**Poster**

327. Consequences of Alcohol and Drug Exposure During Development

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 327.28/Y21
**Abstract:** The alpha-6 (α6) nicotinic acetylcholine receptor (nAChR) subunit exhibits peak expression in the ventral tegmental area and substantia nigra during adolescence. Adolescence is a critical developmental period (12-18 years in humans, postnatal days 28-42 in rodents) where the maturation of brain neurocircuitry is vulnerable to nicotine exposure and coincides with the initiation of drug use. In humans, a single nucleotide polymorphism (SNP), rs2304297, at position 123 in the 3’-untranslated region (UTR) of the α6 nAChR gene, CHRNA6<sub>C123G</sub>, is associated with enhanced smoking and drug use in adolescence. Our lab has developed a humanized mutant rat line via CRISPR/Cas9 genomic engineering replacing the rat CHRNA6 3’UTR with the human CHRNA6<sub>C123CC</sub> (non-risk allele) and CHRNA6<sub>C123GG</sub> (risk allele) 3’UTR. Previously we illustrated that the CHRNA6<sub>C123GG</sub> genetic variant is functional within SH-SY5Y cells. We hypothesize that the CHRNA6<sub>C123GG</sub> genetic variant is i.) functional in vivo and ii.) will significantly influence drug self-administration, but not baseline behaviors. As a first approach to test our hypotheses, we used our humanized CHRNA6<sub>C123G</sub> 3’UTR rodent line to test the role of the genetic variant in baseline behaviors, including natural food reward, locomotion and anxiety. Given that the α6 subunit does not alter baseline locomotion, anxiety, and food reward, we predicted no differences in these behaviors between the WT and humanized CHRNA6<sub>C123G</sub> 3’UTR rat line. CHRNA6<sub>C123GG</sub>, CHRNA6<sub>C123CC</sub>, and WT adolescent rats were subjected to food reinforcement at a fixed ratio (FR1) schedule for 6 days followed by FR2, FR5, and progressive ratio for 2 days at each schedule. Animals then underwent a locomotor assay, light dark box test, and elevated plus maze for 1 day per test (PN 28-42). Our preliminary data shows that male CHRNA6<sub>C123GG</sub> rats have enhanced food reward by the end of FR1 and during the FR2 schedule. The responding normalizes across genotype and sex by progressive ratio testing. No sex- or genotype-dependent differences were identified in locomotor activity and anxiety across all groups. Our preliminary data indicates that the CHRNA6<sub>C123G</sub> SNP is functional in vivo by influencing the motivation to attain food. Future studies aim to elucidate the role of CHRNA6<sub>C123G</sub> SNP in drug self-administration.

**Disclosures:** A. Cardenas: None. Y. Bai: None. S. Lotfipour: None.
Poster

328. Cocaine Craving

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 328.01/Y22

**Topic:** G.08. Drugs of Abuse and Addiction

**Support:** NIH DA015835

**Title:** NMDA receptors in rat nucleus accumbens are dynamically regulated during withdrawal from cocaine self-administration

**Authors:** *D. T. CHRISTIAN*¹, M. T. STEFANIK², L. A. BEAN³, A. M. WUNSCH⁴, J. R. FUNKE⁵, C. A. BRIGGS⁶, J. LYONS⁸, M. MILOVANOVIC⁸, G. E. STUTZMANN⁹, D. NICHOLSON⁴, K.-Y. TSENG¹⁰, M. E. WOLF⁵;


**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, Ca²⁺-permeable AMPARs (CP-AMPARs) that accumulate in the nucleus accumbens (NAc). Less is known about NMDAR plasticity during incubation, especially in the NAc core. It has been shown that elevated levels of GluN2B-containing NMDARs in the NAc shell, during early withdrawal, are important for subsequent incubation (e.g., Wang et al., 2018). Furthermore, incorporation of GluN3- and GluN2B-containing NMDARs accompanies an 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 various withdrawal times following self-administration. We measured evoked NMDAR-mediated synaptic responses across membrane holding potentials (-80 to +40) in the presence of subtype selective blockers, identifying an increase in GluN2B-containing receptors beginning on withdrawal day 4-5. We also detected atypical NMDARs, not found in saline control animals, that are comprised of GluN3 and GluN2B subunits during a later withdrawal period (day 13-20). These atypical NMDARs persisted in MSN synapses into late withdrawal (withdrawal day 39 and greater). Super-resolution light microscopy immunofluorescence array tomography was used to characterize the expression pattern of NMDAR subunits in both control and incubated
conditions. Differential expression of GluN3-containing NMDARs was found between treatment groups. We then conducted behavioral studies to determine if GluN2B- or GluN3-containing NMDARs contribute to the incubation of cocaine craving. Viral knockdown of GluN3A subunits in early withdrawal disrupted later expression of incubated cocaine craving. 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 maintained throughout the period when incubated drug seeking is maximal.


Poster

328. Cocaine Craving

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 328.02/Y23

Topic: G.08. Drugs of Abuse and Addiction

Support: DA046141
DA015835
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Whitehall Foundation
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Edward Mallinckrot Jr. Foundation

Title: Defining the role of retinoic acid mediated homeostatic plasticity in nucleus accumbens core medium spiny neurons during incubation of cocaine craving

Authors: *A. M. WUNSCH*1,2, D. T. CHRISTIAN3,2, J. R. FUNKE1,2, T. A. GREEN4, E. S. CALIPARI5, L. CHEN6, M. E. WOLF1,2,

Abstract: Profound alterations in glutamate-dependent synaptic plasticity accompany incubation of cocaine craving. In rats that self-administer cocaine, calcium (Ca2+) permeable AMPA receptors (CP-AMPAR) accumulate in excitatory synapses of nucleus accumbens (NAc) core medium spiny neurons (MSN) following ~1 month of abstinence and are required for expression of incubation, whereas CP-AMPAR levels are low in drug-naïve rats. Defining signaling
cascades involved in the long-term synaptic plasticity that drives CP-AMPAR accumulation will clarify mechanisms regulating the strength of cocaine craving. Some data support the idea that cocaine withdrawal leads to reduced activity of MSN at baseline, which may trigger homeostatic plasticity leading to incubation. One form of inactivity-induced synaptic scaling (a type of homeostatic synaptic plasticity) involves activity-dependent regulation of dendritic protein translation by retinoic acid (RA). In hippocampal neurons, blockade of neuronal activity reduces intracellular Ca$^{2+}$, which disinhibits RA synthesis and leads to synaptic insertion of CP-AMPAR. We hypothesize that reduced Ca$^{2+}$ activity during cocaine withdrawal similarly increases RA synthesis in NAc core MSN, leading to increased levels of CP-AMPAR. Several lines of work are underway to test this hypothesis. First, we studied NAc neurons co-cultured with prefrontal cortex neurons and transfected with a RA reporter system. We found that application of the RA synthesis inhibitor DEAB reduced RA activity in MSN, suggesting RA signaling cascades are functional in NAc MSN. Studies are underway to assess whether RA signaling in cultured MSN is dependent on synaptic activity and intracellular Ca$^{2+}$ levels. Second, whole cell patch clamp recordings are performed on NAc core MSN in brain slices from cocaine-incubated rats. Preliminary data suggest that inhibiting RA synthesis with DEAB reduces CP-AMPAR levels in cocaine-incubated rats to those seen in drug naïve rats, suggesting RA may be an important regulator of CP-AMPAR during abstinence. Third, we will use viral vectors to interfere with RA synthesis or degradation to study the role of RA in NAc core in the incubation of craving and regulation of CP-AMPAR levels. Lastly, we are using fiber photometry to directly assess changes in intracellular Ca$^{2+}$ levels in NAc core MSN at baseline and in response to a cocaine-paired cue during incubation of cocaine craving. Ultimately this work has the potential to define novel targets to reduce drug craving and prolong abstinence.


Poster

328. Cocaine Craving

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 328.03/Y24

Topic: G.08. Drugs of Abuse and Addiction

Support: DA009621

Title: Defining sex differences during incubation of methamphetamine craving

Authors: *J. R. FUNKE, A. M. WUNSCH, M. E. WOLF; Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR
Abstract: One of the intractable features of methamphetamine (METH) substance use disorder is the high rate of relapse, which occurs even after prolonged abstinence. While relapse can be induced by a variety of stimuli, one established mechanism involves exposure to drug-paired cues leading to craving and subsequent relapse. This form of cue-induced drug craving can be studied in a rodent model of relapse termed ‘incubation of drug craving’. In this model, rats undergo extended-access self-administration (SA) of methamphetamine (or saline; control condition) for 6 hr/day for 10 days. A light cue is paired with each methamphetamine infusion. Rats then undergo cue-induced seeking tests during abstinence in which rats nose poke for presentation of the METH-paired light cue but do not receive drug. Cue-induced METH seeking was found to increase steadily (or ‘incubate’) during the first week of abstinence and remain elevated for at least 45 days following the last METH exposure in male rats. Furthermore, the accumulation of higher conductance calcium (Ca²⁺)-permeable AMPARs (CP-AMPARs) in the nucleus accumbens core mirrors the time course of METH incubation. The overall goal of this project is to establish the rising and falling phases of METH incubation in both male and female rats. In order to define the time course of incubation of METH craving, male and female rats will undergo extended access METH or saline self-administration. Using a within-subject design, experiments are in progress in which cue-induced METH seeking tests are conducted on withdrawal day (WD) 1, WD7, WD30, WD100, and WD180. Estrous cycle is monitored in the female rats around seeking tests. Future studies will determine if CP-AMPAR levels parallel craving during falling as well as rising phases of incubation. Incubation of craving also occurs in abstinent human METH users, so it is important to define its time course and underlying mechanisms.


Poster

328. Cocaine Craving

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 328.04/Y25

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA015835

Title: The role of protein translation in the nucleus accumbens in the expression of incubation of cocaine craving

Authors: *A. B. KAWA, M. E. WOLF;
Behavioral Neurosci., OHSU, Portland, OR

Abstract: In the ‘incubation of cocaine craving’ model of relapse, rats self-administer cocaine using an extended access procedure, and then experience a prolonged abstinence period. During
abstinence, rats exhibit a progressive intensification (incubation) of cue-induced craving. We have shown that Ca\(^{2+}\)-permeable AMPA receptors (CP-AMPAR), comprised exclusively of the GluA1 subunit, accumulate in the nucleus accumbens (NAc) during abstinence and are required for the expression of incubated craving. We have also shown that maintenance of CP-AMPARs in NAc synapses requires active protein translation, as levels are rapidly normalized by general protein translation inhibitors. Also, intra-NAc treatment with a protein translation inhibitor just before the cue-induced seeking test prevents the expression of incubated craving. However, little is known about the specifics of this critical protein translation. In some conditions, inhibition of general protein translation increases the translation of a subset of mRNA with 5’ upstream open reading frames, such as Oligophrenin-1 (OPHN1). OPHN1 translation is positively regulated by p-eIF2α. In the hippocampus, OPHN1 is necessary for eIF2α-mediated mGluR-LTD and the removal of synaptic AMPARs. In the VTA, this pathway plays a role in bidirectional CP-AMPAR plasticity in cocaine-exposed animals. Thus, in our NAc studies, treatment with protein translation inhibitors may have increased translation of OPHN1, mimicking mGluR-LTD and removing synaptic CP-AMPARs. In order to learn more about the proteins translated in the NAc of ‘incubated’ rats and more specifically the regulation of OPHN1 and its role in incubation we are using several strategies. These include puromycin labeling to identify proteins translated during a seeking test in ‘incubated’ rats and using rats that express Cre recombinase in either D1R or D2R medium spiny neurons to express GFP-tagged ribosomes in a cell specific manner, enabling us to isolate actively translating mRNAs for analysis by PCR. We are also testing the effects of bidirectionally manipulating OPHN1 on incubation and CP-AMPAR expression. The results of these experiments will indicate if OPHN1 is a critical regulator of CP-AMPARs in the NAc after incubation of cocaine craving.

**Disclosures:** A.B. Kawa: None. M.E. Wolf: None.

**Poster**

**328. Cocaine Craving**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 328.05/Y26

**Topic:** G.08. Drugs of Abuse and Addiction

**Support:** NIH Grant DA009621 (MW)

**Title:** Alterations of the endocannabinoid signaling complex after incubation of cocaine craving

**Authors:** *C. H. MURRAY\(^1\)*, M. MILOVANOVIĆ\(^1\), J. A. LOWETH\(^2\), A. D. GAULDEN\(^3\), A. J. CACCAMISE\(^4\), J. R. FUNKE\(^5\), S. PATEL\(^3\), M. E. WOLF\(^6\);

Abstract: Relapse is a major challenge to the treatment of substance abuse disorders. Vulnerability to relapse is exemplified by incubation of drug craving, a progressive increase in cue-induced craving over the course of withdrawal from various drugs of abuse observed in both humans and rodents. We and others have shown that incubation of cocaine craving involves a strengthening of nucleus accumbens (NAc) synapses via post-synaptic accumulation of high conductance Ca\(^{2+}\)-permeable AMPA receptors (CP-AMPARs), which enhances medium spiny neuron (MSN) reactivity to drug-associated cues for the expression of incubation. Additionally, impairment of the pre-synaptic regulation of glutamate release occurs after prolonged cocaine withdrawal, through a loss of the DHPG-induced synaptic depression that is normally mediated by mGlu5 and CB1 receptors. Our previous studies suggest that this loss stems from postsynaptic alterations in the mGlu5-endocannabinoid signaling complex, referred to as the 2-AG signalosome. We investigated the mechanisms underlying this impairment. Co-IP studies revealed that, although the 2-AG signalosome was intact, there was an increase in the physical association of Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII) and diacylglycerol lipase-\(\alpha\) (DGL), which would be predicted, based on earlier studies, to inhibit DGL activity and therefore production of 2-AG. This was confirmed through a DGL activity assay. Future studies will further assess the role of CaMKII in the loss of mGlu5-mediated synaptic depression after incubation of cocaine craving. These studies clarify the nature of impaired mGlu5-mediated synaptic depression during cocaine withdrawal and establish a role for CaMKII in the synaptic alterations associated with incubation of cocaine craving. Support: DA009621 (MW)


Poster

328. Cocaine Craving

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 328.06/Y27

Topic: G.08. Drugs of Abuse and Addiction

Support: Intramural Research Program of National Institute on Drug Abuse fellowship from the National Institute on Drug Abuse–French Institute of Health and Medical Research program

Title: Incubation of cocaine craving after intermittent access self-administration: Sex differences and estrous cycle

Authors: *C. NICOLAS\(^1\), T. I. RUSSELL\(^1\), A. F. PIERCE\(^1\), S. MALDERA\(^1\), A. HOLLEY\(^3\), Z.-B. YOU\(^2\), M. M. MCCARTHY\(^3\), Y. SHAHAM\(^1\), S. IKEMOTO\(^1\);
\(^1\)Behavioral Neurosci. Res. Br., \(^2\)Mol. Targets and Medications Discovery Br., Natl. Inst. On Drug Abuse-Irp, Baltimore, MD; \(^3\)Univ. of Maryland Sch. of Med., Baltimore, MD
Abstract: Studies using continuous-access drug self-administration showed that cocaine seeking increases during abstinence (incubation of cocaine craving). Recently, studies using intermittent-access self-administration showed increased motivation to self-administer and seek cocaine. We examined whether intermittent cocaine self-administration would potentiate incubation of craving in male and female rats and examined the estrous cycle's role in this incubation. In experiment 1, male and female rats self-administered cocaine either continuously (8 hours/day) or intermittently (5 minutes ON, 25 minutes OFF x 16) for 12 days, followed by relapse tests after 2 or 29 days. In experiments 2 and 3, female rats self-administered cocaine intermittently for six, 12, or 18 sessions. In experiment 4, female rats self-administered cocaine continuously followed by relapse tests after 2 or 29 days. In experiments 3 and 4, the estrous cycle was measured using a vaginal smear test. Incubation of cocaine craving was observed in both sexes after either intermittent or continuous drug self-administration. Independent of access condition and abstinence day, cocaine seeking was higher in female rats than in male rats. In both sexes, cocaine seeking on both abstinence days was higher after intermittent drug access than after continuous drug access. In female rats, incubation of craving after either intermittent or continuous drug access was significantly higher during estrus than during non-estrus; for intermittent drug access, this effect was independent of the training duration. In both sexes, intermittent cocaine access caused time-independent increases in drug seeking during abstinence. In female rats, the time-dependent increase in drug seeking (incubation) is critically dependent on the estrous cycle phase.


Poster

328. Cocaine Craving

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 328.07/Y28

Topic: G.08. Drugs of Abuse and Addiction

Title: Central amygdala PKCδ-expressing neurons are critical to inhibition of incubation of methamphetamine craving after social choice-induced voluntary abstinence

Authors: *M. VENNIRO1, T. RUSSELL1, L. R. WHITAKER1, C. RICHIE1, R. O. MESSING2, Y. SHAHAM1;
1Natl. Inst. On Drug Abuse, Baltimore, MD; 2Univ. of Texas, Austin, TX

Abstract: We recently reported that social choice-induced voluntary abstinence prevents incubation of methamphetamine craving. This protective effect was associated with activation of PKCδ-expressing neurons in central amygdala lateral (CeL) and inhibition of Fos expression in central amygdala medial (CeM). Here we used short-hairpin RNA against PKCδ mRNA
(shPKCδ) and immunohistochemistry to determine the causal role of CeL PKCδ in inhibition of incubation of methamphetamine craving after voluntary abstinence. In Experiment 1 we used immunohistochemistry to validate the AAV virus expressing shPKCδ by injecting it (or shScram control) into CeL either 2 or 4 weeks before novel context exposure to induce Fos. In Experiment 2, we trained two group of rats injected with shPKCδ or shScram into CeL to lever press for social interaction (6 d) and then for methamphetamine infusions (12 d). We then assessed relapse to methamphetamine seeking after 1 and 15 abstinence days. Between tests, the rats underwent social-choice-induced voluntary abstinence. After day 15 testing, we assessed Fos, PKCδ and Fos+PKCδ expression in CeL, and Fos expression in CeM. In Exp. 1, we found that shPKCδ but not shScram decreased CeL PKCδ, Fos, and Fos+PKCδ expression. In Exp. 2, we found that shPKCδ but not shScram restored incubation of methamphetamine craving after voluntary abstinence. This effect was associated with decreased PKCδ, Fos and Fos+PKCδ expression in CeL, and increased Fos expression in CeM. Results demonstrate a critical role of CeL PKCδ in inhibition of incubation of methamphetamine craving after social choice-induced voluntary abstinence


Poster

328. Cocaine Craving

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 328.08/Y29

Topic: G.08. Drugs of Abuse and Addiction

Support: This work was supported by NIDA/NIH

Title: Role of ventral subiculum in incubation of oxycodone craving after electric barrier-induced voluntary abstinence

Authors: *I. FREDRIKSSON1, S. APPLEBEY1, A. MINIER-TORIBIO1, C. CIFANI2, J. M. BOSSERT1, Y. SHAHAM1;
1Behavioral Neurosci. Br., IRP-NIDA, NIH, Baltimore, MD; 2Univ. of Camerino, Sch. of Pharm., Camerino, Italy

Abstract: In humans, abstinence is often self-imposed, and relapse typically involves a conflict situation where addicts choose between the desire to experience the drug’s rewarding effects and adverse consequences of drug seeking. To mimic this human condition, we recently developed a rat model of incubation of oxycodone craving after electric barrier-induced voluntary abstinence and showed time-dependent increases in drug seeking on abstinence day 15 and 30 compared to day 1 (incubation of oxycodone craving) that was more pronounced than the classical incubation
of craving after homecage forced abstinence. Here, we examined the role of ventral subiculum (vSub) in incubation of oxycodone seeking after electric barrier-induced abstinence, using the activity marker Fos and muscimol + baclofen (GABA A+B receptor agonists) inactivation. We trained male and female rats to self-administer oxycodone (0.1 mg/kg/infusion, 6-h/d) for 14 days. We then introduced an electric barrier near the drug-paired lever of increasing intensity (0.1 to 0.4 mA) that causes cessation of oxycodone self-administration. Next, we tested the rats (n=6-7/group) for relapse to oxycodone seeking (extinction tests) in the absence of shock and drug on abstinence day 15 and extracted the brains for Fos-immunohistochemistry or tested the rats (n=10-12/group) after vSub injections of vehicle or muscimol + baclofen. Relapse after electric barrier-induced abstinence was associated with increased Fos expression in vSub and local inactivation of vSub decreased “incubated” oxycodone seeking. Together, these data demonstrate a role of vSub in incubation of oxycodone craving after cessation of drug taking due to adverse consequences of drug seeking.


Poster

328. Cocaine Craving

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 328.09/Y30

Topic: G.08. Drugs of Abuse and Addiction

Title: Operant social reward decreases incubation of heroin craving in male and female rats

Authors: *T. I. RUSSELL, M. ZHANG, Y. SHAHAM, M. VENNIRO; Natl. Inst. On Drug Abuse, Baltimore, MD

Abstract: Background: We recently reported that operant social choice-induced voluntary abstinence prevents incubation of methamphetamine craving. Here, we determined whether social choice-induced voluntary abstinence would prevent incubation of heroin craving. We also introduce a fully-automatic social reward self-administration model that eliminates the intense workload and rat-human interaction of the original semi-automatic model. Methods: In Exp. 1, we trained male and female rats for social self-administration (6 d) and then for heroin self-administration (12 d). Next, we assessed relapse to heroin seeking after 1 and 15 abstinence days. Between tests, the rats underwent either forced or social choice-induced abstinence. In Exp. 2, we developed a fully-automatic social self-administration procedure by introducing a screen between the self-administration chamber and the social-peer chamber; the screen allows physical contact but prevents rats from crossing chambers. Next, we compared incubation of craving in rats with a history of standard (no-screen) or automatic (screen) social self-administration and social choice-induced abstinence. Results: The time-dependent increase in heroin seeking after
cessation of drug self-administration (incubation of craving) was lower after social choice-induced abstinence than after forced abstinence. There were no differences in social self-administration, social choice-induced abstinence, and incubation of craving in rats trained in the standard semi-automatic procedure versus the novel fully-automatic procedure. **Conclusions:** Our study demonstrates the protective effect of rewarding social interaction on heroin self-administration and incubation of heroin craving, and introduces a fully-automatic social-choice self-administration procedure that can be used to investigate the role of volitional social interaction in drug addiction and other psychiatric disorders.

**Disclosures:** T.I. Russell: None. M. Zhang: None. Y. Shaham: None. M. Venniro: None.

**Poster**

**328. Cocaine Craving**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 328.10/Y31

**Topic:** G.08. Drugs of Abuse and Addiction

**Support:** NIH DA 033404
NIH DA 040965

**Title:** Incubation and cocaine cue-induced changes in medial prefrontal cortex parvalbumin and perineuronal nets after long access to cocaine in rats

**Authors:** *J. ANGUIANO*¹, J. M. BLACKTOP⁴, J. WINGERT⁵, L. CHURCHILL², B. A. SORG³;
¹Neurosci., Washington State Univ., Vancouver, WA; ²Program in Neurosci., Washington State Univ., Pullman, WA; ³Integrative Physiol. and Neurosci., Washington State Univ., Vancouver, WA; ⁴Dept. of Integrative Physiol. and Neurosci., ⁵Neurosci., Washington State Univ. Vancouver, Vancouver, WA

**Abstract:** Extracellular matrix aggregations called perineuronal nets (PNNs) surround mainly fast-spiking, parvalbumin (PV)-containing GABAergic interneurons and importantly contribute to plasticity during development, learning and memory. We previously reported that PNNs in the medial prefrontal cortex (mPFC) contribute to cocaine-associated memories (Slaker et al., J Neuro 2015), and that the intensity of PNNs and their underlying PV cells changes after acute and repeated cocaine (Slaker et al., eNeuro 2018). Here we measured changes in the intensity of PV and PNNs (the latter using *Wisteria floribunda* agglutinin, WFA) after 6 hr access to cocaine in rats followed by either 1 or 30 days forced abstinence or 2 hr after cocaine cue-induced reinstatement. Rats were allowed 6 hr access to cocaine (0.5 mg/kg/infusion) for 14 days followed by 1 or 30 d withdrawal or 10 days extinction and then cue-induced reinstatement. The intensity of PV in WFA+ cells was not different for saline controls after 1 d abstinence, but was
increased compared to saline controls after 30 d abstinence. The intensity of WFA around PV+ cells was slightly decreased at 1 d abstinence but increased after 30 d abstinence. Cue-induced reinstatement decreased PV intensity but, paradoxically, increased WFA intensity. These findings indicate a potential incubation effect on both PV cells and their surrounding PNNs as well as conditioned effects of the cue on PV and PNNs. The results suggest that there may be a compensatory increase in inhibitory output from PV to pyramidal cells to dampen enhanced excitatory drive from the mPFC to the nucleus accumbens during incubation.


Poster

328. Cocaine Craving

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 328.11/Y32

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant DA033641

Title: Decreased N6-methyladenosine (m6A) levels in the prefrontal cortex are associated with incubation of cocaine craving

Authors: *D. K. FISCHER1, S. E. SWINFORD-JACKSON2, J. STOUTE3, F. LIU4, R. C. PIERCE2;
1Grad. Group in Neurosci., 2Dept. of Psychiatry, 3Grad. Group in Biochem. and Molecuclar Biophysics, 4Dept. of Biochem. and Biophysics, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Understanding epigenetic mechanisms, such as RNA modifications, that underlie cocaine relapse-like behavior may lead to the development of new therapies for cocaine addiction. N6-methyladenosine (m6A) is the most prominent mRNA modification. m6A regulates RNA splicing, translation, and stability which can alter gene expression that dynamically influences neuronal function and behavior. m6A and its regulatory enzymes, methyltransferases and demethyltransferases, are regulated in the prefrontal cortex by different experiences and stimuli. Neuronal plasticity in the prefrontal cortex, an important region within the mesocorticolimbic reward system, contributes to the incubation of cocaine craving, a rodent model of relapse-like behavior. The extent to which m6A regulatory enzymes and total levels of m6A within the prefrontal cortex are associated with cocaine seeking has not been investigated. Here, male Sprague Dawley rats were trained to self-administer cocaine and controls self-administered sucrose or received yoked-saline infusions (14 days, 3 hr/day) followed by 1 day or 30 days of forced abstinence, at which time the prefrontal cortex was harvested. Mass-spectrometry was used to assess m6A levels and RT-qPCR was performed to measure the
expression of m⁶A regulatory enzymes. We observed lower m⁶A levels in the prefrontal cortex of rats that self-administered cocaine and underwent 30 days of forced abstinence compared to saline-yoked controls. Cocaine self-administration and forced abstinence for either 1 or 30 days were both associated with significant increases in the expression of Fto, the main demethyltransferase of m⁶A, relative to animals that underwent sucrose self-administration. These data suggest that forced abstinence following cocaine self-administration decreases overall levels of m⁶A in the prefrontal cortex, which may contribute to increases in cocaine-seeking behavior. The data also suggest that Fto may have a reinforcer-specific effect independent of forced abstinence duration. In future studies, we will experimentally alter m⁶A levels within the PFC and assess cocaine-seeking behavior to determine whether m⁶A contributes to the incubation of cocaine craving.


Poster

328. Cocaine Craving

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 328.12/Y33

Topic: G.08. Drugs of Abuse and Addiction

Support: 5RO1DA037257-05
5R21DA044486-02

Title: Accumbal endocannabinoid signaling mediates cocaine craving following prolonged abstinence

Authors: P. H. GOBIRA¹, S. MITRA², J. A. MARTIN³, C. T. WERNER⁴, *D. M. DIETZ⁵; ¹Univ. of São Paulo, Ribeirao Preto, Brazil; ²Pharmacol. & Toxicology, ³Dept. of Pharm and Tox; Res. Inst. on Addictions; Program in Neurosci., ⁴Dept. of Pharmacol. and Toxicology; Program in Neurosci., State Univ. of New York at Buffalo, Buffalo, NY; ⁵State Univ. of New York, Buffalo, NY

Abstract: Cue-induced cocaine craving is a cardinal characteristic of cocaine use disorder. Cocaine craving progressively intensifies during abstinence leading to perpetual relapse vulnerability. Endocannabinoid signaling in the nucleus accumbens (NAc) mediates synaptic depression associated with rewarding properties and contributes towards cocaine-induced behavioral attributes; however, the role of accumbal endocannabinoid signaling in incubation remains to be investigated. In the present study we found dysregulation of enzymes that mediate synthesis (diacylglycerol lipase-DAGL) and breakdown (monoacylglycerol lipase-MAGL) of endocannabinoid 2-Arachidonoylglycerol (2-AG) following prolonged abstinence from
extended-access cocaine self-administration (SA). DAGL was increased, while MAGL was decreased in the NAc on abstinence day 30 (AD30) but not AD1 following extended-access cocaine SA. Intra-accumbal microinjection of DAGL antagonist DO-34 attenuated, while MAGL antagonist URB-602 increased, cocaine seeking on AD30, demonstrating that 2-AG-mediated endocannabinoid signaling regulates cocaine seeking during prolonged abstinence. Dephosphorylation of eukaryotic initiation factor 2 (eIF2α) was previously shown to mediate cocaine seeking during prolonged abstinence, and we found that inhibiting MAGL resulted in dephosphorylation of eIF2α in the NAc, suggesting that cocaine seeking during prolonged abstinence is mediated through a mechanism involving altered phosphorylation of eIF2α (p-eIF2α) by 2-AG. Together, these results demonstrate bidirectional regulation of cue-induced cocaine craving by endocannabinoid signaling in the NAc during prolonged abstinence.


Poster

328. Cocaine Craving

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 328.13/Y34

Topic: G.08. Drugs of Abuse and Addiction

Support: R01 DA034097

Title: Differential microRNA expression in nucleus accumbens shell after prolonged cocaine abstinence in environmentally-enriched rats

Authors: *A. VANNAN1,2, G. L. POWELL2, A. M. H. MOKBEL2, A. L. ESQUER2, M. DELL’ORCO3, N. PERRONE-BIZZOZERO2, J. L. NEISEWANDER1,2;
1Interdisciplinary Grad. Program in Neurosci., 2Sch. of Life Sci., Arizona State Univ., Tempe, AZ; 3Dept. of Neurosciences, Univ. of New Mexico HSC, Albuquerque, NM

Abstract: MicroRNAs (miRNAs) have emerged as “master regulators” of hundreds to thousands of genes, and recent research has linked several miRNAs to substance abuse and addiction. We used a miRNA microarray (nanoString nCounter Rat v1.5) to investigate miRNA expression changes in the nucleus accumbens shell (NAcSh) in relation to incentive motivation for cocaine. Isolate-housed, male Sprague-Dawley rats were trained to self-administer cocaine (0.75 mg/kg/IV infusion) on a variable ratio 5 (VR5) schedule of reinforcement. After rats achieved stable cocaine intake, they were placed into 21 days of forced abstinence while either remaining in isolation or being placed into environmental enrichment (EE) which is known to attenuate motivation for cocaine. Rats then underwent cue reactivity testing in which a cue light and tone that were previously paired contingently with cocaine were presented on a fixed ratio (FR) 1
schedule in the absence of drug, and these operant responses were used as a measure of cocaine-seeking behavior. Immediately following the cue reactivity test, rats (n=12) were sacrificed and their brains were flash-frozen for RNA analyses. As expected, EE rats showed significantly less cocaine-seeking behavior in the cue reactivity test than isolated rats. Differential expression analysis using nSolver™ Analysis Software identified 16 miRNAs significantly upregulated in EE rats compared to isolated rats. Some of these miRNAs, including several from the let-7 family, have previously been implicated in addiction, while others are novel and predicted to target large number of addiction-related genes (Knowledgebase for Addiction Related Genes, karg.cbi.pku.edu.cn/karg.cbi.pku.edu.cn/). All but one of the upregulated miRNAs were negatively correlated with cocaine seeking behavior. These findings enhance our understanding of the neurobiological benefits of EE and reveal several therapeutic targets for attenuating drug craving.


Poster

328. Cocaine Craving

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 328.14/Y35

Topic: G.08. Drugs of Abuse and Addiction

Title: Altering the gut microbiota affects cocaine-seeking in adult but not adolescent male rats

Authors: *G. J. SUESS, J. KASIAH, B. ANTHONY, B. F. WILLIAMS, B. CHASSAING, K. J. FRANTZ; Georgia State Univ., Atlanta, GA

Abstract: Research on the gut-brain axis has revealed that gut dysbiosis is associated with several neuropsychiatric disorders, including substance abuse. Using a cocktail of antibiotics in the drinking water of rats, we sought to determine whether antibiotic treatment decreases bacterial abundance in an age-dependent manner and whether antibiotic-induced microbial depletion differentially affects cocaine-seeking in adolescent vs. adult male Wistar rats. After catheter implantation, rats were given antibiotics and self-administered cocaine for two weeks. At the end of self-administration, animals were taken off cocaine and antibiotics, placed into forced abstinence for 30 days, and then underwent extinction testing followed by cue-induced reinstatement. Animals were sacrificed, with brains and abdominal organs collected. Microbial abundance was characterized by conducting qPCR with universal primers. Inflammation in the gut was assessed by quantifying lipocalin-2, a neutrophil marker. During cocaine self-administration, neither age nor antibiotic treatment influenced drug taking. Adults treated with antibiotics reinstated more to cocaine-seeking than water-treated counterparts, however, an effect
not seen in adolescents. In terms of microbial abundance, the antibiotic cocktail depleted microbiota levels in both age groups. In adults, microbiota levels failed to return to baseline over the 30-day forced abstinence, although they did return to baseline in adolescent-onset counterparts. Cecum mass and size were greater in all antibiotic treated animals, compared to age-matched controls, and a further increase was associated with cocaine intake in adults but not adolescents. Neither age, drug, nor antibiotic altered lipocalin-2. Taken together, the present results suggest that microbial depletion is associated with heightened sensitivity to cocaine-related cues in adult rats, but not rats that took cocaine during adolescence. The present results should be complemented by a more thorough investigation of microbial diversity and pro-inflammatory cytokines, perhaps revealing a cocaine-related microbiota profile. These results also suggest that future studies on the impact of probiotics to restore gut health may ultimately suggest adjunct therapies in the treatment of drug addiction.


Poster

328. Cocaine Craving

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 328.15/Y36

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA14328

Title: Cocaine intake dependent plasticity in nucleus accumbens mGluR5 receptor signaling leads to differential regulation of drug seeking

Authors: *M. GHASEMZADEH, D. KRAVTSOV, S. GIWANI, R. VARELA, J. BOROVICKA, O. BURGOS, L. KELBLE;
Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: A major obstacle in the treatment of addiction has been the propensity to relapse, often mediated by drug-associated cues, even after prolonged period of abstinence from drug use. Repeated exposure to cocaine leads to enduring alterations in glutamatergic signaling in the brain reward circuitry that play an important role in long-lasting molecular, cellular and behavioral neuroadaptations. Therefore, glutamate signaling has been investigated as a target for the development of treatment for addiction. Recent studies suggest that group I metabotropic glutamate receptors (mGluR1/5) play important roles in drug reinforcement and drug seeking and, therefore, have been pursued as promising targets for therapeutic development. Here, we examined the role of mGluR1/5 receptors in abstinence drug seeking using animal models of cocaine self-administration. Male Sprague-Dawley rats were trained to self-administer cocaine
(FR1; 1.0 mg/kg/200 µl/inf) during either 2-hr (ShA) or 6-hr sessions (LgA) for 14 days. Subsequently, animals were left undisturbed in the home cage for 3, 10, or 60 days. Following abstinence period, rats were tested under context-primed extinction condition for cocaine seeking after systemic administration of either saline or an mGluR1/5 receptor antagonist (MTEP or JNJ16259685). Following a short abstinence period (3 or 10 days), the blockade of mGluR5 receptor reduced drug seeking only in ShA subjects without affecting the LgA animals, while mGluR1 receptor blockade was effective in reducing drug seeking in both groups. However, after a long abstinence period (60 days), the systemic blockade of either of receptors significantly reduced drug seeking in ShA and LgA rats. Using separate groups of rats, it was demonstrated that intracerebral infusion of MTEP (3µg/side) after 10 days of abstinence into either NAcortex or NAs shell led to a decrease in drug seeking in ShA rats. However, our preliminary results suggest that it was not effective in reducing drug seeking LgA rats. These results suggest that exposure to cocaine produce a transient intake dependent plasticity in mGluR5, but not in mGluR1, signaling in the brain. Moreover, our data point to Nucleus accumbens as the anatomical substrate contributing to the selective modulation of mGluR5 signaling in LgA rats. Understanding the mechanism of cocaine mediated effects on group I metabotropic glutamate receptors may reveal new molecular targets for the treatment of cocaine addiction.

Disclosures: M. Ghasemzadeh: None. D. Kravtsov: None. S. Giwani: None. R. Varela: None. J. Borovicka: None. O. Burgos: None. L. Kelble: None.

Poster

328. Cocaine Craving

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 328.16/Y37

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH (NIDA) Grant R01-DA031734

Title: CRF in the nucleus accumbens core amplifies drug anticipation: Recruitment of CRF-R2 in cocaine-seeking behavior

Authors: *M. Z. LEONARD1, H. E. COVINGTON, III2, K. A. MICZEK2;
2Psychology, 1Tufts Univ., Medford, MA

Abstract: Dopamine signaling within the nucleus accumbens core (NAcC) is critical to guiding motivated behavior in both appetitive and aversive contexts. The present studies examine accumbens-specific actions of the stress peptide corticotropin releasing factor (CRF) as a candidate mechanism for maladaptive arousal evoked by drug-predictive stimuli. Initial microdialysis studies show that CRF is released in the NAcC when rats are exposed to
environmental contexts that predict an aversive outcome (i.e. social defeat) or drug-reward opportunity (i.e. cocaine availability), and moreover, intra-NAcC infusion of exogenous CRF elicits a robust increase in extracellular dopamine. We subsequently aimed to assess the behavioral impact of NAcC-CRF on instrumental responding directed towards cocaine procurement. To that end, male Long-Evans rats were trained to self-administer cocaine under a chained schedule of reinforcement (FI-FR) in order to dissociate appetitive (‘drug-seeking’) from consummatory (‘drug-taking’) behavior. Accordingly, completion of a fixed interval (5 min.) was followed by 5 min of continuously reinforced responding (0.4mg/kg cocaine; FR1) on another lever. Under these conditions, intra-NAcC microinfusion of CRF dose-dependently increased responding during the fixed-interval component of the procedure, but notably did not affect subsequent cocaine intake. Both the behavioral and neurochemical actions of NAcC-CRF were each prevented by selective CRF-R2 blockade with Astressin-2B, but not the CRF-R1 antagonist CP376395. However, the CRF-R2-mediated effects on dopamine and reward-anticipation were absent in drug-naïve animals trained to self-administer saccharin. Taken together, these data suggest a recruitment of CRF-R2 within NAcC circuits that may contribute to maladaptive cocaine-seeking behavior, perhaps via modulation of dopamine transmission.

Disclosures:  M.Z. Leonard: None. H.E. Covington: None. K.A. Miczek: None.

Poster

328. Cocaine Craving

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 328.17/Y38

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA R01-DA031734

Title: Continuous social stress: Individual effects on CRF and cocaine reinstatement in mice

Authors: *D. T. ARENA, M. Z. LEONARD, K. A. MICZEK;
Psychology, Tufts Univ., Medford, MA

Abstract: Vulnerability to the detrimental effects of social stress varies across individuals. In C57BL/6J mice, continuous exposure to social stress can either intensify or suppress behavioral correlates of reward processing, such as sucrose preference, social interactions and cocaine self-administration. It is hypothesized that the divergent changes to social and reward-related behavior after continuous social defeat arise from different plasticity within stress signaling systems, such as corticotropin releasing factor (CRF). The present experiments aimed to characterize pharmacodynamic changes that correspond with stress vulnerability after continuous social defeat. Separate groups of male C57Bl/6J mice were studied after 10 days of either continuous social defeat stress, or no social stress. Following a 10-day period, mice were
examined for social interaction and sucrose preference in order to establish stress phenotype. Mice were then implanted with IV catheters and trained to administer cocaine under a fixed ratio 1 schedule of reinforcement (0.3 mg/kg/inf) for 10 days. After 10 days of self-administration, mice were subjected to 10 days of forced home-cage abstinence, followed by a context-induced reinstatement test. Systemic administration of CP376395 given immediately prior to reinstatement attenuated drug seeking behavior and this was most pronounced in stressed individuals that displayed heightened responding for cocaine during self-administration. Ongoing microdialysis studies indicate effects of CRF on drug seeking after exposure to continuous social stress. These data provide suggestive evidence that the divergent effects of continuous social stress on cocaine-seeking may arise from adaptive changes to CRFR1 signaling in the VTA of mice.


Poster

328. Cocaine Craving

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 328.18/Y39

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA033344
NIH Grant AA024146
NIH Grant AA006420
NIH Grant AA022249
NIH Grant AA026999

Title: Orexin-A administration in the posterior paraventricular nucleus of the thalamus promotes cocaine-seeking behavior via recruitment of the central amygdala

Authors: *R. MARTIN-FARDON, A. MATZEU;
The Scripps Res. Inst., La Jolla, CA

Abstract: Hypothalamic orexin (Orx) neurons that project to the paraventricular nucleus of the thalamus (PVT) have received growing interest because of their role in drug-seeking behavior. OrxA administration in the posterior PVT (pPVT) reinstates extinguished cocaine-seeking behavior in rats that had long access (LgA) to cocaine (6 h/day) after an intermediate period of abstinence (I-Abst, 2-3 weeks). Considering the long-lasting nature of drug-seeking behavior, we examined whether the priming effect of OrxA is preserved after protracted abstinence (P-Abst, 4-5 weeks). Substantial evidence indicates that the central nucleus of the amygdala (CeA) and bed nucleus of the stria terminalis (BNST) are strongly engaged during drug-seeking behavior, and the pPVT projects to both the CeA and BNST. Thus, a secondary objective of the study was to
monitor activation patterns (i.e., Fos+ cells) of the CeA and BNST following intra-pPVT OrxA injections. Male Wistar rats were trained to self-administer cocaine in LgA sessions for 21 days. After training, the animals were either subjected to 14-21 days of 2 h/day extinction training (I-Abst) or placed for 14 days in the vivarium followed by extinction training for 14-21 days (P-Abst). Once the animals’ behavior was extinguished, they received intra-pPVT injections of OrxA (0.5 µg) and were then placed in operant chambers under extinction conditions for 2 h. Immediately following the behavioral tests, the animals were euthanized, and their brains were prepared for Fos immunostaining. The data showed that OrxA reinstated cocaine-seeking behavior at I-Abst but not at P-Abst. The reinstatement of cocaine-seeking behavior was associated with activation of the CeA and BNST. To test whether CeA and BNST activation is necessary for OrxA-induced cocaine-seeking behavior at I-Abst, a separate group of rats was tested at I-Abst. Following extinction, the rats were simultaneously injected with OrxA in the pPVT and a combination of muscimol+baclofen in the CeA or BNST. Transient inactivation of the CeA with muscimol+baclofen prevented OrxA-induced cocaine-seeking behavior, whereas inactivation of the BNST did not affect the ability of OrxA to reinstate cocaine-seeking behavior. Altogether these findings indicate that the HYP->pPVT->CeA circuit is strongly recruited shortly after abstinence in animals with a history of cocaine dependence, and OrxA transmission in the pPVT participates in the reinstatement of cocaine seeking at I-Abst. The differential behavioral outcomes that were observed at I-Abst vs. P-Abst suggest that cocaine abuse perturbs the function of Orx receptors and connectivity with the pPVT as abstinence progresses.

Disclosures: R. Martin-Fardon: None. A. Matzeu: None.

Poster

328. Cocaine Craving

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 328.19/Y40

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH GRANT F31-DA048527

Title: Cell type specific role of HDAC3 within the NAc in regulating cocaine-induced plasticity

Authors: *R. R. CAMPBELL¹, E. KRAMAR¹, J. BEARDWOOD¹, A. L. LOPEZ², O. CHITNIS¹, J. BANIHANI¹, L. PHAM¹, D. MATHEOS¹, M. WOOD¹; ¹Univ. of California, Irvine, Irvine, CA; ²Vanderbilt Univ., Nashville, TN

Abstract: Cocaine engages mechanisms of synaptic plasticity and transcription within the nucleus accumbens (NAc) to promote drug-seeking behaviors. Recent work from the field demonstrates that this occurs in a cell-type specific manner, often differentially affecting
mechanisms of plasticity within the two major output cell types of the NAc: dopamine D1- (D1R) vs D2-receptors (D2R) medium spiny neurons (MSNs). Consistent with this, activation of D1R- and D2R- MSNs drive opposing behavioral responses to cocaine. However, it is unclear how cocaine affects epigenetic mechanisms within D1R- vs D2R- MSNs to promote cocaine-associated behaviors. Prior work from our lab demonstrates that cocaine disengages histone deacetylase 3 (HDAC3) within the NAc to promote cocaine-induced gene expression and cocaine-associated memory formation. Here, we have examined the specific role of HDAC3’s deacetylase activity in cocaine-induced behaviors and cellular activity within the NAc. In addition, we have investigated cocaine’s cell-type specific effects on histone acetylation within D1R- vs D2R-MSNs. Lastly, we have examined how HDAC3 activity within these distinct cell types regulates cocaine-induced cellular and behavioral responses. Together, these results illustrate how cocaine alters mechanisms of histone acetylation to induce cell-type specific changes in gene expression and synaptic plasticity that promote drug-associated behaviors.


Poster

328. Cocaine Craving

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 328.20/Y41

Topic: G.08. Drugs of Abuse and Addiction

Support: DA048436
DA042111
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Whitehall Foundation
The Brain and Behavior Research Foundation
Edward Mallinckrot Jr. Foundation

Title: Activity-dependent epigenetic alterations underlying cocaine self-administration


Abstract: Substance use disorder (SUD) is a behavioral disorder characterized by cycles of abstinence, drug seeking, and relapse. SUD is primarily a behavioral disorder that involves aberrant learning processes after repeated exposure to drugs of abuse. At the core of this
phenotype is the persistence of symptoms long after the cessation of drug use. The neural basis of these behavioral changes has been linked to receptor-based changes in neural circuits across the brain, however, the molecular drivers that allow for these changes to persist beyond the life-span of any individual protein remain opaque. Work from our lab identified a self-administration-induced remodeling of the transcriptome as well as a novel transcriptional signature that is induced by cocaine following a history of self-administration that predicts addictive behaviors. To understand SUD, it will be critical to define the link between neuronal activation, and longer-term changes in transcription that control drug seeking. Epigenetic adaptations - where DNA-protein interactions are modified to alter the probability of targeted transcription - have been implicated in the resilient nature of drug-seeking behavior. Histone acetylation, a generally permissive epigenetic mark, is induced following re-exposure to cocaine and cocaine-associated cues, suggesting that the epigenetic enzymes regulating histone acetylation are key regulators for drug-induced gene networks. KAT2A, a histone acetyltransferase known to regulate activity-dependent transcription and hippocampal memory. While several KAT2A histone targets are modulated by cocaine, the role of KAT2A in drug-associated behaviors remains unknown. As such, we hypothesize cocaine-induced gene networks are underlied by recruitment of various histone modifications, a subset of which are regulated via KAT2A. To test cocaine-induced changes in KAT2A function, mice received cocaine (20mg/kg, I.P.) and NAc collected following 1, 7.5, 15, 30, and 60 min. Using western blot and co-immunoprecipitation, we identified changes in both KAT2A regulation of H3 and subsequent changes in H3 modifications. To further link KAT2A function with cocaine-associated behaviors, mice were implanted with jugular catheters and trained to self-administer cocaine (1mg/kg/infusion, I.V.) or saline. Following IVSA, NAc tissue was collected and analyzed for histone modifications induced by: 1) acute cocaine, 2) repeated cocaine, or 3) persisted 24 hours following IVSA. Together, we find widespread changes in epigenetic modifications across the nucleosome highlighting the complex remodeling that is induced by drug exposure.


Poster

328. Cocaine Craving

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 328.21/Y42

Topic: G.08. Drugs of Abuse and Addiction

Support: 1R01DA042283

Title: Single-cell RNA-sequencing of mouse nucleus accumbens reveals a subtype of D1 medium spiny neurons
Authors: *W. CHEN*¹, R. CHEN¹, M. DJEKIDEL¹, A. BHATTACHERJEE¹, L. M. TUESTA¹, Y. ZHANG²;

Abstract: As one of the most important entry points of the basal reward circuitry, the nucleus accumbens (NAc) has been suggested to play a crucial role in motivational behaviors. However, our knowledge of its cellular heterogeneity is limited. Here, we performed a high-throughput single-cell RNA-seq to reveal the cellular complexity of the NAc. Our results show a rich cellular heterogeneity of interneurons and medium spiny neurons (MSNs) in the NAc. As a proof-of-concept, we focused on the MSNs and found that the tachykinin 2 (Tac2)-positive neurons in the NAc is a molecularly distinct subtype of D1-MSNs. To characterize the Tac2 clusters as a subtype of D1-MSNs, we conducted multi-color FISH, neuronal tracing and behavioral assays, and found that Tac2 is selectively expressed in the Drd1+ MSNs but not in the Drd2+ MSNs. We also found that the NAc Tac2 clusters are preferentially projected to the midbrain. In addition, we manipulated the Tac2 cluster using chemogenetics and found that activation of the NAc Tac2 clusters potentiated, while inhibition repressed cocaine sensitization. However, such manipulation had no obvious effects on anxiety- and depression-related behaviors. Furthermore, we investigated the role of the NAc Tac2 clusters in mediating reinforcement in a mouse intravenous self-administration (IVSA) and found that inhibition of the Tac2 clusters significantly reduced cocaine intakes. Collectively, our results suggested that the Tac2 cluster in the NAc is a D1 MSN subtype that might play a specific role in cocaine addiction.


Poster

329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 329.01/Y43

Topic: G.08. Drugs of Abuse and Addiction

Support: MINECO Grant SAF2013-49076-P
        MINECO Grant SAF2017-85679-R

Title: Extinction of morphine withdrawal-induced place aversion is related to a decrease in mTORC1 pathway in the basolateral amygdala
Authors: *A. FRANCO*\(^{1,2}\), M. V. MILANÉS\(^{1,2}\), C. NÚÑEZ\(^{1,2}\);
\(^1\)Group of Cell. & Mol. Pharmacol., Univ. of Murcia, Murcia, Spain; \(^2\)Murcia Res. Inst. of Hlth. (IMIB), Murcia, Spain

Abstract: One of the main issues related to opiate addiction treatment is the relapse in drug intake induced by memories paired with the drug use. Alterations in the traditional memory circuits, including dentate gyrus and basolateral amygdala (BLA), have been shown to be involved in the misleading learning and memory processing in the addicted brain. Several studies have demonstrated that the mammalian (or mechanistic) target of rapamycin (mTOR) complex 1 (mTORC1) participates in synaptic plasticity through several protein synthesis regulation, such as Arc, Homer1, GluR1 and GluR5. The alteration of this pathway seems to be the common physiopathological basis of neurological diseases, including addiction to opiates. We aimed to assess the possible changes in the mTORC1 pathway in BLA during the retrieval of aversive memories associated to morphine withdrawal and their extinction. In order to accomplish our objective, we used the CPA paradigm in morphine-dependent and control rats. After behavioral procedures, brain samples were analyzed by means of western blot and immunofluorescence assays to determine the expression of phospho (p)-mTOR and the mTORC1 target p-S6 ribosomal protein, respectively. Our results revealed a decrease in the levels of p-mTOR in the BLA of rats showing aversion to the morphine withdrawal-associated environment. Likewise, control and morphine dependent rats exhibited lower levels of p-mTOR in BLA after extinction training and test. Accordingly, we observed lower expression of p-S6 in glutamategic neurons in BLA after naloxone-induced CPA and after extinction training and test. These data agree with recent findings showing that inhibition of S6 phosphorylation promotes neurite outgrowth *in vitro*, which has been related to new memory formation. Further studies are needed to untangle the mechanisms underlying opiates-associated memory formation, retrieval and extinction processes in the addicted brain.


Poster

329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 329.02/Y44

Topic: G.08. Drugs of Abuse and Addiction

Support: 4189913-IN2BA

Title: Mu opioid receptors on vGluT2-expressing glutamategic neurons regulate aversion to oxycodone
Authors: *K. C. JONES*¹, M. KUBE¹, B. K. ATWOOD², B. MUÑOZ², B. FRITZ¹, D. HAGGERTY¹;
¹Stark Neurosciences Res. Inst., Indianapolis, IN; ²Dept. of Psychiatry, Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Prescription opioid drugs such as oxycodone produce reward and exert largely through mu opioid receptors (MORs). We previously demonstrated that oxycodone alters the ability of MORs to modulate corticostriatal glutamatergic transmission. We also found that MORs regulate thalamostriatal glutamatergic transmission. Using conditional MOR knockout mice (MORflox-vGluT2cre), we now demonstrate the localization of these MORs as being in vGluT2-expressing thalamic neurons. Whereas our previous work found that MORs are expressed on small populations of cortical glutamatergic neurons, indicating a likely more behavioral restricted role of these receptors, MORs are more broadly expressed in vGluT2 neurons in the brain. Therefore, we decided to explore the role of MORs on vGluT2 neurons in oxycodone-related behaviors. Eight-week-old male and female MORflox-vGluT2cre and wild-type controls underwent behavioral tests including oxycodone-induced conditioned place preference (CPP), home cage 2-bottle choice drinking, and locomotion testing. Whereas wild-type mice showed CPP to oxycodone, MORflox-vGluT2cre mice displayed conditioned place aversion. MORflox-vGluT2cre mice showed decreased locomotor activity compared to controls upon initial oxycodone exposure. In 2-bottle choice drinking, MORflox-vGluT2cre mice consumed less and showed decreased preference for oxycodone compared to controls, but did not differ in their consumptions of sucrose and quinine. These data reveal for the first time that MORs on vGluT2 neurons play a significant role in opioid reward circuitry, specifically modulating aversion to oxycodone. Current work is determining what other opioid-related behaviors are mediated by MORs on this class of glutamatergic neurons.


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329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 329.03/Z1

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant ZIADA000566

Title: Heroin-induced respiratory depression and subsequent brain hypoxia are triggered from the periphery
Authors: *E. A. KIYATKIN, D. PEREKOPSKIY, A. AFZAL;
Behavioral Neurosci., NIDA-IRP, NIH, DHHS, Baltimore, MD

Abstract: In this study, we employed high-speed electrochemical monitoring of oxygen in the brain (nucleus accumbens or NAc) and subcutaneous space in freely-moving rats to examine the role of peripheral opioid receptors in mediating respiratory depression and subsequent brain hypoxia induced by intravenous (iv) heroin. As shown previously and confirmed here, heroin administered at a low, human-relevant dose (0.1 mg/kg) induces a biphasic NAc oxygen response, with an initial rapid drop followed by a more tonic increase above baseline. As confirmed by oxygen recording in the subcutaneous space, a highly vascularized location with no metabolic activity of its own, the initial decreasing component of brain oxygen response results from respiratory depression, while the subsequent brain oxygen increase is caused by cerebral vasodilation that enhances diffusion of oxygen into brain tissue independently of its blood levels determined by respiration. Naloxone-HCl, a highly potent opioid antagonist that easily crosses the blood-brain barrier (BBB), fully blocked both components of oxygen response, but naloxone-methiodide, which cannot cross the BBB, fully blocked the heroin-induced NAc oxygen drop but did not affect the subsequent oxygen increase. Naloxone-methiodide also had minimal inhibiting effects on the hyperthermic effects of heroin, which were fully blocked by naloxone-HCl. Therefore, in contrast to the traditional dogma that respiratory depression is mediated via the direct interaction of opioid drugs or/and their metabolites with opioid receptors in the CNS, our study suggests the critical role of peripheral opioid receptors in triggering this adverse effect of opioid drugs.

Disclosures:  E.A. Kiyatkin: None. D. Perekopskiy: None. A. Afzal: None.

Poster

329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 329.04/Z2

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA-IRP Grant ZIADA000566

Title: 6-monoacetylmorphine (6-MAM) but not morphine is responsible for the rapid neural effects of intravenous heroin

Authors: *D. PEREKOPSKIY, E. A. KIYATKIN;
Behavioral Neurosci., NIDA-IRP, NIH, DHHS, Baltimore, MD

Abstract: Although heroin rapidly enters the CNS, it is quickly broken down into metabolites and byproducts. While heroin is metabolized into 6-monoacetylmorphine (6-MAM) and then to
morphine, it is generally believed that morphine interacting with brain mu-opioid receptors is responsible for the neural effects of heroin. However, recent assessments of pharmacokinetics of heroin and its primary metabolites in blood and brain extracellular space questioned this view. While brain levels of 6-MAM increased very rapidly and strongly, peaking at 2-4 min after iv heroin injection, morphine levels increased much weaker, peaking at ~20 min after heroin injection. These data point to 6-MAM as the primary metabolite responsible for heroin-induced neural effects. To clarify this issue, we used oxygen sensors coupled with amperometry to examine changes in NAc oxygen levels induced by heroin, 6-MAM, and morphine delivered intravenously in freely moving rats. Consistent with our previous data we found that iv heroin at an optimal self-administering dose (100 μg/kg) induces a biphasic change in NAc oxygen, with the initial rapid decrease resulting from respiratory depression and more delayed rebound-like increase resulting from cerebral vasodilation. The same biphasic oxygen response occurred when heroin was delivered at a higher dose (400 μg/kg), but with an enhanced initial oxygen decrease. 6-MAM delivered at an equimolar dose mimicked this pattern of oxygen response and its dose-dependent changes, but the effects were slightly weaker and more rapid than those for heroin. This lesser potency of 6-MAM to decrease NAc oxygen levels can be due to the effects of heroin itself before degradation or/and the fast degradation of 6-MAM. In contrast to heroin and 6-MAM, morphine delivered at two equimolar doses failed to decrease oxygen levels, inducing only weak and transient increases. Therefore, it appears that 6-MAM, a primary heroin metabolite, is the major contributor for the acute neural effects induced by intravenous heroin.

Disclosures: D. Perekopskiy: None. E.A. Kiyatkin: None.

Poster

329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 329.05/Z3

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant U01 S07201

Title: Opioid withdrawal shifts amygdalar transcriptome and is correlated with gut dysbiosis

Authors: *S. J. O'SULLIVAN¹, E. MALAHIAS¹, J. PARK¹, B. A. S. REYES², E. J. VAN BOCKSTAELE², R. VADIGEPALLI¹, J. S. SCHWABER¹;

Abstract: Opioid withdrawal syndrome consists of severe negative physical and emotional symptoms. Avoidance of these negative physical and emotional symptoms contributes to dependence by negative reinforcement. The amygdala is a well-established set of limbic nuclei
involved in threat detection, fear, panic, and memory that has demonstrated primary involvement in opioid dependence. Moreover, recent findings have connected gut microflora to mood and emotions. We assayed a subset of the transcriptome of single neurons, microglia, and astrocytes in the central nucleus of the amygdala in morphine-dependent and morphine-withdrawn rats using high-throughput microfluidic RT-qPCR. General and cell type-specific transcriptional shifts that lend insight into the underlying substance dependence pathophysiology were observed including increased Tnf expression and TNFα protein in withdrawal. Astrocytes, in particular, demonstrated high transcriptional activity. Additionally, opioid withdrawal decreased the Firmicutes to Bacteroides ratio in gut microflora suggesting gut dysbiosis. We speculate that the transcriptional changes measured in the amygdala and the microfloral changes measured in the gut are related.


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329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 329.06/Z4

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH grant F32 MH116574
Univ of MD School of Medicine Dean's Challenge Award

Title: Fentanyl abstinence causes structural and molecular changes to nucleus accumbens D1 medium spiny neurons

Authors: *M. E. FOX*¹, A. B. WULFF³, C. A. CALARCO¹, S. A. AMENT², M. ENGELN¹, M. LOBO¹;
¹Anat. and Neurobio., ²Inst. for Genome Sci. and Dept. of Psychiatry, Univ. of Maryland Sch. of Med., Baltimore, MD; ³Univ. of Maryland Baltimore, Baltimore, MD

Abstract: Opioid abuse has risen dramatically over the last decade. Potent, synthetic opioids like fentanyl are responsible for nearly half of opioid-related deaths, yet synthetic opioid abuse remains broadly understudied. Opioids, like other drugs of abuse, engage and alter dopaminergic circuitry to promote continued use and eventual relapse. Neurons in the Nucleus Accumbens (NAc) play a key role in drug abuse and receive dopaminergic input from the midbrain. NAc medium spiny neurons (MSNs) express either dopamine D1 or D2 receptors, and manipulation of their activity can oppositely regulate drug-related behaviors. While it is known that morphine exposure causes a loss of dendritic spines on NAc MSNs, it is unknown if this is true for synthetic opioids like fentanyl, and if the changes are specific to D1- or D2- MSNs. Here we
show that after homecage fentanyl exposure and abstinence, both male and female mice show increased social-withdrawal and reduced dendritic complexity specific to D1-MSNs. To identify the molecular mechanisms behind cell-type specific dendritic remodeling, we used D1- or A2A-Cre mice crossed with Ribotag mice to isolate ribosome-associated mRNA in specific cell types after fentanyl. We performed RNA sequencing of the D1- and D2-MSN translatome and found >1,000 differentially expressed genes. Preliminary weighted correlation network analysis (WCGNA) indicates the most strongly down-regulated genes are associated with Wnt signaling, neurogenesis, and ion channels. Ongoing experiments aim to delineate the precise molecular mechanisms underlying the social behavior deficits and neuron subtype specific atrophy, as well as to characterize changes to NAc function following operant fentanyl self-administration.


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329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 329.07/Z5

Topic: G.08. Drugs of Abuse and Addiction

Support: Novo Nordisk
NIDA drug supply program
The Groff Charitable Trust

Title: Exploring liraglutide as a potential treatment for oxycodone use and abuse

Authors: *C. M. PORTO, III¹, M. M. CLASEN²;
¹Program in Neuroscience, Dept. of Psychology, ²Psychology Department, Program in Neurosci., Williams Col., Williamstown, MA

Abstract: The lack of successful treatments for drug use and abuse has led to a number of novel approaches to study and treat this chronic and relapsing brain disease. One promising therapeutic endpoint lies within a neural system known to regulate food intake, obesity, and type 2 diabetes. In this vein, pharmaceutical drugs designed to enhance the glucoregulatory actions of the incretin hormone, glucagon-like-peptide-1 (GLP-1; i.e., liraglutide), have been shown to reduce food intake and adipose tissue in both preclinical and clinical populations, while producing long term improvements in cardiovascular health. Following this initial research investigating the role of the GLP-1 system in food intake and body weight regulation, researchers studying drug dependence hypothesized that GLP-1 agonism might also decrease the consumption of abused drugs given that the modulation of other peptide systems known to regulate food intake, also regulate drug intake. Interestingly, these researchers found that peripheral administration of the
GLP-1 agonist exendin-4 reduces alcohol and cocaine intake in male mice and rats, respectively. While these preliminary investigations suggest that long-term GLP-1 agonism reduces drug intake, additional research is required to evaluate whether this system modulates the intake of other abused drugs (i.e., opioids, nicotine, psychostimulants, cannabinoids, etc.) and whether males and females differ in the magnitude of these effects. To this end, the present research sought to investigate whether the long-term GLP-1 agonist liraglutide modulates the intravenous self-administration (IVSA) of oxycodone in male and female Sprague-Dawley rats. Here, we report that liraglutide (10, 100, 250, 500, 1000 μg/kg; IP) dose dependently reduces oxycodone IVSA (0.056 mg/kg/infusion) and that a single low-dose of liraglutide (250 μg/kg; IP) decreases the reinforcing properties of a number of doses of oxycodone (0.01, 0.032, 0.1, 0.32 mg/kg/infusion) in both male and female Sprague-Dawley rats. Together, these recent data suggest that liraglutide might be a novel target for the pharmacological treatment of oxycodone use and abuse. Ongoing research from our lab is investigating whether liraglutide impacts the escalation of oxycodone IVSA.

Disclosures: C.M. Porto: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Novo Nordisk, NIDA drug supply program. M.M. Clasen: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Novo Nordisk, NIDA drug supply program.

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329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 329.08/Z6

Topic: G.08. Drugs of Abuse and Addiction

Support: Startup Funds from Department of Psychology University of Maryland

Title: Role of orbitofrontal cortex in incubation of oxycodone craving in male and female rats

Authors: *E. S. YANG, M. HAILE, I. R. DAVIS, S. RAZAVI, S. A. COLDREN, X. LI; Dept. of Psychology, Univ. of Maryland Col. Park, College Park, MD

Abstract: Background: Drug seeking progressively increases after withdrawal from drug self-administration (incubation of drug craving). Previous studies have shown that this incubation generalizes across different drug classes in both rats and humans. Here, we examine neural mechanisms underlying incubation of craving to oxycodone, a commonly abused prescription opioid, and we focus on orbitofrontal cortex (OFC), a brain region previously implicated in incubation of heroin craving.

Methods: We trained male and female adult Sprague Dawley rats to self-administer oxycodone (0.1 mg/kg/infusion, 6-h/d for 10 d). Next, we either tested (Relapse-test) or did not test (No-test)
rats for oxycodone seeking (2-h) under extinction condition on withdrawal day 1 or withdrawal day 15. Immediately following the tests, we perfused rats for immunohistochemistry to measure c-Fos, a neuronal activity marker, in OFC.

Results: We found that rats escalated oxycodone intake during training, and exhibited higher oxycodone seeking on withdrawal day 15 than on withdrawal day 1. We observed no sex differences either during the self-administration training or relapse test. Furthermore, c-Fos expression increased in the Relapse-test group compared with the No-test group on withdrawal day 15.

Conclusions and future studies: Results demonstrate that incubation of oxycodone craving occurs in both adult male and female rats after withdrawal day 15 from oxycodone self-administration and is associated with neuronal activation in OFC. Studies are underway to examine the causal role of OFC in incubation of oxycodone craving by pharmacological and chemogenetic approaches.

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329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program#/Poster #: 329.09/Z7

Topic: G.08. Drugs of Abuse and Addiction

Support: R01DA035958
R00AA022937

Title: Effects of acute and chronic morphine on lateral paracapsular amygdala circuitry

Authors: *S. J. WERNER1, N. LEWIS2, H. WADSWORTH2, S. STEFFENSEN2, N. GRAZIANE3, Y. SILBERMAN3, J. YORGASON2;
1Neurosci. Ctr., 2Physiol. and Developmental Biol., Brigham Young Univ., Provo, UT; 3Dept. of Neural & Behavioral Sci., Penn State, Hershey, PA

Abstract: Basolateral amygdala (BLA) activity is thought to underlie anxiety-like behavior that may be associated with stress induced drug seeking. Excitatory activity of BLA pyramidal neurons is regulated by local and paracapsular interneurons. The lateral paracapsular interneurons (LPCs) border the external capsule, and are optimally localized for receiving dense cortical input and providing feed-forward inhibition onto BLA principle neurons. The LPCs also uniquely express high concentrations of mu-opioid g-protein coupled receptors (MORs). Therefore, the effects of morphine on LPC activity and local GABA release were examined. Fluorescently double labeled LPCs were observed in GAD65-mcherry/GAD67-GFP transgenic...
mice. Whole-cell electrophysiology experiments demonstrated that acute exposure to DAMGO (a synthetic selective mu-opioid receptor agonist), reduced sIPSC frequency in LPCs with no apparent effect on sEPSCs. In VGAT-ChR2 mice, light evoked GABA release onto BLA was reduced with acute application of DAMGO, and reversed with naloxone application. Furthermore, BLA fEPSP amplitude increased in BLA neurons, supportive of reduced inhibition onto BLA principle neurons. Chronic morphine-injected mice (10mg/kg/day, across 5 days, 1-2 days off) had increased sIPSCs compared to saline-injected controls. Current injection induced firing in LPC neurons, but less effectively than in saline controls. Together these data suggest that morphine acutely reduces GABA release onto LPC neurons, but long term opiate exposure results in greater inhibition onto LPCs and subsequent decreases in LPC excitability. These data have implications for increases in anxiety observed during opiate withdrawal.


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329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 329.10/Z8

Topic: G.08. Drugs of Abuse and Addiction

Support: NARSAD Young Investigator Award (NMG)
NIDA Drug Supply program

Title: Increased excitability of PVT neurons following morphine exposure is light/dark cycle-dependent

Authors: *D. S. MCDEVITT, N. M. GRAZIANE;
Pennsylvania State Univ. Col. of Med., Hershey, PA

Abstract: The paraventricular nucleus of the thalamus (PVT) is a midline thalamic nucleus that is reciprocally connected to the major circadian pacemaker of the central nervous system, the superchiasmatic nucleus of the hypothalamus (SCN) and projects to several regions of the greater reward circuit including the nucleus accumbens, amygdala, and medial prefrontal cortex. This anatomical architecture suggests that the PVT is a functionally important relay between arousal and motivated behaviors. In agreement with this, it has been shown that PVT neurons express diurnal variations in activity, that cocaine alters the firing properties of PVT neurons, and that the PVT regulates drug-seeking behaviors. Given this information, we investigated whether i) opioids alter PVT activity, ii) whether opioids alter PVT activity differentially when administered during the day (light cycle) versus at night (dark cycle), and iii) whether opioid administration during the light cycle versus the dark cycle influences the acquisition of
morphine-induced conditioned place preference. Our results show that five day, repeated morphine injections (10 mg/kg i.p.) in mice, during the light cycle, followed by one day forced abstinence, increases the number of tonically firing neurons in the PVT, while the same morphine treatment given during the dark cycle has no effect (due to the already increased tonic firing at baseline). Similarly, morphine treatment during the light cycle elicits increases in excitatory transmission at PVT neuronal synapses (measured using AMPAR/NMDAR ratios), but this effect is not observed following morphine treatment during the dark cycle due to the increased excitatory transmission at baseline. Furthermore, we found that five day, repeated morphine injections (10 mg/kg i.p.) during the light cycle or during the dark cycle elicit a strong conditioned place preference suggesting that the rewarding properties of opioids are intact when administered during the day (light cycle) or during the night (dark cycle). Overall, our results show that morphine-induced changes in PVT neuronal function express diurnal variations. However, there are no diurnal variations observed in the acquisition of morphine-induced CPP.

Disclosures: D.S. McDevitt: None. N.M. Graziane: None.

Poster

329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 329.11/Z9

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R00 DA037279

Title: Interruption of continuous opioid exposure exacerbates drug evoked adaptations in brain function and behavior

Authors: *L. BYSTROM¹, E. LEFEVRE², M. T. PISANSKY², T. J. Y. KONO³, P. E. ROTHWELL²;
¹Col. of Biol. Sci., Univ. of Minnesota Twin Cities, Minneapolis, MN; ²Dept. of Neurosci., ³Minnesota Supercomputing Inst., Univ. of Minnesota, Minneapolis, MN

Abstract: Clinical use of opiate-based analgesics often leads to negative consequences including dependence and addiction. Due to high prescription rates for the treatment of chronic pain, the United States is now faced with an ever rising opioid abuse epidemic. Our central hypothesis posits that maladaptive changes in brain function occur when otherwise continuous opioid exposure is interrupted by brief cycles of withdrawal. To test this hypothesis, male and female C57BL/6J mice were implanted with Alzet osmotic pumps to continuously deliver morphine (63 mg/kg/day), or saline as control, over the course of 7 days. In order to interrupt the continuous morphine administration, mice were given twice daily naloxone (10mg/kg, s.c.) injections (n=18/group). Locomotor activity recorded on the first and last days of treatment revealed that
continuous morphine exposure produced tolerance to the psychomotor activating effects of morphine. In contrast, interruption of morphine exposure with naloxone caused psychomotor sensitization, an addiction-related behavior related to adaptations in the mesolimbic circuitry. A series of low dose (2mg/kg, s.c.) morphine challenges revealed this sensitization to be persistent and enduring up to 4 months withdrawal after initial treatment. We then sought to identify the neurobiological impact of these divergent patterns of morphine administration in key reward-related brain regions. Using our established paradigm, mice (male only; n=6/group) were euthanized on the 6th day of drug treatment and nucleus accumbens and dorsal striatum tissue were collected for next generation RNAseq. After controlling for false discovery rate, we found no genes that were significantly regulated by continuous morphine exposure in either brain region. In contrast, interrupted morphine significantly regulated 687 transcripts in the nucleus accumbens and 407 transcripts in the dorsal striatum. Ingenuity Pathway Analysis identified unfolded protein response, endoplasmic reticulum stress and protein ubiquitination canonical pathways were significantly regulated by interrupted morphine in both striatal subregions. These pathways were all contributed to by the up-regulation of several heat shock proteins. Increased transcription of heat shock proteins therefore represents a unique consequence of interrupted morphine exposure, as previously reported after daily injections of morphine, but not after continuous morphine exposure. This study demonstrates that opioid-evoked adaptations in brain function and behavior are critically dependent on the pattern of drug administration, and exacerbated by interruption of continuous exposure.


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329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 329.12/Z10

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA025674

Title: Gene by environment interactions influence naloxone precipitated withdrawal in mice

Authors: *D. J. MOORE, C. F. DAVIDSON, T. D. PATTON, A. M. TOORIE, D. N. TECENO, F. M. VASSOLER, E. M. BYRNES;
Dept. of Biomed. Sci., Tufts Univ., North Grafton, MA

Abstract: Drug related deaths involving opioids have increased 200% between the years of 2000-2014. With this continuing rise in opioid use, identifying factors that modify an individual’s response to opioids may help both prevention and treatment efforts. Preclinical
models have documented significant strain differences in opioid withdrawal, implicating a genetic contribution to this effect. The extent to which experiential factors interact with genetic vulnerabilities to influence opioid withdrawal remains unknown. The purpose of the current study was to determine the effect of limited exposure to morphine during adolescence on measures of acute opioid withdrawal in adulthood using two mouse strains that demonstrate significant differences in acute naloxone precipitated jumping, a behavioral measure of opioid withdrawal (C57BL/6 - high responder)/ (129/Sv1 - low responder). Beginning at 30 days of age females were injected daily with morphine for a total of 10 days using an increasing dosing regimen with doses increased every other day (10, 20, 30, 40, 50 mg/kg, s.c.) with age-matched control animals receiving saline. Animals were then drug-free until testing ~10 weeks later. On the day of testing subjects were injected with either morphine (50 mg/kg, s.c.) or saline; 3h later they received naloxone (30 mg/kg, s.c.). Mice were then immediately placed in test chambers and jumping behavior was recorded for 15 min. One hour later all mice were sacrificed. Trunk blood was tested for glucose and brains were harvested. Significant strain differences in naloxone precipitated jumping and glucose levels were observed. Modest effects of adolescent treatment on naloxone precipitated jumping were observed in C57 but not 129 mice. However, prior adolescent exposure decreased the effects of naloxone on glucose in both strains, although these effects were more robust in C57 mice. Such findings suggest that the effects of endogenous opioids on glucose regulation can be dissociated from effects on behavioral measures of withdrawal. Additional studies examining neuronal activation using cFos labeling in brain regions that regulate naloxone precipitated withdrawal are ongoing. Overall, these findings indicated that prior exposure to opioids in adolescence alters the adult endogenous opioids system and that these effects are moderated by strain.


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329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 329.13/Z11

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH-NIDA#05010

Title: Generation of knock-in mu opioid receptor-Cre mice

Authors: *J. BAILLY1, *N. DEL ROSSI1, M.-C. BIRLING2, E. DARCQ1, B. L. KIEFFER1; 1Psychiatry, McGill University, Douglas Res. Ctr., Montréal, QC, Canada; 2Inst. de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch Graffenstaden, Strasbourg, France
Abstract: The mu opioid receptor (MOR) is the primary molecular target for medicinal opioids, and is broadly expressed throughout the nervous system. Preclinical studies have demonstrated that MOR mediates both the unrivaled analgesic properties of opioids in pain treatment, as well as their numerous adverse effects such as notably their strong addictive potential. Under physiological conditions, MOR is activated by opioid peptides and modulates various physiological systems at the periphery and in the brain, including nociceptive pathways, respiration centers, as well as the processing of natural rewards and mood. Recently, tools have been developed to map and characterize sites of MOR expression at the cellular level, and identify neuronal populations responding to endogenous and exogenous opioids. These reporter knock-in lines are instrumental to localize the receptor, and speculate about circuit mechanisms underlying MOR function. A further step to better understand MOR physiology, and neural dysfunctions associated to opioid drug use, misuse and abuse, is to study and manipulate the activity of MOR-expressing neurons. To this aim, we have generated a new MOR-Cre line using a knock-in strategy allowing the expression of a detectable and functional eGFP-Cre recombinase under the control of the MOR promoter. Here, we present the characterization of the novel Cre driver mouse line at molecular and behavioral level. We also demonstrate that optogenetic stimulation of MOR-positive neurons in the ventral tegmental area is sufficient to induce strong avoidance behaviour, as anticipated from the literature. This MOR-Cre line represents a unique tool of general interest in areas of pain, addiction and mood disorders.


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Poster
329. Opioids, Dependence, Withdrawal, and Reward
Location: Hall A
Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM
Program #/Poster #: 329.14/Z12
Topic: G.08. Drugs of Abuse and Addiction
Support: NIH-NID#05010
NIH-NIAA#16658
Title: Whole brain connectivity alterations upon protracted morphine abstinence: A mouse fMRI study
**Authors:** *L. WELSCH*¹, T. M. D. NASSEEF², J. PUNEET SINGH², E. DARCQ², B. L. KIEFFER¹;
¹Psychiatry, McGill Univ., Montréal, QC, Canada; ²McGill/Douglas Res. Ctr., Montréal, QC, Canada

**Abstract:** An opioid epidemic has emerged in North America: overprescribing of opioids pain relievers has led to a rise in overdose deaths at alarming rates. Drug addiction is defined as a chronic relapsing brain disorder, characterized by a cycle of preoccupation, binging, and withdrawal/negative affect [1]. Opioid abstinence enhances negative feelings, resulting in profound dysphoria, irritability, emotional pain and anxiety. Therefore, understanding the neural adaptations occurring during protracted abstinence that drive the negative emotional state is essential to minimize drug craving and social isolation, and prevent relapse. Our previous reports have shown that mice experiencing a 4-weeks period of spontaneous withdrawal from chronic morphine [2] or heroin [3] develop depressive-like behaviors and social withdrawal, and that these emotional deficits can be both prevented and reversed [4] by fluoxetine (5HT-uptake inhibitor) or norBNI (kappa opioid receptor antagonist). Here we study long-term consequences of the chronic morphine treatment on whole brain connectivity using mouse fMRI. As in our previous studies, mice were exposed to escalating doses of morphine or saline during 6 days and underwent 4 weeks of spontaneous withdrawal. Resting-state fMRI was performed on the morphine abstinent and vehicle control mice as described previously [5]. After scanning, mice were tested behaviorally and showed social deficit and more passive coping in the classical forced swim test as we previously reported. We will present both data-driven and seed-based analyses of fMRI datasets. By combining new knowledge on the long-term effects of chronic opioid exposure at behavioral and functional connectivity levels, we hope to elucidate brain networks underlying the negative affect of morphine abstinence at whole brain scale.


**Disclosures:** L. Welsch: None. T.M.D. Nasseef: None. J. Puneet Singh: None. E. Darcq: None. B.L. Kieffer: None.
Poster

329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 329.15/Z13

Topic: G.08. Drugs of Abuse and Addiction

Title: Craniofacial mucosal application of NOP agonists for the treatment and prevention of addiction

Authors: *A. HUNTER, M. KLUKINOV, D. YEOMANS; Anesthesia, Stanford Univ., Palo Alto, CA

Abstract: Activation of the mesolimbic reward system by opioid drugs of abuse is believed to play a major role in the development of addiction and is a target of ongoing research. Past evidence has indicated the role of Nociceptin Orfanin FQ (NOFQ) as an endogenously occurring polypeptide that attenuates addictive behavior when administered directly into the brain by suppression of dopaminergic neuron activity in the ventral tegmental area. However, these previous studies have featured intracerebroventricular injection as the mode of delivery, an immensely invasive technique that does not translate well for wide-spread human use. In order to further explore the potentially “anti-addictive” characteristics of nociceptin with a method that can translate to human treatment, we have developed a method for intranasal application of NOFQ with the end goal of attenuating addictive behavior in opioid-addicted subjects. We used a customized Conditioned Place Preference (CPP) paradigm in order to model addictive behavior. Male adult Sprague Dawley rats were randomly paired with one of two visually and tactiley distinct chambers. Subjects received either morphine (5mg/kg, S.C.) or saline vehicle and were immediately placed in the assigned chamber for 40 minutes every other day for a total of four doses. On days following drug administration rats received an equivalent volume of saline vehicle and were placed in the opposite chamber for 40 minutes every other day for a total of four doses. On days following drug administration rats received an equivalent volume of saline vehicle and were placed in the opposite chamber for 40 minutes. Preference was determined using a combined custom video analysis system and MATLAB analysis program to record and calculate time spent in each chamber. Subjects then received 1 dose of NOFQ (5mg/kg) or saline vehicle via intranasal (I.N.) delivery once per day for a total of 6 doses. IN delivery took place under isoflurane anesthesia with rats in a supine position. 50μL of NOFQ or saline vehicle was delivered via 6.25μL droplets directly into alternating nostrils at two-minute intervals for 16 minutes. Performance on the CPP test was recorded 24 hours following 3 and 6 doses of NOFQ. Our results show that rats subjected to 3 doses of NOFQ, but not saline demonstrated a significant reduction in preference for the morphine-paired chamber compared to rats given I.N. saline under the same addiction regimen. I.N. NOFQ had no significant effect on CPP in the absence of morphine treatments, implying that it is not aversive and does not contaminate CPP results in subjects treated with morphine. This early evidence suggests that I.N.
administration of NOFQ has promise as a viable drug delivery method with therapeutic potential for opioid addiction.


Poster

329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 329.16/Z14

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH-NIDA project #1P01DA041307-01

Title: The role of cannabinoid and opioid combinations on pain and respiratory depression in rodents

Authors: *B. M. Wiese¹, E. Liktor-Busa², A. Levine¹, S. Nikas⁴, A. Makryannis⁴, T. M. Largent-Milnes⁵, T. W. Vanderah³;
¹Pharmacol., ²Pharmacology, ³Univ. of Arizona, Tucson, AZ; ⁴Northeastern Univ., Boston, MA; ⁵Univeristy of Arizona, Tucson, AZ

Abstract: With over 100 million Americans suffering from chronic pain each year, and the scope of the opioid epidemic remaining a massive problem with accidental overdose fatalities at an all-time high, it is imperative that a safer alternative for treatment of chronic pain is found. Recent studies have shown that cannabis and cannabinoids may hold the key as a viable analgesic, on their own or in adjunct with opioids, and provide a wider safety profile with the absence of respiratory depression. As more states legalize cannabis for medicinal and recreational use, it is important to understand the mechanisms of action of cannabinoids on pain relief and respiratory depression. In this study we sought to understand the impact of cannabinoid 1 receptor (CB1R) and cannabinoid 2 receptor (CB2R) activation on respiratory depression using both synthetic and available CB1/CB2 agonists alone and in combination with morphine. Furthermore, we sought to evaluate if the analgesic properties of the combinations would remain. Results suggest that CB1 and CB2 activation do not induce respiratory depression on their own. In addition, cannabinoids can mitigate opioid induced respiration depression. Additionally, when tested for antinociception, these combinations retained their analgesic properties. These data support further research of cannabinoids as potential therapeutic analgesics/adjuncts in the face of the opioid epidemic.

Poster

329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 329.17/Z15

Topic: G.08. Drugs of Abuse and Addiction

Title: Differential effects of various opioids on affect and anxiety-like states

Authors: C. A. MADISON, P. J. WELLMAN, *S. EITAN;
Texas A&M Univ., College Station, TX

Abstract: Opioid misuse and overdose deaths are rising exponentially in the United States, reaching levels characterized by public health officials as an epidemic. This rise in opioid-related adverse outcomes has been driven, in part, by the use of opioids to treat pain disorders in the clinical population. However, the choice of which opioids to be prescribed has largely not been guided by research, due to the assumed inseparability of analgesic functions from adverse outcomes. Recent studies demonstrate that, despite binding to and activating the same receptors, different opioids signal preferentially via differential signaling pathways. This implies that opioids cannot be made equivalent by mere dose adjustment. Our recent research demonstrates that at equianalgesic doses, different opioids could have different benefit/risk outcomes related to drug-specific differences in producing opioid-induced hyperalgesia (OIH), managing burn pain early after injury and in treating/preventing chronic pain. Lastly, various opioids have differential effects on the activity of the D2 dopamine receptors (D2DRs) which have been shown to be related to many disorders, including predisposition to substance abuse as well as mood and anxiety disorders. A connection between substance abuse and mood and anxiety disorders has been well-established in humans; however, there is a lack of experimental research establishing this connection in animal models. For the present study, male mice (PND 27) were tested for baseline levels of sociability, anxiety, and pain sensitivities, as well as for acute opioid-induced locomotion. Then they were administered with escalating doses of various opioids (20mg/kg, 40mg/kg), or saline for two weeks and tested for the development of locomotor sensitization. Levels of sociability, anxiety, and pain sensitivities were retested on the 5th week. This was followed by examining the acquisition, extinction, and reinstatement of conditioned place preference (CPP), and retesting for sociability, anxiety, and pain sensitivities levels. The present data show different effects of various opioids on affect, specifically in inducing anxiety-like states. Differences in the magnitude of inducing sensitization and in CPP were also observed. These results suggest that in a clinical setting a more careful selection of specific opioid drugs over others can provide similar pain relief while reducing opioid-associated risks.
Disclosures:  **C.A. Madison:** None. **P.J. Wellman:** A. Employment/Salary (full or part-time); Texas A&M University. **S. Eitan:** A. Employment/Salary (full or part-time); Texas A&M University.

**Poster**

**329. Opioids, Dependence, Withdrawal, and Reward**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 329.18/Z16

**Topic:** G.08. Drugs of Abuse and Addiction

**Support:** NIDA Grant DA048385

**Title:** circRIMS2 expression is differentially impacted in acute and chronic oxycodone exposure

**Authors:** *V. E. LEHMANN,* A. LACHMANN, H. KRONMAN, A. RAMAKRISHMAN, P. J. KENNY;
Icahn Sch. of Med. at Mount Sinai, New York, NY

**Abstract:** Opioid addiction is a major public health crisis in the United States, leading to over 47,000 opioid-related deaths in 2017 alone (CDC, 2018). Additionally, just under one-third of Americans who are prescribed opioid painkillers (such as oxycodone) will eventually become dependent on them. One important facet of addiction research is the function of noncoding RNAs on the development of drug dependence. Circular RNAs (circRNAs) are an emerging class of noncoding RNAs that are formed by backsplicing non-adjacent intronic or exonic sequences. Though circRNAs are expressed throughout the body, they are particularly enriched in the brain. circRNA expression is further localized to synapses, where their mechanism of action is currently unknown. We obtained an RNAseq dataset from isolated DRD1-positive nuclei from the nucleus accumbens, which were further processed using a circRNA analysis pipeline (Circexplorer2), through which we were able to identify raw counts of circRNA transcripts in each individual subject. RIMS2, a synaptic calcium tethering protein, was identified as a host gene for several circRNAs. We chose to further investigate the link between RIMS2 and the striatum, as there is evidence to suggest that RIMS2 is required for dopaminergic exocytosis (Liu et al, 2018). Though a given host gene can have many circular transcripts, the most common RIMS2-derived circular transcript in our dataset has also been identified in other published studies. We chose to investigate the effects of oxycodone withdrawal following 7 days of chronic injections of oxycodone on circRIMS2 expression in striatal synaptosomes as well as total RNA. We observed a significant increase in striatal circRIMS2 expression in synaptosomes 24 hours after the final oxycodone injection. Conversely, a single acute injection of oxycodone was shown to decrease circRIMS2 expression 24 hours post injection. This suggests that circRIMS2 may initially decrease following acute oxycodone exposure, but eventually increase as a compensatory effect of prolonged oxycodone. If circRIMS2 does indeed modulate neural
responses to oxycodone exposure, then manipulation of circRIMS2 in vivo could mitigate the addictive properties of opioids.


Poster

329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

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Topic: G.08. Drugs of Abuse and Addiction

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Title: Optical subcellular pharmacology with fluorescent biosensors of nicotine and opioids

Authors: *A. K. MUTHUSAMY¹, A. V. SHIVANGE¹, P. M. BORDEN³, A. L. NICHOLS¹, T. M. CHIN¹, C. H. KIM¹, K. BERA¹, A. KAMAJAYA¹, J. JEON¹, J. S. MARVIN³, E. K. UNGER⁴, B. N. COHEN¹, M. J. MULCAHY¹, H. BAO⁵, E. R. CHAPMAN⁶, L. TIAN⁴, D. A. DOUGHERTY², L. L. LOOGER³, H. A. LESTER¹;

Abstract: The chronic effects of neural drugs may be explained in part by multiple modes of intracellular ligand-receptor interactions. Nicotine and some opioids bind to their respective nascent receptors in the endoplasmic reticulum (ER) to increase successful export and trafficking via pharmacological chaperoning. Additionally, some opioid drugs effect G-protein signaling from within organelles. To quantify the extent and dynamics of these drug actions with imaging in live cells, we employ genetically encoded fluorescent biosensors of nicotine and opioids. These data then encourage further studies of molecular and cellular mechanisms that underpin addiction. The visualization of the drug in real-time can be combined with other optical or physiological measurements to achieve a comprehensive picture of cellular responses and
adaptations to the drug. We began by creating a family of “intensity-based nicotine-sensing fluorescent reporters” (iNicSnFRs) meeting the criterion of ΔF/F₀ > 0.3 at 1 μM. The iNicSnFRs showed directly that nicotine itself enters the ER in clonal cell lines, in primary hippocampal cells, and in human dopaminergic neurons differentiated from iPSCs, within 10 s of application at the sub-micromolar concentrations relevant to CSF levels in a smoker or vaper. Moreover, varenicline, a smoking cessation drug, shows only slightly slower kinetics, potentially explaining its strengths and weaknesses in human use.

Opioids are potent analgesics but pose an inherent risk for addiction and overdose. The cellular mechanisms of tolerance that drive users to take increasing amounts of opioids are not well understood. We screened 16 biosensor mutants with 40+ opioids representing μ, δ, and κ agonists, antagonists, and allosteric modulators. We found biosensor hits for fentanyl, morphine, methadone, and tapentadol among other ligands that we have partially evolved for increased dynamic range and affinity. The data show these opioids entering the ER of HeLa cells within a factor of 4 of the concentration found at the PM. We are now developing AAV-packaged biosensors for use in human iPSC-derived neuronal models for subcellular pharmacokinetic studies of opioids.

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Poster

329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

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Title: Transcriptional regulation of EGR3 in the prefrontal cortex mediates oxycodone-induced pain relief
Abstract: Opiates, like oxycodone (oxy), are extremely effective in their analgesic properties but have also been abused by users for non-medical purposes, highlighting the need to better understand the neurobiology between the pain and exposure to such substances. The goal of this study was to investigate transcriptional changes in the prefrontal cortex (PFC), a region of the mesolimbic dopamine system targeted by drugs of abuse and is involved with pain processing. Oxycodone treatment upregulated EGR3 expression regardless of the pain state. EGR3 overexpression in the PFC was found to potentiate mechanical pain relief with oxycodone. EGR3 expression is mediated in part by the transcription factor NFAT. Pharmacological inhibition of NFAT and subsequent downstream EGR3 expression resulted in decreased oxycodone-induced analgesia. Taken together, this demonstrates a potential role for the NFAT-EGR3 pathway in mediating oxycodone-induced pain relief.

death, but the effects of carfentanil on brain oxygen concentrations have not been studied. Here, we employed oxygen biosensors combined with fixed-potential amperometry to measure changes in oxygen concentration in the nucleus accumbens of freely-moving rats. Male rats received surgically-implanted intravenous (i.v.) catheters and intracerebral biosensors under pentobarbital anesthesia. After 4 days of recovery from surgery, rats received i.v. doses of carfentanil at a range of doses. Doses of 0.1 and 0.3 ug/kg carfentanil induced slight increases in oxygen, whereas 1.0 ug/kg carfentanil elicited a slight drop in oxygen followed by an increase. After an i.v. dose of 3 ug/kg, carfentanil induced strong and prolonged brain hypoxia that could be blocked with the MOR antagonist naloxone. The respiratory depressant effects of 3 ug/kg carfentanil mimicked the effects of 6.4 mg/kg morphine, suggesting that the former drug is at least 2,000-fold more potent than the latter. Our studies demonstrate the robust suppression of brain oxygen levels by carfentanil and highlight the risk to drug users who are unknowingly exposed to this ultrapotent opioid agonist.

Disclosures: E. Solis: None. M.H. Baumann: None.

Poster

329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 329.22/Z20

Topic: G.08. Drugs of Abuse and Addiction

Support: 1P20GM103653

Title: The effect of traumatic stress on mu opioid receptor dynamics in brain regions associated with emotional learning and addiction

Authors: *N. MOHAMMADMIRZAEI, D. KNOX;
Univ. of Delaware, Newark, DE

Abstract: The onset of post-traumatic stress disorder (PTSD) often precedes and increases the risk for subsequent development of substance use disorder. Individuals with opioid dependence have the highest prevalence of PTSD (33%) compared with all other substance abuse. Hyperarousal and heightened anxiety are common symptoms of PTSD which are frequently addressed through opioidergic drug prescriptions. Opioidergic systems are also implicated in facilitating emotional reactivity, including the modulation of fear, the suppression of affective defense behavior, and anxiolysis. Since, endogenous opioids have inhibitory and modulatory roles in emotional responses, we hypothesized that Mu opioid receptor dysregulation in brain regions associated with emotional learning and memory may be particularly sensitive to the effects of traumatic stress. In order to test this hypothesis, we used the single prolonged stress (SPS) model of PTSD in rats. SPS is a procedure consisting of serial stressors (restraint, forced
swim, and ether exposure) applied over a three-hour window which mimics the behavioral and neurological symptoms of PTSD. Rats were exposed to SPS or control stress, euthanized, and brain tissue were collected 7 days later. The medial prefrontal cortex (mPFC), amygdala, and dorsal hippocampus, were dissected out of coronal brain sections and the cytosolic and membrane proteins from these brain regions separated. We then assayed Mu opioid receptors in all samples using western blot. Other aspects of Mu opioid receptor function were assayed including total Mu opioid receptor expression and changes in cAMP production due to Mu opioid receptor activation. We also examined general changes in neural function in emotional substrates and throughout the reward circuit (e.g. D1/D2 ratios). The study is ongoing, but preliminary data showed decreased membrane Mu opioid receptors in mPFC and dorsal hippocampus with an increase in cytosolic Mu opioid receptors of these regions; a pattern of results indicative of changes in Mu opioid receptor internalization. However, Mu opioid receptors of both membrane and cytosol decreased in amygdala regions, which suggests a down-regulation of the Mu opioid receptors. Thus far, the preliminary data suggests that SPS exposure desensitizes Mu opioid receptor function in brain regions associated with emotional learning and memory.

Disclosures: N. Mohammadmirzaei: None. D. Knox: None.

Poster

329. Opioids, Dependence, Withdrawal, and Reward

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Title: β-arrestin2 conditional knockout alters morphine-induced excitability changes in D2 spiny projection neurons

Authors: *A. K. PETKO¹, K. A. PORTER-STRANSKY², C. LILES², D. WEINSHENKER³, C. A. PALADINI⁴;
¹Univ. of Texas at San Antonio, San Antonio, TX; ²Emory Univ., Atlanta, GA;³Dept Human Genet., Emory Univ. Sch. Med., Atlanta, GA;⁴UTSA Neurosciences Inst., UTSA, San Antonio, TX

Abstract: Opiates such as morphine exert their physiological effects by binding to the G protein-coupled (GPCR) μ-opioid receptor (MOR). In addition to transducing canonical G protein signaling, GPCRs also couple to β-arrestin2 (βarr2), which is involved in receptor desensitization and trafficking, and independently signals downstream effectors. Within the nucleus accumbens (NAc), MORs expressed on D1 and D2 spiny projection neurons (SPNs) are subject to
modulation by βarr2. Global deletion of βarr2 alters behavioral sensitivity to morphine, but the cellular subtype and underlying mechanisms responsible are not known. Here, we examined morphine responses in mice with a conditional βarr2 knockout in either D1 or D2 cells. Using slice electrophysiology, we demonstrate that morphine similarly decreases maximum firing rate and slope of the FI curve of D1-expressing SPNs in control and D1 βarr2 KO mice. Morphine also decreases maximum firing rate of D2-expressing SPNs, and this effect is enhanced in those lacking βarr2. Together, these results suggest that MOR-induced inhibition of D2 SPNs, but not D1 SPNs, is modulated by βarr2.


Poster

329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 329.24/Z22

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH DA005010

Title: Mu opioid receptors in dopamine D1 expressing neurons are required for oxycodone-induced locomotor sensitization in the presence of chronic pain

Authors: J. A. ALDERETE1, H. NASEF1, A. L. SEVERINO3, S. A. LEE1, W. M. WALWYN2, C. J. EVANS1, *C. M. CAHILL1;

2Dept Psychiat & Biobehav Sci., 1UCLA, Los Angeles, CA; 3Psychiatry and Biobehavioral Sci., Univ. of California Los Angeles, Los Angeles, CA

Abstract: Chronic pain affects 1 in 4 Americans, making it a public health crisis and the most common cause of disability in the US. Existing non-addictive treatments are limited, which in part has led to the opioid epidemic. Mu opioid receptor (MOR) expression in the mesolimbic circuitry is responsible for rewarding properties of opioids. Our research aimed to investigate the contribution of MOR in D1 and D2 dopamine receptor expressing neurons via assessment of oxycodone-induced hyperlocomotion in pain naïve and chronic pain (sciatic nerve injury) male and female mice. We ablated MOR in dopamine receptor expressing neurons by breeding D1-cre, D2-cre and A2A-cre with MORloxP mice. Oxycodone produced a dose dependent (1-10 mg/kg, s.c.) increase in locomotor activity, which was not altered by chronic pain. Ablating MORs from D1 or D2 dopamine receptor neurons did not alter dose-dependent increases in locomotor activity in either pain naïve or chronic pain states. Locomotor sensitization (10 mg/kg oxycodone x 4 days) was evident in both pain naïve and chronic pain animals. Ablation of MORs from D2 dopamine receptor expressing neurons (using D2-cre or A2A-cre) mice did not
affect locomotor sensitization. However, oxycodone-induced locomotor sensitization was prevented by ablation of MORs from D1 neurons in chronic pain, but not pain naïve mice. This extended to cocaine-induced effects where cross sensitization to cocaine (5 mg/kg) was blocked by ablation of MORs from D1 but not D2 neurons in pain but not pain naïve mice. Cocaine cross sensitization was enhanced in mice where MOR was ablated from A2A neurons. These studies suggest that chronic pain alters opioid-induced effects in D1 expressing neurons. Since locomotor sensitization is a proxy for changes in reward, future studies will investigate how opioid reward is altered in pain and no pain following manipulations of MOR expression.


Poster

329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 329.25/Z23

Topic: G.08. Drugs of Abuse and Addiction

NIDA/Intramural research program

Title: Activation of the striatal MAPK-CREB pathway during early withdrawal from escalated oxycodone self-administration

Authors: *C. A. BLACKWOOD, M. T. MCCOY, B. LADENHEIM, J. L. CADET; Mol. Neuropsychiatry Br., DHHS/NIH/NIDA/IRP, Baltimore, MD

Abstract: The continuing opioid epidemic has prompted research aimed to elucidating potential mechanistic substrates of compulsive oxycodone taking behaviors in rodents. The therapeutic and rewarding effects of oxycodone are both due to its binding to opioid receptors in the brain and periphery. In the present study, we sought to identify biochemical cascades that might be associated with early withdrawal from escalated and non-escalated oxycodone self-administration in rats. To reach that goal, we trained separate groups of rats to self-administer oxycodone using short-access (3h) and long-access (9h) paradigms that lasted for 20 days. Rats were euthanized at 2 hours after the last day of training. Striatal tissues were collected to measure the expression of proteins involved in cellular phosphorylation signaling cascades. Rats given long access to oxycodone escalated their intake of oxycodone to differential degrees, with some rats (LgA-H) taking greater amount of oxycodone than others (LgA-L). Short Access (ShA) rats did not escalate their intake. Biochemical analyses revealed increased abundance of several phosphoproteins of the MAPK-ERK signaling pathway only in the LgA-H animals. Activation of that cascade also led to CREB phosphorylation and histone H3 phosphorylation. These observations suggest that oxycodone SA may cause escalation of drug intake via increased
expression of genes that are downstream targets of this activated cascade. Identification of these genes and their protein products may inform the development of better therapeutic agents to suppress oxycodone abuse and subsequent addiction.

Disclosures: C.A. Blackwood: None. M.T. McCoy: None. B. Ladenheim: None. J.L. Cadet: None.

Poster

329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

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Program #/Poster #: 329.26/Z24

Topic: G.08. Drugs of Abuse and Addiction

Title: Oxycodone self-administration under punishment: Differential immediate early gene expression in the dorsal striatum of compulsive oxycodone takers and abstinent rats

Authors: *M. T. MccoY, C. A. Blackwood, B. Ladenheim, J. L. Cadet; Mol. Neuropsychiatry Br., DHHS/NIH/NIDA/IRP, Baltimore, MD

Abstract: Oxycodone is a prescribed opioid drug that is abused in the USA due to over-prescription. Approaches to treatment of opioid addiction are often difficult because of repeated relapses to drug taking behaviors even after long periods of abstinence. These difficulties in developing successful treatment may be related to lack of understanding of the molecular neurobiology of opioid addiction. To provide partial elucidation of these mechanisms, our laboratory has used footshocks as adverse consequences during oxycodone self-administration (SA) to model one of the criteria of substance use disorders (SUDs). For this presentation, we have used this model to measure potential changes in the expression of immediate early genes (IEGs) in the dorsal striatum of rats during early withdrawal times from oxycodone SA and footshocks.

Male Sprague-Dawley rats were trained to self-administer oxycodone (0.1 mg/kg/injection, i.v.) for 6-9-hour training sessions for 22 days. After training, the escalated rats were administered 9-hour foot-shocks with increasing intensity over a period of 6 days. The rats were euthanized 2h after the last session. We then extracted RNA from the dSTR, made cDNA, and ran quantitative polymerase chain reaction (PCR) to measure the expression of Fos-, Jun-, and Egr- IEG families. Rats, given access to 9 hours of oxycodone escalated their intake of oxycodone over the time of the behavioral experiment. The introduction of contingent foot-shocks produced two distinct oxycodone SA phenotypes: (1) shock-resistant (SR) rats that continued to press the lever for oxycodone despite punishment and (2) shock-sensitive (SS) rats that significantly reduced their lever pressing. Quantitative PCR revealed significant decreases in FosB and Egr2 expression in the sensitive group compared to the control and resistant groups. In addition, c-jun and Egr1 mRNA levels were significantly decreased in the SS phenotype in comparison to control rats.
These observations suggest that abstinence from oxycodone self-administration in the presence of contingent punishment may be related to decreased expression of genes that are down-stream targets of several IEGs. This model that mimics the criterion of compulsive drug taking in the presence of adverse consequences may provide greater insight into the molecular neurobiology of oxycodone addiction.

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Poster

329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 329.27/Z25

Topic: G.08. Drugs of Abuse and Addiction

Title: Opioid overdose and rescue impairs risk-based decision-making and behavioral flexibility

Authors: *H. J. CARMON, S. M. THOMPSON, M. S. MCMURRAY;
Psychology, Miami Univ., Oxford, OH

Abstract: The opioid epidemic has become a growing issue throughout the United States. In the year 2016, synthetic opioids such as fentanyl were among the most common drugs involved in overdose deaths in the United States. Fentanyl induces a hypoxic state during an overdose, in which the brain becomes deprived of oxygen and can be damaged. Overdose rescue via naloxone is relatively new and prevents opioids from binding to opioid receptors, saving an overdosing individual. Thus, recent individuals who have overdosed on opioids are more likely to survive due to the increased use of naloxone; however, the consequences of overdose-induced brain damage are unknown. The purpose of this study is to determine the long term neurobehavioral effects of a fentanyl overdose and naloxone recovery on decision-making, which if affected, could explain reports of increased likelihood of relapse following overdose. To do this, we first developed a novel rat model that accurately depicts the timing and extent of physiological events that occur when an individual overdoses on fentanyl. Next, we used this model to compare the effects of overdose and naloxone recovery to naloxone alone on two decision-making paradigms: probability discounting and probabilistic reversal learning. Preliminary results suggest that animals experiencing fentanyl OD demonstrated an elevated risk preference on the probability discounting task, and reduced behavioral flexibility on the reversal learning task. These preliminary results suggest that deficits in decision-making may be associated with fentanyl overdose, increasing the likelihood of a second potential overdose and complicating treatment of addiction.

Poster

329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 329.28/Z26

Topic: G.08. Drugs of Abuse and Addiction

Title: Neurotoxic consequences of opioid overdose and rescue

Authors: *S. M. THOMPSON¹, D. N. TAPP¹, H. CARMON¹, E. M. BYRNES², M. S. MCMURRAY¹;

Abstract: Widespread use of naloxone, an opioid antagonist, has rescued thousands of opioid overdose victims. However, little is known about the long-term effects of opioid overdose. Postmortem studies have indicated neuropathologies in a number of brain regions after lethal opioid overdose, which could complicate treatment if present in survivors. Here, we determine if cell death is present in the rat brain after overdose and naloxone rescue. Regions associated with the maintenance of addiction and reported to be damaged during overdose were specifically investigated, including the prefrontal cortex, ventral tegmental area, and nucleus accumbens. Jugular catheters were surgically implanted into Long Evans rats, who were then administered either subcutaneous naloxone alone (control group), or IV fentanyl (2mg/kg) and subcutaneous naloxone (0.25mg/kg) 10 minutes later (overdose group). Brains were extracted and rapidly and flash frozen for slicing. Neuropathologies (cell death, shrinkage, malformation, etc) were visualized by fluorescent imaging and manually counted with Image J. Damage to these brain regions suggest that they maybe involved in relapse and are permanently affected by overdose in survivors, complicating treatment and contributing to future risks. Additionally, other cognitive domains may also be affected by opioid overdose, including reward processing, decision-making, and potentially memory.

Title: Small molecule inhibitors of PSD95-nNOS protein-protein interactions suppress opioid-induced reward

Authors: *S. A. SABERI, C. RANGEL-BARAJAS, T. GUTIERREZ, V. IYER, Y. Y. LAI, P. M. KULKARNI, S. GARAI, G. A. THAKUR, J. D. CRYSTAL, G. V. REBEC, A. G. HOHMANN; 1Dept. of Psychological and Brain Sci., Indiana Univ., Bloomington, IN; 2Dept. of Pharmaceut. Sci., Northeastern Univ., Boston, MA

Abstract: Excessive glutamate signaling through N-methyl-D-aspartate receptors (NMDARs) is implicated in altered forms of neuronal plasticity including opioid reward and dependence. NMDAR antagonists can block these effects, but their clinical use is limited by adverse side-effects. However, such side-effects can potentially be circumvented by interrupting NMDAR activity downstream. Excessive NMDAR activity activates the enzyme neuronal nitric oxide synthase (nNOS) to produce the signaling molecule nitric oxide (NO). nNOS is tethered to NMDARs via the scaffolding protein postsynaptic density 95 kDa (PSD95). Thus, disruption of the protein-protein interaction between PSD95 and nNOS may provide a viable therapeutic route to inhibit NMDAR-dependent opioid reward without unwanted side-effects of NMDAR antagonists. We previously reported that small-molecule inhibitors of PSD95-nNOS protein-protein interactions (IC87201, ZL006) inhibited NMDAR-dependent NO formation and suppressed pathological pain without impairing memory or motor function (Neuropharm 97 (2015) 464-75; Behav Brain Res (2016) 305:23-29). However, whether PSD95-nNOS inhibitors themselves lack abuse liability or interfere with NMDAR-dependent opioid reward remains unknown. We used a three-chamber conditioned place preference (CPP) assay to evaluate the impact of IC87201 and ZL006 on opioid reward. Morphine produced robust CPP whereas IC87201 and ZL006 failed to induce either place preference or aversion. Both IC87201 and ZL006 blocked CPP to morphine at doses that lacked intrinsic rewarding or aversive properties. Similar results were observed using CPP paradigms that involved either repeated or single pairings. Our results support the hypothesis that PSD95-nNOS inhibitors exhibit limited abuse liability. Our findings also highlight a previously unrecognized role for PSD95-nNOS disruptors...
as a novel non-opioid therapeutic strategy that shows promise for suppressing opioid reward. Support: DA042584 and CA200417 (to AGH).


Poster

329. Opioids, Dependence, Withdrawal, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 329.30/Z28

Topic: G.08. Drugs of Abuse and Addiction

Support: DA042584
DA027113
EY024717
DA041229

Harlan Scholars Program

Title: Negative allosteric modulation of CB1 cannabinoid receptor signaling suppresses opioid-mediated reward and dopamine efflux

Authors: *V. IYER\textsuperscript{1,2}, C. RANGEL-BARAJAS\textsuperscript{1,2}, J. D. CRYSTAL\textsuperscript{1,2}, A. KULKARNI\textsuperscript{4}, G. A. THAKUR\textsuperscript{4}, G. V. REBEC\textsuperscript{1,2}, A. G. HOHMANN\textsuperscript{1,2,3};
\textsuperscript{1}Program in Neurosci., \textsuperscript{2}Dept. of Psychological and Brain Sci., \textsuperscript{3}Gill Ctr. for Biomolecular Sci., Indiana Univ., Bloomington, IN; \textsuperscript{4}Dept. of Pharmaceut. Sci., Northeastern Univ., Boston, MA

Abstract: In recent years, the pervasiveness of the opioid crisis has underscored the urgent need to identify novel compounds that circumvent opioid abuse liability while sparing their therapeutic efficacy. Opioid reward and reinforcement require crosstalk between cannabinoid type 1 receptors (CB1R) and mu-opioid receptors (MOR). While CB1R modulate the motivational properties of opioids via dopaminergic mechanisms and are well-positioned to control critical aspects of opioid abuse, the clinical use of CB1R agonists and antagonists have been limited by adverse side effects. Allosteric modulation represents an indirect strategy to modulate endocannabinoid signaling by targeting a site distinct from that which binds agonists like Delta-9-tetrahydrocannabinol (THC) or the natural ligands (endocannabinoids) of CB1R. Positive and negative allosteric modulators (PAM and NAM) increase or decrease the affinity and/or efficacy of orthosteric agonists, respectively and may produce an optimal and circumscribed spectrum of physiological effects compared to the indiscriminate effects of agonists or antagonists. The present study used GAT358, a CB1-NAM to test the hypothesis that negative allosteric modulation of CB1R would block opioid reward by decreasing dopaminergic
transmission, while bypassing the unwanted side effects of direct CB1R blockade. In vivo voltammetry findings suggest that GAT358, a CB1-NAM can attenuate morphine-induced increases in dopamine signaling in the nucleus accumbens shell, a key component of the mesocorticolimbic reward pathway. GAT358 also blocked morphine mediated conditioned place preference without producing reward or aversion when administered alone. Our results support the therapeutic potential of negative allosteric modulation of CB1R and highlight their role in fine-tuning the undesirable on-target effects of MOR signaling.


Poster

330. New Methods for Studying Cognition

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 330.01/Z29

Topic: H.01. Animal Cognition and Behavior

Support: NSF 1707316
NSF 1545858

Title: Micro-LED optoelectrodes for high-spatiotemporal-resolution in-vivo opto-electrophysiology (optoEphys) in freely moving animals

Authors: *K. Kim¹, J. Seymour¹, Y. Wu¹, G. Buzsaki², E. Yoon¹;
¹Univ. of Michigan, Ann Arbor, MI; ²New York Univ., New York, NY

Abstract: Micro-LED optoelectrode, whose first prototype was presented in [1], is a Michigan Probe with monolithically integrated cell-sized (10 × 15 μm) micro-LEDs (μLEDs). Its capability of high-spatial-resolution optical stimulation combined with matching resolution for extracellular electrical recording enables a variety of experiments for the study of micro-circuits inside the brain. A number of improvements have been made to the μLED optoelectrode so that the tool can meet a variety of specifications for an ideal tool for in-vivo optical stimulation combined with electrical recording, or opto-electrophysiology (optoEphys). Optoelectrodes are currently being disseminated through our NSF funded hub, NeuroNex MINT, and actively utilized in experiments to answer the fundamental neuroscience questions. We present specifications of the μLED optoelectrode and introduce some of the exciting neuroscience experiments that it enables. Furthermore, we are seeking new collaborators to help us guide the design toward greater future impact.

Poster 330. New Methods for Studying Cognition

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 330.02/Z30

Topic: H.01. Animal Cognition and Behavior

Title: Transcranial radio frequency stimulation (TRFS): A novel noninvasive contactless neuromodulation technique based on radio frequency waves

Authors: *O. YAGHMAZADEH, M. VÖRÖSLAKOS, L. ALON, B. ZHANG, D. K. SODICKSON, G. BUZSAKI;
New York University, Sch. of Med., New York, NY

Abstract: Affecting brain activity by means of external stimuli provides various possibilities for research and clinical applications. In this context, non-invasive neuromodulation techniques sit in a unique position due to their potential low level of side effects. Here we introduce a novel non-invasive contact-less neuromodulation technique based on Radio Frequency (RF) waves. We demonstrate entrainment of neuronal activity, at single cell and neural network levels, by RF exposure in rodent models. We apply RF stimulation at around 1GHz by TEM cell or patch antenna. The RF stimulation intensities that we apply induce electric field and SAR values that are well below the threshold set by safety regulations. We demonstrate that our stimulation paradigm does not induce a significant increase in the body and brain temperature (<0.5°C; n=4 rats and n=2 mice). While applying these ‘temperature-safe’ intensities, we record in-vivo brain activity in freely moving rodents with silicon probes (hippocampal and cortical regions). Radio-Frequency stimulation is able to modulate the ongoing activity of approximately 30% of neurons, including pyramidal cells and interneurons in both mice and rats. In addition, RF stimulation can affect the ongoing network activity, such as the power and occurrence rate of hippocampal sharp-wave ripples. We demonstrate entrainment of neurons’ action potential firing with low frequency (1-20Hz) amplitude modulated RF stimuli. We also provide evidence for RF dose- and direction-dependent response in neural firing in head fixed anesthetized rats. Finite-element physics-based electrodynamic simulations demonstrate the possibility of achieving the induction of similar electric field levels to what we have used in our pilot animal studies into the human brain, suggesting the potential use of this novel method in clinical applications, hence promising the birth of a novel neuromodulation technique: Transcranial Radio Frequency Stimulation (TRFS).

Poster

330. New Methods for Studying Cognition

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 330.03/Z31

Topic: H.01. Animal Cognition and Behavior

Support: NSF #1707316

Title: Hardware and software for driving high-density optogenetic µLED probes in freely moving animals

Authors: *N. Slager1, A. Mendrela1, D. James1, K. Kim2, H. Ye1, J. P. Seymour1, E. Yoon1;
1Dept. of Electrical Engin. and Computer Sci., 2Univ. of Michigan, Ann Arbor, MI

Abstract: Recent advances have allowed for chronic recording and optogenetic stimulation in freely moving animals via high-density monolithically integrated µLED (10 x 16 µm, 460nm peak emission wavelength) probes. In this poster we present an open-source hardware/software implementation for precisely controlling the individual µLEDs independently by user-defined customizable waveforms. The open source hardware (133 x 93 mm) consists of low cost ($450), discrete, commercially available components. Based on the Texas Instruments’ DAC8750 (digital to analog converter), the system includes 12 independent stimulation channels (compatible with a 12 µLED probe), all controllable from field programmable gate array (FPGA) and MATLAB code. The system also incorporates trigger functions (in/out) for external control and communication. All documentation, printed circuit board (PCB) gerber files, and code will be open sourced. However, as the density of µLEDs increases, it becomes difficult to scale up the number of discrete components on a PCB. Therefore, work has been done to design, build and test integrated circuits for current-based independent driving of 36 µLEDs. Similar to the discrete circuit design, this includes custom waveform generation to avoid artefacts induced by step changes in driving current. In addition, this design features open source FPGA and MATLAB code for complete control, communication and interfacing via trigger functions on all 36 channels. Future work involves increasing the channel count available for driving high-density µLED probes up to 128 and beyond.

Title: Rapid customization and distribution of carbon fiber array prototypes via the multimodal integrated neural technologies (MINT) hub

Authors: *J. RICHIE, P. R. PATEL, E. J. WELLE, E. DELLA VALLE, J. D. WEILAND, E. YOON, J. P. SEYMOUR, C. A. CHESTEK;

Abstract: The BRAIN Initiative is directed toward accelerating the development and application of innovative technologies to help understand the nervous system. Traditional electrodes are fabricated using silicon based structures. Our group has developed low-cost carbon fiber electrode (diameter = 8um) arrays that are directly mounted to printed circuit boards. While the boards do require many manual fabrication steps, an unexpected benefit to this is the ability to rapidly prototype design changes. Recently, through the NSF funded NeuroNex MINT Hub at University of Michigan, we have established a comprehensive service center for designing and fabricating custom arrays. This center, the carbon fiber core, connects users to the design options and use of this high-density technology. While the basic form factor is similar to previous carbon fiber devices produced (Patel 2015), primary capabilities of the core include changing the number of populated fibers, length of electrodes, tip geometry, and tip coating material. Electrode site numbers are currently limited to up to 16 channels, but can be arranged in varying configurations. The length of the electrode itself can be between 100um-500um and maintain its ability to self-insert into brain tissue without temporary stiffening agents. The shank of the probe can also be selected (10mm or 3mm) depending on the needs of an experiment. Tips coatings can either be optimized for dopaminergic recordings (laser ablation and plasma ashing) or for electrophysiology (PEDOT:pTS or platinum coatings). Users are able to request probes tailored to their experimental needs, troubleshooting assistance, and receive updates about new devices and modifications that can be requested. As of April 2019, our lab has distributed 162 devices to 22 labs in just over a year with successful, reproducible results. Of those labs, 13 are external to University of Michigan and have received roughly 40% of all devices and services provided by our core, with the other 60% having been distributed to labs internal to University of Michigan. Carbon fiber have been requested for use in mammalian (rodent and feline), insect, and Aplysia neural systems. Based on a cost analysis study for a batch of 8 probes, a quarter of the cost of the
arrays are labor followed by the combined cost of the Omnetics connector and PCB. This eases customization as the labor per device remains about the same for each modification. In small volume production, a university based service center could allow for these rapid modifications with a relatively low cost of production per device.

**Disclosures:** J. Richie: None. P.R. Patel: None. E.J. Welle: None. E. della Valle: None. J.D. Weiland: None. E. Yoon: None. J.P. Seymour: None. C.A. Chestek: None.

**Poster**

**330. New Methods for Studying Cognition**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 330.05/Z33

**Topic:** H.02. Human Cognition and Behavior

**Support:**
- DFG JA 1999/5-1
- DFG GE 3008/3-1
- ERC StG MEMCIRCUIT
- Else Kroener-Fresenius Foundation

**Title:** Intraoperative large scale extracellular recordings for studying the cellular and microcircuit basis of human cognition

**Authors:** *V. M. EISENKOLB, J. GEMPT, B. MEYER, S. N. JACOB;
Dept. of Neurosurg., Tech. Univ. of Munich, Munich, Germany

**Abstract:** The mammalian frontoparietal association cortex is crucial for intelligent behavior. As a center for executive control, these regions are responsible for grouping of sensory stimuli into abstract categories, online storage in working memory and selection of behaviorally relevant over distracting information. It is unknown how individual neurons and neuronal networks in the human association cortex control these higher brain functions. Commonly used methods in human cognitive neuroscience such as EEG or fMRI provide large coverage, but suffer from substantial spatial and temporal summation of neuronal signals which precludes the investigation of cognitive functions at the cellular and microcircuit level. To overcome these obstacles, we have established techniques to implant high-density, multi-channel microelectrode arrays (Utah arrays) into functional parts of the left prefrontal and posterior parietal cortex of awake neurosurgical patients undergoing resection of left-hemispheric brain masses (e.g. tumors). The array can be placed flexibly within the craniotomy allowing us to record with broad cortical access from many locations on the lateral convexity. During sleep and awake phases of the surgery, we record stable local field potentials (LFPs) and spiking activity of individual neurons on up to 96 channels simultaneously. These acute, large-scale extracellular cortical recordings can be combined with cognitive tasks requiring e.g. the categorization of abstract numerical
information. With adequate training, patients achieve satisfactory to very good intraoperative performance in the demanding setting of awake brain surgery. The combination of multi-channel recordings directly from the human association cortex with controlled behavioral tasks opens up new possibilities to study species-independent principles of cognitive functioning and to address neuronal mechanisms that govern human-specific cognition (e.g. language) on a microcircuit level.

Disclosures: V.M. Eisenkolb: None. S.N. Jacob: None. J. Gempt: None. B. Meyer: None.

Poster

330. New Methods for Studying Cognition

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 330.06/Z34

Topic: H.01. Animal Cognition and Behavior

Support: University of Catania intramural funds

Title: A novel modular behavioral apparatus to standardize experimental context in recognition memory assessment

Authors: *M. TROPEA¹, W. GULISANO¹, D. PUZZO¹;²
¹Biomed. and Biotechnological Sci., Univ. of Catania, Catania, Italy; ²Oasi Res. Institute-IRCCS, Troina, Italy

Abstract: The object recognition test (ORT) exploits the natural curiosity of rodents for novelty to assess recognition memory. Mice are presented with two similar objects during the first session (training), and one of the two objects is replaced by a new object during a second session (testing). The amount of time taken to explore the new object provides an index of memory. The easy technical execution, the lack of aversive stressful stimuli and the possibility to repeat the test on the same mice, make the ORT one of the most commonly used behavioral test to study learning and memory in mouse models. However, mice exploration is strongly influenced by the environment and the high variability of protocols used might critically affect the reliability of the results. To standardize the experimental context, we designed a customized behavioral apparatus by using the SolidWorks software. The apparatus consisted in a frame made by assembling aluminum profiles equipped with 3D-printed components to insert the Acrylic glass panels constituting the arena. Lights and a webcam to record mouse behavior were allocated on the lid of the frame. An anti-glare film was applied on the panels and light sources were located to assure a homogeneous illumination. We first tested different light intensities to assess the optimal environmental conditions. Next, we investigated the preference and/or discrimination capability for objects characterized by different colors, materials, shapes or dimensions. We found that mice tested by our customized behavioral apparatus presented a basal increase of
exploration time and less variability in latency to explore the object for the first time, and exploration time, suggesting a high repeatability of the experimental setting and an improvement of the context. Moreover, the preference/discrimination test provided us with an unbiased set of items to use during the ORT, as ethological characteristics of mice, influencing the natural curiosity and propensity to exploration for a specific object, have been taken into account. Thus, our customized apparatus might be useful when designing a behavioral experiment in order to improve the scientific outcomes and minimize possible biases. Moreover, besides its low cost, the modular design can be easily applied to perform different behavioral tests when the exploration of an arena is required (e.g. open field test, novel object location test).


Poster

330. New Methods for Studying Cognition

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 330.07/Z35

Topic: H.01. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation
AG055544
MH109548

Title: Using network metrics to characterize network functionality in hippocampal CA1

Authors: *D. T. GUENTHER¹, N. DELROCCO², C. HERDEGEN³, N. M. DICOLA¹, D. R. MILLER¹, H. KHOSHOUBEI¹, S. N. BURKE¹, K. DIBA⁶, R. VACCA⁴, A. P. MAURER⁵;
¹Neurosci., ²Biostatistics, ³McKnight Brain Inst., ⁴Sociology, ⁵Evelyn F. McKnight Brain Inst., Univ. of Florida, Gainesville, FL; ⁶Dept of Anesthesiol., Univ. of Michigan, Ann Arbor, MI

Abstract: Structure and function have a dynamic interplay in the brain, organizing the flow of activity in support of behavior. While functional connectivity parameters have been widely studied in human imaging data, less is known regarding the neuronal dynamics of local networks underlying these parameters. Recently, advances in single-cell neurophysiology have enabled the measurement of several neurons simultaneously. Convergent development in graph theory has allowed for the intersection between theory and application of network metrics to the single cell level in freely behaving animals. The current study used hippocampal CA1 recordings from populations of individual CA1 neurons, a subpopulation of which had an additional bilateral hippocampal CA1 implants. Recordings were made immediately before, during, and after rats traversed a linear track to quantify functional CA1 network organization. Neurons were considered connected in the network when the cross-correlogram between two neurons had a significant short-time peak within 5 milliseconds. Across rats, during rest and run epochs,
interneurons showed a higher degree (number of connections) relative to pyramidal cells, indicating their role as hubs. The hub organization of interneurons, however, was largely confined within a hemisphere. The majority of inter-hemispheric connectivity was between pyramidal neurons. This suggests that local network structure is largely governed by the local interneurons while interactions between distal regions is primarily driven through pyramidal neurons. When performing on the task, more neurons were recruited within the network relative to the pre- and post-behavior rest periods. Therefore, the reorganization in network structure may be a result from reorganization during learning.


Poster
330. New Methods for Studying Cognition

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 330.08/Z36

Topic: H.01. Animal Cognition and Behavior

Support: NIH/NIA R01 AG049722
McKnight Brain Research Foundation

Title: Translational rat model of cognitive aging using a touchscreen operant platform to test visual discrimination and association

Authors: *S. ZEQUEIRA¹, S. A. JOHNSON¹, A. HAMPTON², A. P. MAURER¹, J. L. BIZON¹, S. N. BURKE¹;
¹Neurosci., ²Psychology, Univ. of Florida, Gainesville, FL

Abstract: Animal models play an essential role in testing treatments for cognitive dysfunction linked to advanced age and Alzheimer’s disease (AD). Within animal models, behavioral paradigms are designed to elucidate neurobiological underpinnings of these cognitive impairments; however, tasks administered to rodents often do not parallel those used in humans. Rodent cognition is frequently assessed in mazes using 3-dimensional object stimuli, while human participants are more often tested with 2-dimensional image stimuli displayed on computer monitors. The Cambridge Neuropsychological Test Automated Battery (CANTAB), a non-verbal cognitive assessment tool administered on interactive touchscreens, has been widely implemented to gauge cognitive dysfunction in humans and non-human primates (Robbins et al. 1994; Roberts 1996) and, more recently, in rats and mice (Bussey et al. 2012). In particular, the CANTAB Paired Associates Learning (PAL) task has been adapted for screening rodent visuospatial learning (Talpos et al. 2009). In the task, three distinct visual stimuli (A, B, and C)
are associated with three touchscreen locations (1, 2, and 3, respectively). On each trial, rats choose between a correct stimulus-location conjunction (e.g. A1) and a stimulus presented in one of two incorrect locations (e.g. B3). Impaired PAL performance is observed in humans with AD (Égerházi et al. 2007) and in young adult rats following disruption of neural activity in brain regions associated with AD (Talpos et al. 2009). However, PAL performance has not yet been assessed in rat models of cognitive aging. Another test sensitive to cognitive dysfunction in older adults and AD is the Mnemonic Similarity Task (MST) (Yassa et al., 2010; Stark et al., 2013), which measures participants’ abilities to discriminate between image stimuli that share overlapping features. In our rodent adaptation of the MST, two image stimuli are displayed on the touchscreen: a previously learned target image and a lure that ranges in similarity to the target. On each trial, rats choose between the rewarded target and an incorrect lure. The current study assessed performance of adult (10-12 months) and aged (26-30 months) F344 x Brown Norway F1 hybrid rats on touchscreen PAL and MST tasks. Performance was compared across tasks to identify potential differences in visual discrimination and associative memory in aged animals. Future research utilizing these cross-species behavioral paradigms will advance our understanding of neurobiological aging and guide development of effective therapeutic interventions for cognitive decline in advanced age and AD.


Poster

330. New Methods for Studying Cognition

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 330.09/Z37

Topic: H.01. Animal Cognition and Behavior

Support: DARPA Contracts Management Office Grant No. HR0011-17-2-0019.

Title: A methodology pipeline for CLARITY and Arc imaging following vagus nerve stimulation

Authors: *M. MELTON\(^1\), S. BURKE\(^1\), M. ASH\(^1\), D. LAMB\(^1\), L. BRATTAIN\(^2\), K. OTTO\(^1\), B. SETLOW\(^1\), J. BIZON\(^1\), A. MAURER\(^1\), K. OLCZAK\(^1\), E. DIRR\(^1\);
\(^1\)Univ. of Florida, Gainesville, FL; \(^2\)MIT, Lexington, MA

Abstract: Methods to augment learning rates are of significant interest. Manipulation of neuromodulatory brain systems with pharmaceuticals have been shown to enhance attention, perception, memory, and other cognitive abilities; yet the mechanisms of these systemic treatments can produce non-specific effects that eclipse the cognitive benefits of such interventions. Direct nervous system interfaces offer an intriguing cognitive enhancement
alternative because of the precise spatial and temporal control opportunities, yet they are difficult to realize due to the necessary invasiveness. On the contrary, peripheral nervous system neuromodulation is possible via non-invasive transcutaneous stimulation. Critically, the mechanisms by which peripheral nerve stimulation modulates brain function need to be elucidated to optimize the ability of these types of interventions to enhance cognitive function. We have developed a pipeline for examining the ability of cervical vagus nerve stimulation to modulate the expression of the immediate-early gene Arc in cleared brain tissue. Rats (4-7 months old) were surgically implanted with a stimulating electrode cuff (Qualia) around the cervical branch of the vagus nerve. After a recovery period, rats received high-frequency (50 Hz) vagus nerve stimulation paired with object exploration for 15 min; they were sacrificed 30 min later and perfused via hydrogel solution. After fixation, and subsequent polymerization, the brain tissue was passively cleared for 1-2 months. After sufficient clearing, the sample was incubated with an ARC and tyrosine hydroxylase antibody and nuclei were stained with DAPI. Samples were imaged using a Zeiss lightsheet Z.1 with complimentary ARIVIS Vision 4D modular software. This pipeline will enable the visualization of plasticity networks engaged by vagus-nerve stimulation.


Poster

330. New Methods for Studying Cognition

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 330.10/Z38

Topic: H.01. Animal Cognition and Behavior

Support: DARPA HR0011-17-2-0019

Title: Automated multichannel centroid detection and coincident analysis of cleared brain tissue following vagus nerve stimulation

Authors: D. G. LAMB1, M. MELTON2, J. L. BIZON3, M. ASH4, *S. N. BURKE3, K. J. OTTO4, B. SETLOW5, A. P. MAURER6, E. DIRR4, L. BRATTAIN7;
1Psychiatry, Univ. of Florida, Gainesville, FL; 2Univ. of Florida, Gainesville, AL; 3Neurosci., 5Dept. of Psychiatry, 6Evelyn F. McKnight Brain Inst., 4Univ. of Florida, Gainesville, FL; 7MIT, Lexington, MA

Abstract: Stimulation of peripheral nerves offers an opportunity to non-invasively modulate brain function in order to treat disease as well as to enhance cognitive performance in healthy adults. In particular, Vagus Nerve Stimulation (VNS) has been of interest due to its broad
involvement in autonomic regulation, metabolism, and sensory/motor integration between the brain and viscera. Moreover, as VNS has been shown to induce network modification, efforts are underway to map the induction of plasticity across the brain in response to VNS by labeling the expression of immediate-early genes. Immediate-early genes are transcribed by neurons following patterned neural activity associated with active behavior and can therefore be used to identify cellular populations undergoing plasticity. While large-scale (Terabyte +) 3D fluorescence multichannel z-stack images of cleared rat brain tissue can be used for VNS-induced network plasticity reconstruction and analysis, there is currently no effective tool for automated plasticity analysis at scale. Such an analysis requires the identification of cell bodies and a fluorescently labeled protein of interest, as well as the spatial coincidence of these two signals. Image analysis challenges include the time it takes to extract images from the data acquired by the imager and the lack of robust neuron component detection algorithms that can process TB+ datasets efficiently. To tackle this unmet need, we are developing an automated multichannel centroid detection and coincident analysis pipeline, which consists of three main steps: 1) fast image extraction and compression directly from the microscopy outputs, 2) centroid detection using image processing techniques combined with deep learning 3) coincidence analysis among multiple channels, each corresponding to a particular fluorescent marker. The pipeline is implemented on a high-performance CPU cluster using parallel programming (pMatlab), and is able to process multiple tissue blocks simultaneously. An interactive front-end user interface is also being developed for quick reviews of both the raw data and results, allowing for editing of the centroid detections. Accuracy is currently being assessed by experts and will be reported in the poster. In the future, we plan to leverage deep learning to further improve our algorithm performance.


Poster

330. New Methods for Studying Cognition

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 330.11/Z39

Topic: H.01. Animal Cognition and Behavior

Support: ALS Society Canada
BrainsCAN

Title: Thinking inside the box - Using automated touchscreen tasks to evaluate cognition in FTD/ALS mouse models
Univ. of Western Ontario, London, ON, Canada

Abstract: TAR-DNA-binding protein 43 (TD-P43) misfolding and aggregation is a major pathological hallmark of Fronto-Temporal Dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS) but, has also impact in Alzheimer’s and Parkinson’s. FTD and ALS are characterized by motor and cognitive impairments. However, robust cognitive phenotypes related to TDP-43 proteinopathy have not yet been explored in mouse models of FTD/ALS. In this study, we used the Bussey-Saksida touchscreen technology for assessing executive function in the transgenic FTD/ALS mouse model TDP-43Q331K_low. It has been reported that these mice show motor impairments at 12 months of age. However, cognitive dysfunction has not yet been evaluated in this mouse line. Attention and learning/cognitive flexibility (major constructs affected in FTD/ALS) were assessed in TDP-43Q331K_low male mice using the Five Choice Serial Reaction Time Task (5-CRSTT) and Pairwise Visual Discrimination (PVD) task. In 5-CRSTT, TDP-43Q331K_low mice (5-6-month-old) present greater number of omissions and compulsive-like behaviour. Interestingly, in PVD task TDP-43Q331K_low mice were impaired in the acquisition and reversal phase of the task, suggesting that these mice present learning and potentially cognitive flexibility deficits. These results indicate that the TDP43Q331K-low mice present attentional, learning and cognitive flexibility deficits, similar to the cognitive impairments observed in humans affected by these diseases. Also, reveal the usefulness of the touchscreen tasks for the development or new therapeutic approaches for FTD/ALS. All the touchscreen results are deposited into an open-access database (www.mousebytes.ca) we have developed that allows for search, upload, storage, and re-analysis of touchscreen data from mouse models of neurodegeneration.


Poster

330. New Methods for Studying Cognition

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 330.12/Z40

Topic: H.02. Human Cognition and Behavior

Title: A task that allows analyzing separately the different stages of decision making: An electroencephalographic study
Authors: *P. CORTES ESPARZA, J. GARCÍA-HERNÁNDEZ, M. RAMÍREZ-RENTERÍA, M. HERNÁNDEZ-GONZÁLEZ, M. GUEVARA;*  
Inst. De Neurociencias, Guadalajara, Mexico

Abstract: Decision making is the ability to process multiple alternatives and choose an optimal course of action to achieve one’s goals in a given situation. This definition underlies a temporally structured process that many authors have sought to describe. Ernst and Paulus proposed a theoretical model that divided decision making into 3 temporally different stages based on distinct cognitive process and functionality of several brain structures. These stages were defined as follows: 1) input y process (forming preferences for each stimulus); 2) output (executing an action); and 3) feedback (experiencing the outcome of the action). Although there are cognitive processes specific to each stage, some as motivation, attention and working memory are present during the whole decision making difficult the individual analysis of each stage. Especially, the individual analysis of stage 2 has been challenging since most experimental paradigms can’t temporally divide stage 1 from 2 or stage 2 from 3. Because of this, little is understood of how stage 2 integrates cognitive processes into a well-defined motor plan to achieve a set goal. With this in mind, we design an experimental task aimed at temporally separating the 3 stages of decision making proposed by Ernst and Paulus. Against this background, the objective of this study was to characterize the electrical activity of the prefrontal, temporal and parietal cortices during the execution of the proposed task in the different stages of decision-making. In thirty healthy, right-handed men aged 20-35, the electroencephalographic activity (EEG) was recorded from the frontopolar (Fp1-Fp2), dorsolateral (F3-F4), temporal (T3-T4) and parietal (P3-P4) cortices during stages 1, 2 and 3 of the decision-making task. Absolute power (AP) was analyzed in the following EEG bands: theta, alpha1, alpha2, beta1, beta2 and gamma. The stages 1 and 3 showed a similar EEG pattern characterized by a higher AP of alpha and beta bands in dorsolateral and parietal areas, while stage 2 showed a higher AP of theta band in posterior areas (temporal and parietal) and gamma in the left frontopolar area. Alfa and beta bands are related to visual stimuli processing and attentional components, so its higher activation during the stages 1 and 3 could be related to the stimuli-outcome association while theta activation in stage 2 could be related to information processing and motivational components of the task execution. These results show a different cortical activation for each stage (especially stage 2) of decision making thus the proposed task could be a useful tool to study the temporal divisions of the decision-making process.

Disclosures:  
**P. Cortes Esparza:** None.  
**J. García-Hernández:** None.  
**M. Ramírez-Rentería:** None.  
**M. Hernández-González:** None.  
**M. Guevara:** None.

Poster

330. New Methods for Studying Cognition

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 330.13/Z41
Title: Nelpy: A powerful toolset for the analysis of electrophysiology data

Authors: *J. CHU, E. ACKERMANN, C. KEMERE;
Rice Univ., Houston, TX

Abstract: Recent advances in neural recording technology have enabled hundreds to thousands of channels. As bottlenecks shift away from data acquisition toward data analysis, it is imperative to have fast, flexible tools for such experiments. Here we introduce the nelpy ecosystem, a collection of Python software packages that enable the neuroscientist to process data with a convenient object-oriented syntax and perform common analyses. The core nelpy package supports a range of data typically encountered by electrophysiologists, including sampled analog signals, spike trains, and binned spike trains, with each of these objects allowing fast time or indexed-based data access. In addition to convenient containerization, nelpy provides a highly customizable plotting library for quick data visualization and exploration. The nelpy ecosystem provides a comprehensive environment by supplying the user with multiple options for different kinds of analyses. To showcase this power and flexibility, we give examples of neural spike train decoding with a variety of decoders, including the common Bayesian methods, and we demonstrate spectral analyses with our nelpy-compatible package ghost. Ghost augments nelpy's capabilities by being one of the first Python packages to implement the state-of-the-art generalized Morse wavelet transform, and it offers additional features such as multitaper spectrum estimation and cross-frequency coupling analysis. Nelpy reduces frequently experienced bottlenecks by handling the heavy lifting of data representation and implementing a variety of analyses in a fast, principled manner. We thus believe that nelpy will be an invaluable tool for many neuroscientists and will aid the reliability and reproducibility of results from electrophysiology experiments.

Disclosures: J. Chu: None. E. Ackermann: None. C. Kemere: None.

Poster

330. New Methods for Studying Cognition

Location: Hall A

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Program #/Poster #: 330.14/Z42

Topic: H.01. Animal Cognition and Behavior

Support: NSF CAREER award (CBET-1351692)
NSF BRAIN EAGER award (IOS-1550994)
HFSP Young Investigator’s award (RGY0088)
Ken Kennedy Institute for Information Technology
Rice Undergraduate Scholars Program

Title: Computational methods for advancing sharp-wave ripple detection and disruption

Authors: *A. K. FELDMAN, S. DUTTA, C. T. KEMERE;
Rice Univ., Houston, TX

Abstract: Hippocampal sharp-wave ripples (SWRs) arise from the synchronous firing of neural populations in CA3 and present as transient 150-250 Hz oscillations in CA1 pyramidal layer accompanied by sharp-waves in CA1 stratum radiatum, lasting approximately 60-150 ms. These events have been shown to be highly correlated with memory consolidation, recall and decision making, as they represent sequential reactivation of cells based on previous experience. Thus, selective interrogation of SWR events provides an attractive opportunity to modulate memory formation, relying on fast, effective algorithms for detection and disruption. While state-of-the-art closed loops systems have enabled perturbation of these events, latency between SWR onset and disruption ranges from 35-60 ms based on more liberal canonical ripple boundaries; essentially, these systems only allow for the interrogation of the latter 40-60% of the SWR event. Here, we look to improve detection accuracy and enable further access to SWR events. Through incorporation of false detection channels, temporal detection requirements and data from multiple hippocampal regions, we show improved detection accuracy. Additionally, we quantify performance of our detection algorithm during active animal behavior with varying canonical SWR frequency definitions in order to isolate SWRs from fast-gamma and ultra fast-gamma bursts. Lastly, we look to incorporate machine learning methods (cascade classifiers, deep neural networks, etc.) in order to enable interaction with a larger portion of SWR events. We combine these features with our current modular real-time ripple detection system, thereby increasing user specificity concerning preferential investigation. Overall, we expect our improved SWR detection algorithm will facilitate a wider range of memory consolidation inquiries, including that of in vivo SWR generation.


Poster
330. New Methods for Studying Cognition

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 330.15/AA1

Topic: H.02. Human Cognition and Behavior

Title: Neuroimaging decoder of cognitive load under stress
Abstract: Introduction - Technological advancements and increased complexity in work-environments have highlighted nowadays the need for objective indication of Cognitive Load (CL) under stress. One of the difficulties in studying CL, known to increase under stress, is that it cannot be directly observed, as task demands and performance are not equivalent for subjective experience of load. However, advancements in decoding otherwise unobservable internal states using neuroimaging, along with accumulated knowledge on executive functions and stress in the brain may help outline these processes. We developed a novel fMRI task that ecologically depicts CL. Using this task, we aim to elucidate the neural substrates of CL experience under stress.

Methods - 50 healthy male participants underwent an fMRI scan in which they performed a flight task, incorporating sub-tasks probing executive functions (e.g. working memory and cognitive inhibition) and stress induced by social and physical threat. A sliding window was applied to the extracted whole brain BOLD time series for each window under parcelled brain regions. Classification and regression algorithms were used to infer which regions were associated with the CL in accordance with individual performance, task demands and induced stress (i.e. CL index). Kernel SVMs, recurrent neural network and Ensemble methods were trained and their prediction performance was evaluated by cross validation.

Results - CL index continuous measure was estimated by a recurrent neural network (R=0.32, MSE=0.056), with classification (Gaussian kernel SVM) resulted in accuracy of 71% (AUC: 0.78). Higher CL index was characterized by higher values of the following regions; Intraparietal Sulcus, vent ant Cingulate Cortex, dorsal posterior Cingulate Cortex, L. Dorsolateral Prefrontal Cortex and R. Amygdala. Lower CL index was associated with higher values of the R. Temporoparietal Junction, R. IFG/ant Insula and the R. Putamen.

Conclusions - Our computational approach depicted a distributed pattern of functional indices in both executive function associated areas, as well as limbic and salience areas related to CL under stress. Our study provides a unique platform for studying the complex relation between cognition and stress with several novel aspects - developing an ecological multi-domain paradigm to probe CL in neuroimaging setting; defining CL index based on task demands and individual performance, as well as induced stress, rather than on task demands alone; and using a machine learning approach for associating mental states with brain activation patterns.

Evaluating translational neurophysiological measures to improve efficacy of preclinical therapeutic target discovery

Authors: *D. J. G. GREGG¹, J. F. CAVANAGH², G. LIGHT³, J. W. YOUNG³, J. L. BRIGMAN³;
¹Dept. of Neurosciences, ²Dept. of Psychology, Univ. of New Mexico, Albuquerque, NM; ³Dept. of Psychiatry, UCSD, San Diego, CA

Abstract: Preclinical studies can provide many potential therapeutic targets for neuropsychiatric disorders. However, many such targets prove ineffective during clinical trials. The failure to convert preclinical findings into clinical treatments suggests that behavioral similarity without biomarkers demonstrating similar brain function is not sufficient. Using pharmacology and EEG-like behavioral recordings we assessed the translational validity of several cognitive tasks measuring specific Research Domain Criteria (RDoC) which can be performed by both humans and mice. In phase one of the current study we evaluated three tasks, 5-choice continuous performance task (5C-CPT) which was previously shown to have cross-species similarity as accuracy was increased in both species following amphetamine administration, progressive ratio breakpoint task (PRBT), and probabilistic learning task (PLT). Male and female C57BL/6J mice were trained and assessed in a touch-screen based operant system. Once training criteria was reached, mice were fitted with dura-resting leads targeting mPFC, PPC and M1 with a ground centered over the cerebellum. During each recording session oscillatory activity was captured via a multichannel acquisition processor and assessed. Comparable data were obtained from healthy male and female human participants using non-invasive EEG procedures. Analysis of activity during each rodent task found oscillatory activity which correlated strongly with that seen in EEG of healthy human volunteers tested on a reverse-translated variant of the rodent touch-screen tasks. During 5C-CPT, theta and low-beta frequencies during target and particularly non-target trials correlated between species. For PRBP, an increase of alpha power was observed in both humans and mice as they neared their breakpoint. Lastly in PLT, the reward positivity event related potential (ERP) component scaled with the degree of positive prediction error, varying in strength according to probability ratio in both species. In phase two of the current study we administered varying doses of amphetamine (Mouse: 1.0 mg/kg, 0.3 mg/kg, 0.1 mg/kg, and vehicle), (Human: 20 mg/kg, 10 mg/kg, and vehicle) during recording of PRBP and PLT. The
correlation observed in phase one was seen again across amphetamine doses along with a correlation in dose dependent changes in behavioral performance. Taken together, these data support the use of EEG-like recording to examine neurophysiological biomarkers in rodents and humans to enhance the translatability of potential therapeutic targets.


Poster

331. Attention

Location:  Hall A

Time:  Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #:  331.01/AA3

Topic:  H.01. Animal Cognition and Behavior

Title:  Neural correlates of uncertainty parameters in temporal prediction

Authors:  *M. GRABENHORST¹, G. MICHALAREAS³, L. T. MALONEY⁴, D. POEPPEL²;  
¹Neurosci., ²Max-Planck-Institute For Empirical Aesthetics, Frankfurt, Germany; ³Max Planck Inst. for Empirical Aesthetics, Frankfurt Am Main, Germany; ⁴New York Univ., New York, NY

Abstract:  The anticipation of events in time, based on sensory input, allows for action preparation before an event has occurred. These well-timed responses to salient cues require estimation of several parameters of temporal uncertainty: i) the probability that an event will happen at all - a parameter likely related to resource allocation, ii) the probability density function (PDF) of events, of which the hazard rate (HR) is a prominent model of behavior and related neural activity, and iii) the uncertainty in time estimation which has been suggested to scale with elapsed time, irrespective of event probability (scalar property, a.k.a. temporal blurring). Here we investigate these three important sources of uncertainty in temporal prediction. We conceptualize event anticipation as an inferential process involving a transfer function that relates the brain's stochastic sensory input to its reaction time output. In a series of visual and auditory MEG and psychophysiological experiments, we find consistent results across the two sensory modalities. We propose new computational models for all three uncertainty parameters: i) the effect of event probability on RT is captured by an exponential function that weights time based on event probability (probabilistic weighting), which - similar to a cost function - describes the effect of the probability of event occurrence on anticipation in time; ii) the brain uses the reciprocal PDF of events but not the commonly employed HR to model event probability over time; and iii) uncertainty in time estimation scales directly with the inverted event PDF (probabilistic blurring) and is not merely based on elapsed time itself. Analyses of MEG data characterize modality-independent and modality-specific neural activity as correlates of anticipatory processes. We relate these electrophysiological findings to the different
computational models. The results suggest that the neurobiological implementation of temporal prediction involves specific computations based on the reciprocal PDF of events in time.

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**Poster**

**331. Attention**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 331.02/AA4

**Topic:** H.01. Animal Cognition and Behavior

**Support:** European Research Council (ERC-CoG 770951)

**Title:** Fiber photometry of anatomically-defined claustrum populations in awake behaving mice

**Authors:** *G. ATLAN1, N. PERETZ-RIVLIN1, E. SHEINBACH2, A. CITRI1,2,3;
1Edmond and Lily Safra Ctr. for Brain Sci., 2Inst. of Life Sci., Hebrew Univ. of Jerusalem, Jerusalem, Israel; 3Program in Child and Brain Develop., Canadian Inst. for Advanced Res., Toronto, ON, Canada

**Abstract:** The claustrum is a thin neuronal structure, located deep in the mammalian brain. Enclosed between the insular cortex and the striatum, it is reciprocally interconnected with sensory cortices, frontal regions, and subcortical nuclei. We have recently proposed that by regulating cortical gain, the claustrum may suppress task-irrelevant sensory stimuli in support of attentional processes (Goll et al. 2015, Atlan and Terem et al. 2018). Studies of natural activity of the claustrum in vivo have been limited in scope (Remedios et al. 2010). As such, while recent work shows that optogenetic claustral activation can have dramatic effects on the cortex (Jackson et al. 2018), it remains unclear whether such activity occurs naturally, and if so, under which circumstances. To understand the natural dynamics of claustral activity, we utilized retrogradely transported viruses and fiber photometry to record calcium transients from anatomically-defined subpopulations of claustral neurons in awake and behaving mice. During quiet wakefulness, spontaneous claustro-cortical output is characterized by low-frequency large bursts of activity. Sensory stimuli, however, elicited differential responses that depended on the alertness state of the mouse. As the claustrum receives substantial input from sensory cortices (Atlan et al. 2017, Wang et al. 2017), these results indicate that the claustrum is kept under a blanket of inhibition during wakefulness, a finding supported by the extensive inhibitory network of claustral interneurons (Kim et al. 2016). We next trained mice on an auditory figure-background segregation task, requiring mice to identify a target tone within a distracting background. Training was done in automated home-cages, minimizing the number of sessions required to achieve successful performance during recordings. Initial analysis indicates that claustrum
population activity is recruited selectively during the different phases of the task. We are currently analyzing these results to define distinct behavioral epochs during which the claustrum is active, as well as to describe how this activity develops with learning. Determining the particular sensory events that evoke activity in the claustrum during behavior is a necessary step to achieve a better understanding of this central structure, which will help direct future studies that selectively suppress or excite claustrum activity.


Poster

331. Attention

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 331.03/AA5

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant EY026924
         NIH Grant EY014800
         Research to Prevent Blindness Inc., New York, NY

Title: The global influence of visual signals in guidance of saccadic eye movements

Authors: *K. HUBBARD*¹, B. NOUDOOST²;
         ¹Biomed. Engin., ²Ophthalmology and Visual Sci., Univ. of Utah, Salt Lake City, UT

Abstract: The transformation of sensory signals into motor outputs is at the heart of our visually-guided behavior. How the information within visual areas, such as V4 and middle temporal, is used by motor areas, such as the Frontal Eye Field, is not known. We studied the characteristics of sensorimotor integration in rhesus monkeys during visually guided eye movements. In our visually guided saccade task, after fixating on a central point, a peripheral random dot motion (RDM) target appears, spanning 3-4 degrees of visual angle. The animal needs to maintain fixation until the fixation point disappears. After this go cue, in 25% of trials the animal makes an eye movement toward the RDM (Toward condition). In 75% of trials, simultaneously with the go cue a stationary circle appears as an alternative target to which the animal must make an eye movement to receive a reward (Away condition). In each trial, the RDM target motion was in one of four directions (45, 135, 225, or 315 degrees). First, we confirmed that the direction of motion biased the endpoint of saccades in the Toward condition. We compared the landing points of saccadic eye movements to the RDM target for different directions of motion. We quantified the visual guidance using the area under the curve (AUC) in the receiver operating characteristics method, for saccades to RDM targets with opposite motion directions. AUC values were significantly different than 0.5, indicating the presence of the classic visual guidance phenomenon. In order to examine the spatial specificity of RDM’s
influence on biasing saccade vectors, we assessed the AUC in the Away condition. We found that the presence of an RDM stimulus is sufficient to influence the saccade endpoints, even when that RDM stimulus is not the target of the eye movement. These results suggest that the transformation of sensory information into motor commands involves an integration of sensory signals from sources beyond the current target of the eye movement.

Disclosures: K. Hubbard: None. B. Noudoost: None.

Poster

331. Attention

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Program #/Poster #: 331.04/AA6

Topic: H.01. Animal Cognition and Behavior

Support: Shanghai Municipal Science and Technology Major Project Grant 2018SHZDZX05
Strategic Priority Research Program of the Chinese Academy of Sciences Grant XDB32010100

Title: Improve the learning of olfactory working memory tasks by suppressing the delay activity of lateral orbitofrontal cortex

Authors: *Q. CHENG*¹,², H. FAN³, Z. CHEN³, R. HOU³, C. T. LI¹,²;
¹Inst. of Neuroscience, State Key Lab. of Neuroscience, Chinese Acad. of Sciences, CAS Ctr. for Excellence in Brain Sci. and Intelligence Technology, Shanghai Ctr. for Brain Sci. and Brain-Inspired Technol., Shanghai, China; ²Sch. of Future Technology, Univ. of Chinese Acad. of Sci., Beijing, China; ³Inst. of Neurosci., Shanghai, China

Abstract: Learning ability is of great significance for survival in a competitive daily life. How to improve learning ability and its corresponding neural basis were relatively unexplored fields in neuroscience research. In this study, we report that suppressing the delay-period activity of lateral orbitofrontal cortex (IOFC) could improve the learning of olfactory working memory (WM) tasks in mouse, via an inhibition of task irrelevant distractions. Optogenetically suppressing the lateral, but not the ventral or medial parts of OFC, enhanced the learning rate of WM task. This effect was specific to the delay-period, rather than the decision-period activity. Silencing the delay activity of IOFC in a dual task, but not in a value-based WM task, could also improve the learning rate, which was compatible with the cognitive map hypothesis but not the value-coding hypothesis. Consistently, neurons in IOFC could encode both the task relevant and irrelevant stimuli. Furthermore, inhibiting the IOFC-to-dorsomedial striatum and IOFC-to-basolateral amygdala pathways could also improve the behavioral performance during learning and reduce the coding of distractions. Therefore, suppressing IOFC could improve learning
ability of mouse through reducing the coding of task-irrelevant distractions in WM within a distributed neural network.

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**Poster**

**331. Attention**

**Location:** Hall A

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**Program #/Poster #:** 331.05/AA7

**Topic:** H.01. Animal Cognition and Behavior

**Support:** W911NF-16-1-0368

**Title:** Virtual reality system for the immersive display of visual and audiovisual objects

**Authors:** *S. SABHARWAL-SIDDIDI, A. DUBEY, J. CHOI, J. HAGGERTY, S. QIAO, E. VOINAS, B. PESARAN;
Ctr. for Neural Sci., New York Univ., New York, NY

**Abstract:** As we navigate this world, we use sensory information to react to behaviorally relevant objects. When an object is itself multimodal, our brains enable us to react with optimal speed and accuracy by integrating the sensory inputs. An important question is to understand how and when this integration occurs in the brain. One approach is to present subjects with unimodal and composite multimodal objects, and then to compare reaction times and target selection biases. In attempting to present audiovisual objects, however, investigators are often limited by their experimental equipment. Indeed, it is easy to present a visual object on a 2D monitor, but difficult to precisely co-localize an auditory stimulus. Some circumvent this problem by using real audiovisual objects (speaker with LED light), but this severely limits the range of target properties that can be manipulated in real time. Therefore, to overcome these constraints, we developed a virtual reality (VR) headset for the immersive presentation of visual and audiovisual targets. We displayed visual objects on a modified Oculus Rift gaming headset. A custom cold-mirror assembly for coaxial stimulus delivery permitted stimulus delivery and IR-illuminated eye-tracking. We furthermore refined procedures and equipment for binocular stimulus calibration and implemented corrections for radial distortion and chromatic aberration produced by custom optics. Sounds were artificially localized to defined points in space using a head related transfer function (HRTF) and delivered through earphones. Using a previously published HRTF for the rhesus macaque, we were able to present audiovisual objects to a monkey. The monkey performed a two-alternative forced choice task in which both targets were visually identical but only one was paired with an auditory stimulus. We found that monkey was able to reliably discriminate the audiovisual target from the visual-only target at a statistically significant rate. This suggests that he was able to integrate auditory and visual information in our
virtual reality system, and that this system can be reliably used for future experiments on
multisensory integration.

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**Poster**

**331. Attention**

**Location:** Hall A

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**Program #/Poster #:** 331.06/AA8

**Topic:** H.01. Animal Cognition and Behavior

**Support:**
- NSF/NCS 1734901/1734916
- NIH R00EY025768

**Title:** Cell-type-specific suppression of spontaneous activity by attention in visual cortex

**Authors:** *E. M. SACHSE*¹, A. C. SNYDER¹,²,³,

¹Brain & Cognitive Sci., ²Neurosci., ³Ctr. for Visual Sci., Univ. of Rochester, Rochester, NY

**Abstract:** Multi-unit recordings have helped identify different features of neuronal populations and how they are modulated by attention. Such cellular and circuitry features are important clues to the mechanisms of spatial attention. Information coding in V4 is influenced by complex organization in cortical columns that contain several cell types. Fast-spiking interneurons make up the majority of inhibitory neurons in the cortex and have dense connections to local regular-spiking excitatory neurons. These cell types are known to exhibit different attention dynamics during spontaneous and stimulus-evoked activity. Local inhibitory activity has been shown to alter correlations among excitatory neurons, indicating these inhibitory circuits likely act to regulate activity of local excitatory populations across space and time through changes in neural synchrony. For example, low synchrony may be beneficial during spontaneous activity, as it indicates reduced dependence on internal signals and greater receptivity to stimulation. On the other hand, higher synchrony may effectively transmit information downstream during stimulus processing. To investigate this, we compared attention dynamics of V4 fast-spiking and regular-spiking neurons by recording population activity while monkeys performed an attention task. We measured dynamics of attention modulation for each neuron during spontaneous activity and stimulus-processing and classified neurons as fast-spiking or regular-spiking based on their action potential waveform. For all neuron pairs with significant attention effects, we calculated average synchrony among different cell types and as a function of location on our Utah array. We found that patterns of attention dynamics co-segregated with cell classes and that average synchrony is influenced by local connectivity and pair type. Together, these suggest that fast-
spiking and regular-spiking cell classes play distinct functional roles in the dynamic allocation of attention across sensory stimulation contexts.

**Disclosures:**  
**E.M. Sachse:** None.  
**A.C. Snyder:** None.

**Poster**

**331. Attention**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 331.07/AA9

**Topic:** H.01. Animal Cognition and Behavior

**Support:**  
Riken  
HHMI  
JPB foundation

**Title:** Locus coeruleus stimulation averts stress-induced attentional deficits and anxiety

**Authors:**  
*A. BARI*¹², S. XU¹², D. TAKEUCHI³, J. MARTIN¹², S. TONEGAWA¹⁴₂;  
¹The Picower Inst. for Learning and Memory, Dept. of Brain and Cognitive Sci., ²RIKEN-MIT Ctr. for Neural Circuit Genet., ³Dept. of Biol., ⁴MIT, Cambridge, MA

**Abstract:** The locus coeruleus / noradrenergic (LC/NE) system regulates a wide array of cognitive and emotional processes, including attention and anxiety. Selective attention allows focusing on task-relevant information, and the inhibition of distracting stimuli and intrusive thoughts. On the other hand, anxiety is characterized by distractibility and impulsiveness, both of which increase non-selective responding to internal and external stimuli. Stress is known to induce anxiety, which in turn hinders the efficiency of selective attention. Although the interplay between attention and anxiety is generally accepted, it is currently unclear how the LC/NE system activity regulates both, ultimately promoting the optimization of behavioral output. Attention deficits, impulsivity and anxiety often co-occur in several brain disorders such as depression, attention deficit / hyperactivity disorder (ADHD) and Alzheimer’s disease. Moreover, these symptoms are all relieved by increasing norepinephrine (NE) synaptic levels via pharmacological blockade of the NE transporter (NET) in certain patient populations, which is suggestive of common neurochemical underpinnings. Because stress is known to activate the LC/NE system, the clinical efficacy of NET blockers on attention deficits and anxiety symptoms seems paradoxical. To better understand the reciprocal influence between attention and anxiety, and their modulation by the LC/NE system, we used double transgenic mice that allow the selective optogenetic manipulation or recording of NET+ neurons activity. We first confirmed the long-hypothesized causal role of LC neurons activation on ameliorating attention and impulsive behavior. More specifically, our results suggest that stimulation of the LC/NE system is necessary and sufficient for the implementation of selective attention, in that, on the contrary,
its inhibition increases distractibility and impulsivity. We also assessed the effects of LC stimulation on tests of anxiety-like behavior. Contrary to a widespread belief, we found that LC activation decreases both baseline and stress-induced anxiety. Finally, we tested the effects of stress on attentional performance with or without LC stimulation. Stressed animals were anxious and displayed marked attentional deficits and impulsive responding. Activation of the LC/NE system immediately before the attentional task prevented these deficits. In summary, our results show that LC neurons stimulation is anxiolytic and completely abolishes the negative effects of stress-induced anxiety by preventing the distractibility and impulsivity that usually follow a stressful experience.


Poster

331. Attention

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 331.08/AA10

Topic: H.01. Animal Cognition and Behavior

Support: CONACyT Problemas Nacionales Grant 2016-2132
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CONACyT LNG Grant 438974

Title: Aging associated attention decline is prevented by D-serine and correlates with a reduction on functional brain connectivity

Authors: L. NAVA-GOMEZ¹, A. CALERO-VARGAS², J. ORTIZ-RETANA³, S. ALCAUTER³, *M. LOPEZ-HIDALGO²;
¹Facultad de Medicina. Univ. Autonoma de Queretaro, Queretaro, Mexico; ²Escuela Nacional de Estudios Superiores-Unidad Juriquilla. Univ. Nacional Autonoma de Mexico, Queretaro, Mexico; ³Dept. de Neurobiologia Conductual y Cognitiva. Inst. de Neurobiologia. Univ. Nacional Autonoma de Mexico, Queretaro, Mexico

Abstract: D-serine is essential for the induction of NMDA-receptor-dependent synaptic plasticity and cognitive processes such as attention. Cerebral D-serine levels are diminished in aged rats which could account for the deterioration of these functions with aging. Results from our lab have shown that chronic administration of D-serine significantly improves the performance of aged rats in attention tasks. However, the cellular mechanisms and neural networks underlying this behavioral effect are not clear. To analyze this, we use resting state functional magnetic resonance imaging to study the effect of D-serine on functional connectivity in the aged rat brain. Using a Bruker 7T MRI scanner, we explored the functional connectivity
between selected anatomical structures, previously related to attention performance, in 18-month-old rats. Specifically, we analyzed the functional connectivity between striatum, hippocampus, insular, cingulate, orbitofrontal, frontal and retrosplenial cortices. Rats treated with D-serine showed decreased functional connectivity when compared to 18-month-old rats that did not receive D-serine. Furthermore, the functional connectivity was negatively correlated with attention performance in the rats treated with D-serine but not in the control group, suggesting that D-serine enhances attention performance through the modification of local and long-range functional networks.


Poster

331. Attention

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 331.09/AA11

Topic: H.01. Animal Cognition and Behavior

Support: NIH IRP ZIAMH002784

Title: A role for adult-born hippocampal neurons in arousal

Authors: *J. MERCURIO\textsuperscript{1,2}, H. A. CAMERON\textsuperscript{3}; \textsuperscript{1}Natl. Inst. of Hlth., Bethesda, MD; \textsuperscript{2}Neurosci., Brown Univ., Providence, RI; \textsuperscript{3}NIMH, NIH, Bethesda, MD

Abstract: During adulthood, new neurons are born and integrated into the pre-existing hippocampal circuitry of the mammalian dentate gyrus (DG). How these immature neurons contribute to downstream circuit activity in the hippocampus and ultimately to behavior remains unclear. By using a transgenic rat line (GFAP-TK rats) to pharmacologically inhibit adult neurogenesis with temporal and cell-type specificity, our lab is studying the function of these adult-born neurons. Previous literature shows that rats with hippocampal lesions have deficits in their ability to orient toward a distracting stimulus while focused on a task (Hendrickson et al., 1969). Our lab has found that rats lacking adult neurogenesis in the DG orient less frequently toward a novel auditory stimulus that is administered during a simple task (i.e. drinking water). The reason rats lacking adult neurogenesis in the DG have this deficit in shifting attention toward a novel stimulus is unclear, but one possibility could be a difference in internal arousal state of the animal, which can affect the sensory input and thus the behavioral output of an animal within its environment. Pupillometry is a non-invasive technique for measuring an organism’s physiological arousal that correlates strongly with the more established measure of hippocampal sharp wave activity (McGinley et al., 2015). The existence of arousal signals in the hippocampus...
suggest that newborn granule cells could potentially affect arousal and in turn modulate the ability to selectively attend stimuli. We are using pupillometry to compare the arousal states of rats with and without ongoing adult neurogenesis and to determine whether differences in arousal underlie the role of new neurons in orientating toward a novel stimulus.

Disclosures:  J. Mercurio: None. H.A. Cameron: None.

Poster

331. Attention

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Program #/Poster #: 331.10/AA12

Topic: H.01. Animal Cognition and Behavior

Support: R01DA045063

Title: Sign-trackers deploy perceptual, but not cholinergic-attentional, mechanisms to respond to salient cues

Authors: *K. B. PHILLIPS, C. AVILA, M. SARTER; Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: Sign-trackers (STs) attribute incentive value to stimuli that predict food and drug rewards and therefore have emerged as a model for studying vulnerability for addiction-like behaviors. Relative to goal-trackers (GTs), who do not imbue discrete predictive stimuli with motivational value, STs also show a reduced capacity for engaging forebrain cholinergic signaling for the processing of behaviorally significant and attention-demanding cues. The greater power of Pavlovian drug cues in STs has been attributed in part to their relatively poor attentional control of such cues. However, when tested in an operant Sustained Attention Task (SAT), STs exhibit only a minor impairment in hit rates but, more robustly, unstable performance over time. These observations raised the question as to the neuro-behavioral or -cognitive mechanisms via which STs perform the SAT. Male and female STs were trained on SAT. The SAT requires the reporting of cues as well as non-cue events via separate levers, yielding four response categories (hits and misses, and correct rejections and false alarms). After reaching criterion, half of STs received bilateral infusions of the cholinergic-selective neurotoxin 192-IgG saporin while the remaining STs received sham-lesions. Following recovery, performance was assessed on the SAT and a version of SAT incorporating a flashing house light distractor (dSAT). Goal-directed (or top-down) attention is thought to maintain and recover performance during dSAT and mediated via increases in cortical cholinergic activity. In STs, neither SAT nor dSAT performance depended on the integrity of the cholinergic system. We therefore hypothesized that STs perform the SAT using model-free, non-attentional mechanisms, perhaps relying largely on trial-biased perceptual processes to detect salient cues. To test this
hypothesis, separate STs and GTs were trained on SAT. The salience of the cue light relative to
the house light was varied across operant chambers. In STs, greater perceptual sensitivity
reductions were observed as a function of relatively weaker cue salience. In contrast, GTs’
perceptual sensitivity did not relate to cue salience. Associated with their relatively unresponsive
cholinergic system, STs rely on perceptual mechanisms, rather than attentional mechanisms, to
perform the SAT. The relative absence of (top-down) attentional control of behaviorally
significant cues, combined with a propensity to attribute incentive value to such cues, renders
STs less likely to reject such cues from guiding their behavior and engaging in alternative action.

Disclosures:  K.B. Phillips: None. C. Avila: None. M. Sarter: None.

Poster

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Program #/Poster #: 331.11/AA13

Topic: H.01. Animal Cognition and Behavior

Support:  CRC-15-04-KIST
        NRF 2017R1A2B3012659

Title: Functional dissociation of EEG theta rhythms between prefrontal and visual cortices and
their synchronization during sustained attention

Authors: *H.-B. HAN1,2, K. LEE1,3, J. CHOI1,4;
1Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; 2Korea Advanced Inst. of Sci. and
Technol., Daejeon, Korea, Republic of; 3Seoul Natl. Univ., Seoul, Korea, Republic of; 4Korea
Univ. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Over a decade, coherent theta rhythms (4-12 Hz) within the cerebral cortex have been
believed to facilitate information processing by bridging distal brain regions during a task
situation. For example, the theta synchrony between prefrontal cortex (PFC) and visual cortex
(VC) has been known to be associated with enhanced task performance and attention. Recently,
this conceptual framework has been mitigated by a wayward behavior of theta power in VC
during sustained attention task: Spyropoulos et al. (2018) observed that the evoked theta in VC
decreased as the demands for the visual attention raised in a visual task. Likewise, Stitt et al.
(2018) observed that the theta power in high-order VC became lessened by arousal but
maintained its synchrony with the PFC in a sustained attention task. These inconsistent behaviors
of theta rhythms suggest that theta rhythms in PFC and VC might be differently involved during
a task situation. In order to enunciate their different roles, we analyzed EEG signals from mouse
PFC and VC during visual Go/No-Go task with respect to short-term task performance. The
short-term task performance was assessed by the ratio of correct trials obtained through a moving
window over 10 previous trials. We found that PFC and VC theta behaved in an opposite way: PFC (VC) theta was stronger (weaker) in the good-performing epochs compared to the bad-performing epochs. Interestingly, the synchronization between two oscillations increased during the epochs with good performance, despite the suppression of theta in VC. Furthermore, the phase relationship in the fronto-visual phase-locked theta rhythms showed a predominant posterior-to-anterior direction in the order of few milliseconds. Along with the improved synchrony, the delay showed a subtle but systematic decrease, suggesting a boost of information relay from the posterior to the anterior brain region. Our findings not only provide empirical evidence for the distinction between the theta of PFC and VC, but also reveal the overlooked aspect of long-range synchrony between functionally different oscillators in the cerebral cortex.

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**Poster**

**331. Attention**

**Location:** Hall A

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**Topic:** H.01. Animal Cognition and Behavior

**Support:** CRC-15-04-KIST
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**Title:** Cortico-cortical and baso-cortical gamma oscillations represent functionally distinct attentional networks

**Authors:** *K. LEE*[^1],[^2], H.-B. HAN[^2],[^3], J. CHOI[^2],[^4];

[^1]: Seoul Natl. Univ., Seoul, Korea, Republic of;
[^2]: Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of;
[^3]: Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of;
[^4]: Korea Univ. of Sci. and Technol., Daejeon, Korea, Republic of

**Abstract:** Attentional states contribute to task performance. Yet, the neurological correlates that represent attentional states are poorly defined. Temporal analysis of gamma band oscillations (GBO, 30-80 Hz) in the seconds-to-minutes timescale has shown the cortico-cortical GBO network to be pro-cognitive. On the contrary, a recent study observed prominent GBO in the baso-cortical network during the task-negative state in a longer timescale of few-tens-of-minutes. These two opposing observations raise a possibility that GBO networks are differentially correlated with different cognitive states depending on the brain regions and/or timescale. To elucidate this, we analyzed cortico-cortical and baso-cortical GBO network dynamics in the minutes versus seconds timescales using the Go/No-Go paradigm. EEG and LFP were obtained from prefrontal and posterior-parietal cortices and the basal forebrain in head-fixed mice. We characterized GBO patterns that accompany the long-term shifts in behavioral state in addition to
trial-by-trial fluctuations in task performance. Our results showed that the baso-cortical GBO networks reflect long-term attentional state transitions, manifested by the progressive decline in task engagement. On the other hand, independent of the long-term trends, trial-by-trial performance analysis revealed that stronger cortico-cortical GBO network precedes correct trials. Interestingly, one of the baso-cortical networks exhibited stronger connectivity before error trials. This suggests that on top of being involved in the long-term transition into task-negative states, certain activities of the baso-cortical gamma network can trigger immediate errors in behavior, possibly through causing acute disruptions in attention. Thus, cortico-cortical and baso-cortical GBO networks can be interpreted to be involved in functionally distinct attentional networks that influence brain states and behavior on different timescales.

Disclosures:  K. Lee: None. H. Han: None. J. Choi: None.

Poster

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Title: Timing is everything in the attention network: Specific oscillatory patterns in spike timing predict behavioral performance

Authors: *I. C. FIEBELKORN, S. KASTNER;

Abstract: The deployment of spatial attention is associated with both changes in neural activity across multiple nodes of a large-scale network (i.e., the attention network) and improvements in behavioral performance (e.g., faster response times). Although it is often assumed that attention-related changes in neural activity improve subsequent behavioral performance, the specifics of this relationship have remained largely unknown. Previous studies have frequently observed attention-related changes in neural activity among neurons that represent a behaviorally relevant location. These changes in neural activity occur in the absence of sensory stimulation, in anticipation of a subsequent, temporally unpredictable target (i.e., during a cue-target delay). Such preparatory changes in neural activity are thought to enhance the sensitivity and efficiency of sensory processing. Here, we investigated the relationship between attention-related changes
in neural activity and behavioral performance during a spatial cueing paradigm, while simultaneously recording from frontal and parietal nodes of the attention network in macaques. These higher-order cortical regions direct attention-related neural effects in sensory cortex. We specifically focused on the link between different aspects of neural activity (e.g., spike rates and spike timing) and behavioral performance. Spiking activity represents the output of neuronal computations and is the primary means through which neurons interact. Despite large increases in spike rates during the deployment of spatial attention, our results show no difference in spike rates between trials that resulted in either faster or slower response times (or between trials that resulted in either hits or misses). Instead, our results demonstrate that differences in behavioral performance are characterized by differences in spike timing, specifically differences in the oscillatory patterns of spike timing. This temporal organization of spiking activity at different oscillatory frequencies reflects specific functions associated with covert spatial attention (e.g., the suppression of eye movements), as well as enhanced functional connectivity among nodes of the attention network. The anticipatory strengthening of between-region connectivity may serve to facilitate visuo-motor integration in response to targets at an attended location, leading to better behavioral performance. While elevated spike rates in the attention network certainly reflect the deployment of spatial attention at a specific location, changes in neuronal spike timing seem to have more relevance for behavioral outcomes.

Disclosures: I.C. Fiebelkorn: None. S. Kastner: None.

Poster

331. Attention

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 331.14/AA16

Topic: H.01. Animal Cognition and Behavior

Title: Flexible feature-based attention and learning in monkeys

Authors: *S. D. KOENIG¹, T. WOMELSDORF²;
²Dept. of Psychology, ¹Vanderbilt Univ., Nashville, TN

Abstract: Visual attention is directed to objects predicting reward as well as to objects that are informative about possible future rewards. In naturalistic environments, both types of attention go hand in hand to ensure an organism’s fitness to harvest maximal rewards, but how attention-to-reward and attention-for-learning are related is poorly understood. For understanding their relation, it is necessary to vary them independently and test how the efficiency of attention-for-learning predicts attention-to-reward and vice versa. Here, we report findings from two novel task paradigms achieving this goal in up to six nonhuman primates using a complex naturalistic environment consisting of 10,800 unique 3D objects composed of four feature dimensions: color, shape, pattern, and arms. With a feature learning task, we quantify how increasing attentional
filtering demands affect the learning of feature values. Monkeys learn feature values of objects through trial-and-error that are defined by one, two or three features, but only one feature value predicts reward. After a certain number of trials, the rewarded feature value suddenly changes. By increasing the dimensionality of the feature space, we increase the demand for attention-for-learning. With a feature search task, we quantify how efficient attention filters out distractors that increase in the number of features and feature similarity to a target object. Using the feature learning task, we find that learning is significantly slowed down with increased dimensionality (bootstrapped learning curves, p’s < 0.05). Using the same objects in the feature search task allowed us to quantify the efficiency of attention-for-reward as search slopes. We found significant (z-test, all p’s < 0.001) search slopes of 10-33 ms per distractor in a simple feature search but dramatically enhanced search slopes of 88 ms per distractor when feature reward rules were dependent on context. Overall, these results illustrate how to precisely quantify the efficiency of attention-for-learning and attention-for-reward in nonhuman primates in two task paradigms in a naturalistically complex environment. Our findings support the reinforcement learning modeling framework of attention which posits that feature-specific reward prediction errors are continuously updated to ensure fast learning and efficient attentional selection of features in multidimensional environments (Niv, 2015; Leong, 2017; Oemisch, 2019).

**Disclosures:** S.D. Koenig: None. T. Womelsdorf: None.

**Poster**

331. Attention

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**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIH Grant R01 NS088661

**Title:** Cortex-projecting basal forebrain PV neurons in a sustained attention task

**Authors:** *S.-J. LI*¹, B. HANGYA², A. KEPECS¹;
¹Cold Spring Harbor Lab., Cold Spring Harbor, NY; ²Dept. of Cell. and Network Neurobio., Inst. of Exptl. Medicine, Hungarian Acad. of Sci., Budapest, Hungary

**Abstract:** The basal forebrain plays an important role in modulating cortical activity, potentially influencing attention, learning and memory. Although most studies of basal forebrain have focused on the cholinergic neurons, the functional roles of long-range projecting GABAergic neurons are less well understood. Here we studied the projection patterns of basal forebrain PV neurons and recorded their activity in an auditory sustained attention task. Using anterograde and retrograde tracing, we first studied the cortical targets of basal forebrain PV neurons. We found that basal forebrain PV neurons are compartmentalized and nucleus-
specific in terms of their targeting cortical regions. We also recorded optogenetically identified basal forebrain PV neurons and imaged the cortical terminal fields of PV neurons using fiber photometry. We tested mice in a sustained attention task in which mice had to detect “tone A” to earn water reward and ignore “tone B” to avoid airpuff punishment embedded in varying noise background. Mice performed the task well and their accuracy and reaction time varied systematically as a function of signal-to-noise ratio. Previously we demonstrated that basal forebrain cholinergic neurons respond phasically to the presentation of outcomes (reward and punishment) and this response is scaled by the unexpectedness of outcomes (Hangya et al., 2015). Here we found that PV neurons have tonic responses to both the tones and the outcomes. Interestingly, tone-induced increases of PV neuron activity were negatively correlated with the reaction time of the animal’s behavioral response towards the tone. These results suggest that basal forebrain PV neurons may be involved in broadcasting signals important for vigilance and outcome expectation and may be relevant to sustained attention.

Disclosures: S. Li: None. B. Hangya: None. A. Kepecs: None.

Poster

331. Attention

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Topic: H.01. Animal Cognition and Behavior

Support: NIMH 1P50MH109429
        NIMH R01MH064043
        NEI R01EY017699

Title: Corticothalamic networks underlying exogenous and endogenous attention

Authors: *R. CHEN¹, A. B. MARTIN²¹, S. KASTNER¹;

Abstract: Visual information can be selected actively via endogenous attention, depending on its relevance to the current task. Moreover, salient and sudden events also attract spatial attention through exogenous mechanisms. Neural correlates of these attentional modes have been found in the frontal eye fields (FEF) and the lateral intraparietal area (LIP). These cortical areas have overlapping projection zones in the thalamic nucleus of the pulvinar, which is known to modulate cortical attentional responses. However, the interaction of attentional control in these areas when switching between these modes has not been shown. To explore this question, we recorded simultaneous neural activity (SUA and LFP) from these areas in two monkeys who performed a variant of the Eriksen flanker task. While the monkey maintained central fixation, a
spatial cue was flashed to signal the location of a subsequent target shape. The cue was either a salient disk flashed at the target location to attract exogenous attention, or an arrow at the fixation point to direct endogenous attention to the target location. After a variable cue-target delay window, the target shape was presented at the cued location, embedded in a circular array of distractors. Following a target-response delay, a color change of the target indicated that the monkey should saccade to the target location. While accuracy and reactions times for both cue conditions were remarkably similar, microsaccade patterns, which are thought to signify the direction of covert attention, were different. Corroborating previous studies, we found that attentional modulation of SUA was primarily observed in visual and visual-motor neurons, but was absent in oculomotor neurons. Exogenous attentional modulation was apparent across all three regions during both cue-target and target-response delay periods. Endogenous attentional modulation was restricted to FEF during the cue-target delay period, although it was present in all three areas during the target-response delay. Granger causality and coherence analyses were performed to explore functional interactions across the network. The functional connectivity profiles during the two attention modes also showed different patterns. These findings suggest that although exogenous and endogenous spatial attention share an overlapping neural network, influences on neuronal representations and information communication across the network underlying these two attention modes differ.

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Poster

331. Attention

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I01 BX002774
P01 HL095491
R01 MH039683
I01 BX004500
I01 BX001404
I01 BX001356

Title: Optogenetic excitation of basal forebrain parvalbumin neurons quickens reaction times in the rodent psychomotor vigilance task

Authors: F. L. SCHIFFINO¹, J. M. MCNALLY¹, A. N. HASSLER², R. E. BROWN¹, *R. E. STRECKER¹;
¹VABHS & Harvard Med. Sch., West Roxbury, MA; ²Stonehill Col., Easton, MA
Abstract: Deficits in attention are a major feature of neuropsychiatric disorders. Thus, understanding the brain circuitry underlying attention is important to develop novel treatments. Dysfunction and degeneration of basal forebrain (BF) neurons are early features of many disorders. Previous studies have shown that cholinergic BF neurons are important in attention and their loss contributes to attention deficits. However, the majority of BF neurons are non-cholinergic and little is known about their role in cognition. Here, we use optogenetic techniques and behavioral paradigms in mice to test the role of one non-cholinergic subtype, basal forebrain parvalbumin neurons (BF PV), in attention for the first time. Recent work suggests that BF PV neurons may support levels of alertness underlying sustained attention. First, activity of an unidentified population of non-cholinergic neurons predicted reaction time and accuracy in a manner that was interpreted to reflect momentary fluctuations in sustained attention. It is plausible that these were BF PV neurons, as their fast firing rate patterns were well within the appropriate range. Second, excitation of BF PV neurons enhances wakefulness and overall levels of locomotor activity. **Hypothesis:** We predict that BF PV neurons transiently increase alertness to support short latency, goal-directed responses. **Methods:** We used brief (1s) tonic optogenetic excitation of BF PV neurons during an operant signal detection task that assesses sustained attention, the rodent psychomotor vigilance task (rPVT). The rPVT is a simple reaction time (RT) test that requires monitoring of a central stimulus location (lightbulb) for a brief and unpredictable signal (500ms light flash) and performance of an operant response (lever press) to report detection of the signal. The primary measures in the rPVT are RT and number of lapses in attention (omissions, failure to respond within a specified amount of time: 1500ms). **Results:** In N=3 mice, we found that 1s tonic BF PV excitation beginning 500ms prior to the 500ms signal quickens RT (Stim: 494 ± 33ms vs Baseline: 632 ± 20ms, p < 0.02). To control for nonspecific arousal (Ctrl Stim), we delivered 1s tonic BF PV excitation 5s after the outcome of each trial (correct or omission) during the intertrial interval (ITI: ~23s), which did not improve performance (Ctrl Stim: 603 ± 11ms vs Baseline: 632 ± 20ms, p > 0.09). **Conclusion:** Brief BF PV excitation prior to signals leads to a transient enhancement of attention. This improved performance is not due to nonspecific arousal. **Significance:** This is the first demonstration of a role for BF PV neurons in attention.


Poster

331. Attention

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 331.18/AA20

Topic: H.01. Animal Cognition and Behavior

Support: NIDCD R03
**Title:** Neuromodulatory- and prefrontal- sensory cortical interactions underlying motivated shifts in attentional effort

**Authors:** Z. H. MRIDHA, J. DE GEE, Y. SHI, H. RAMSAYWAK, A. BANTA, W. ZHANG, *M. J. MCGINLEY;*  
Baylor Col. of Med., Houston, TX

**Abstract:** Humans and other animals constantly adapt their allocation of cognitive resources to changes in the environment. In sensory domains, the brain is capable of enhancing the processing of difficult-to-perceive stimuli when they are important (Kahneman, 1973). In the context of perceptual decision-making, this is referred to as attentional effort (Sarter et al., 2006). In contrast to the extensive study of e.g. the selective aspect of attention (Desimone and Duncan, 1995), the neural circuit mechanisms of attentional effort are poorly understood. Key candidate mechanisms are direct neuromodulation of sensory cortex (Aston-Jones & Cohen, 2005), and bidirectional interactions of sensory and frontal cortical regions (Miller & Cohen, 2001). Here, we seek to determine the neural circuit basis of attentional effort. We developed an attentional effort (AE) task for head-fixed mice. In the AE task, mice lick for sugar-water reward to report detection of the unpredictable emergence of temporal coherence in an ongoing tone cloud, analogous to coherent motion in common visual attention tasks. Perceptual difficulty is parametrically and unpredictably varied, trial-by-trial, through partial degradation of the coherence. To manipulate attentional effort, we alternate between a large and small reward volume in blocks of 60 trials. Thus, mice are motivated to expend more attentional effort in blocks with large rewards. Increased attentional effort in high-reward blocks manifested as increased sensitivity (d’ from SDT) to detect coherence in noise (2-way rmANOVA F₁,₂₁ = 38.5; p < 0.0001; N=22 mice), particularly for weak-coherence targets (interaction F₂,₄₂ = 11.7; p < 0.0001). Mice exhibited >5 effort shifts within each session, tightly time-locked to block changes. In addition, contrary to the trivial prediction that high reward increases global neuromodulator levels (arousal), mice decreased their arousal in high reward blocks, apparent in baseline pupil size (p = 0.002). Thus global neuromodulation doesn’t account for attentional effort. Finally, feedback pupil responses exhibited multiple signals reflecting flexible AE regulation, including: correctness, reward context, and prediction errors. In ongoing 2-photon GCaMP imaging, we are determining the roles of frontal-sensory and neuromodulatory signals in mediating these shifts in overt behavior. 


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**Title:** Non-invasive trigeminal nerve stimulation effect on locus coeruleus response and autonomic nervous system changes

**Authors:** *J. C. TANNER*, S. I. HELMS TILLERY;

1SBHSE, 2Sch. of Biol. and Hlth. Systems Engin., Arizona State Univ., Tempe, AZ

**Abstract:** Neural activity in the midbrain structure locus coeruleus (LC) modulates attention, arousal, working memory, and stress. The structure consists of noradrenergic (NA) cells with widespread projections throughout cortex, cerebellum, and brainstem. Activation of NA modulates synaptic signal transmission for attention and/or sensory stimuli. Using direct (fMRI or PET) and indirect (pupillometry) measures, literature provides evidence that tasks requiring increased exploration/attention correlate with locus coeruleus activity. Being able to affect LC activity could provide access to modulate plasticity, arousal, or working memory. Modulating LC activity is possible through invasive vagus nerve stimulation, and parameters have been characterized for optimal effect. Both the vagus nerve and the trigeminal nerve have common direct projections and respectively project to the LC through the nucleus tractus solitarius and trigeminal nucleus caudalis. In this study, we aim to investigate the tonic and phasic components of LC response to non-invasive trigeminal nerve stimulation, determine optimal stimulation duration and parameters, and observe the effect on the autonomic nervous system via the pupillary light reflex (PLR). The latter model allows an independent and parallel measure of sympathetic and parasympathetic nervous activation and inhibition by measuring pupil constriction and redilation in response to repeated short bursts of light. Through both the LC recordings and the PLR, a parallel observation of the autonomic changes and neural changes can be seen in response to trigeminal nerve stimulation. At present, 10 minute stimulation periods have induced LC responses correlated with activation of the sympathetic nervous system. These responses have a consistent tonic component and a pseudo-phasic increase in activity to stimulation onset and offset, each lasting a few minutes. This leads to an interesting set of experiments where we attempt to harness the separate on-off phasic responses by cycling the stimulation in an attempt to maintain higher LC activity over longer periods of time.

**Disclosures:** J.C. Tanner: None. S.I. Helms Tillery: None.
Poster

331. Attention

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Program #/Poster #: 331.20/AA22

Topic: H.01. Animal Cognition and Behavior

Support: Saint Mary's College SISTAR Grant

Title: Development of a method for assessing divided attention in a rodent model of ADHD

Authors: *A. TWISSELMANN¹, T. M. AUBELE-FUTCH²;
²Psychological Sci., ¹St. Mary's Col., Notre Dame, IN

Abstract: There are four types of attention that allow individuals to focus and process the information available to them, one of which is divided attention. Also known as multitasking, divided attention describes the ability to maintain mental focus on two or more tasks at the same time. Divided attention is used often in humans’ everyday lives; however, the neuropsychological assessment of this type of attention in rodents is currently lacking. In fact, only one method has been published that assesses this specific cognitive task in rodents, (Arnold, Bruno and Sarter 2003), and that paradigm has never been used in the Spontaneously Hypertensive Rat (SHR) model of ADHD. Thus, here, we build off of this previously published method and adapt it for use with this widely accepted rodent model. Briefly, SHR rats and their controls (WKY) were trained on two operant rulesets, learning to discriminate a steady versus pulsing light, and a steady versus pulsing sound by pressing one of two available levers. After each ruleset was learned independently, all four options were presented randomly within a session. The previous method was adapted by changing the rate of light pulsing to accommodate the poor vision of albino rodents, and learning was scaffolded by the addition of corrective trials as animals learned the original rulesets. After these methodological adaptations, two rats (one SHR and one WKY animal) were able to complete the entire paradigm, confirming that the revised method does indeed work. Since such a small number of animals were able to complete the protocol, parametric statistics were difficult to perform; however, assessments within individual rodents showed expected trends in response latency. The development of this paradigm will allow for future research into this attentional realm in the ADHD model animal.

Disclosures: A. Twisselmann: None. T.M. Aubele-Futch: None.
Title: Evaluating “productivity” vs “quality” of performance in rodent assays of goal-directed behavior and cognitive function

Authors: *R. L. NAVARRA¹, C. P. KNAPP¹, D. P. FOX¹, S. B. FLORESCO², B. D. WATERHOUSE¹; ¹Cell Biol. and Neurosci., Rowan Univ. Sch. of Med., Stratford, NJ; ²Univ. British Columbia, Vancouver, BC, Canada

Abstract: A wide variety of neuropsychiatric disorders and brain insults impair aspects of cognitive function. Psychostimulant drugs sometimes restore these processes in models of compromised brain function as well as improve baseline performance in otherwise healthy animals. Sophisticated behavioral assays for use in rodents have been developed to characterize and understand experimentally induced manipulations across multiple dimensions of cognitive performance. Standard measures include accuracy and response latency; however, there are dynamic components of sensory processing, decision making, and motor output that contribute behind the scenes to the behavioral outcomes we are able to quantify in these tasks. The present data exemplify improvements or impairments in response latency following either methylphenidate (MPH) administration or repetitive mild traumatic brain injuries (rmTBI), respectively, without concomitant effects on traditional measures of performance accuracy. First, MPH improved reaction times to make correct responses without affecting overall accuracy during performance of a signal detection task. MPH also reduced latency of visually evoked activity recorded in response to visual cues used to guide performance of the task. Improvements in reaction speed were correlated with faster visually evoked responses, suggesting that enhanced sensory processing was a significant component of the observed enhancement of behavioral performance. Second, in an operant strategy shifting task, MPH selectively improved reaction times on trials where animals made the correct choice without broadly affecting overall accuracy or trials to reach criterion. Lastly, following rmTBI (three closed skull controlled cortical impacts over a period of one week), injured animals demonstrated slower reaction times as compared to sham controls. Impaired reaction times observed following rmTBI were independent of whether animals made correct or incorrect choices, indicating a non-selective
impairment of processing speed and response latency. Numerous factors may impede the ability to detect effects on accuracy of performance, but still reveal important information about productivity vs quality of performance. Together, these data beg further consideration of whether manipulating the speed of information processing and behavioral output are sufficient to contribute meaningful and translationally relevant information towards further understanding alterations in productivity of performance.


Poster

331. Attention

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 331.22/AA24

Topic: H.01. Animal Cognition and Behavior

Support: NIMH MH105592

Title: Involvement of a locus coeruleus-to-prefrontal (LC-mPFC) circuit in a touchscreen variant of the continuous performance test (CPT) in mice

Authors: *H. L. HALLOCK*¹, A. C. DEBROSSE¹, M. NOBACK², H. M. QUILLIAN, IV¹, J. C. BARROW³, G. V. CARR⁴, K. MARTINOWICH⁵;
¹The Lieber Inst. for Brain Develop., Baltimore, MD; ²Johns Hopkins Sch. of Med., Baltimore, MD; ³Lieber Inst. for Brain Develop., Lieber Inst., Baltimore, MD; ⁴Drug Discovery, ⁵Lieber Inst. For Brain Develop., Baltimore, MD

Abstract: Sustained attention is disrupted in a variety of neuropsychiatric disorders, including schizophrenia and attention deficit hyperactivity disorder (ADHD). Much research suggests that interregional communication between subcortical brain areas (i.e., basal forebrain, ventral tegmental area, locus coeruleus) and the PFC may contribute to attention-directed behavior. The exact role of these circuits in sustained attention, however, remains unclear. In humans, the continuous performance test (CPT) is a neuropsychological tool that is frequently used for evaluating sustained attention. In the human CPT, subjects attend to a visual cue on a screen, and either respond or inhibit responding depending on the type of cue that is presented. In order to understand how distinct frontal-subcortical circuits participate in sustained attention and improve translatability of results from rodents to humans, we trained mice to perform a touchscreen-mediated variant of the CPT. In this CPT variant, mice are trained to make a nose poke following the presentation of one visual cue (S+), and inhibit responding following the presentation of a second visual cue (S-). Our previous research has shown that systemic administration of the catechol-O-methyl transferase (COMT) inhibitor tolcapone improves performance of the task in
mice, implicating dopaminergic function in successful CPT performance. To investigate how PFC function contributes to the CPT, we imaged calcium dynamics in medial PFC (mPFC) neurons during CPT performance with a miniature head-mounted microscope. To further understand which frontal-subcortical circuits mediate the CPT, we injected a retrograde virus encoding a fluorescent reporter (tdTomato) into the mPFC of wild-type (w/t) mice, and compared expression of the immediate early gene (IEG) c-Fos in tdTomato+ cells between mice that learned to discriminate the S+ and S-, and mice that only learned to respond to a visual cue for a reward (yoked controls). We found that projection neurons from the locus coeruleus to the mPFC (LC-mPFC projectors) were selectively recruited during CPT performance. Current research focuses on identifying a molecular signature of LC-mPFC projectors, and understanding how these neurons contribute to mPFC population activity during the CPT.


Poster

331. Attention

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 331.23/AA25

Topic: H.01. Animal Cognition and Behavior

Title: Stable cognitive computations in the prefrontal cortex of unrestrained monkeys

Authors: *C. TESTARD1, S. TREMBLAY2, M. PETRIDES3;

Abstract: Neurophysiologists studying the primate caudal lateral prefrontal cortex (area 8A) using standard visual-saccadic paradigms have mostly described neurons encoding visual-attention and eye movement signals in this area. However, we know from lesion studies that bilateral ablation of area 8A produces no deficits in basic visual attention nor in eye movement control. In fact, bilateral lesions of area 8A result only in a specific impairment in using “if-then” instructional cues to select visual objects from the environment. We believe that this discrepancy between neuropsychological (i.e. lesion) and neurophysiological evidence is the result of the low ecological validity of standard visual-saccadic paradigms ubiquitous in monkey neurophysiology. Although these paradigms are useful to study visual areas of the brain, we posit that they offer a limited portrait of higher-order associative areas such as the prefrontal cortex. In the current study, we introduce a new paradigm that combines multi-electrode neurophysiology in monkeys with ecologically valid cognitive testing. By performing intracranial multi-electrode recordings in monkeys that are unrestrained in their head, eye and arm movements, we show that neural ensemble activity in area 8A encodes the cognitive process predicted by lesion studies.
Importantly, we show that this cognitive representation is stable even while the monkey is freely moving and looking around. We expect that this paradigm will provide a more accurate portrait of the neuronal properties of associative areas.

**Disclosures:** C. Testard: None. S. Tremblay: None. M. Petrides: None.

**Poster**

**332. Decision Making: Medial Prefrontal Cortex**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 332.01/AA26

**Topic:** H.01. Animal Cognition and Behavior

**Support:** Wellcome Trust Investigator Award to TWR (104631./Z/14/Z/)

**Title:** Controlling the balance between goal directed and habitual behaviour: Parsing the contribution of primate medial and orbital prefrontal cortex sub-regions and the caudate nucleus

**Authors:** *L. Y. DUAN*¹,⁴, N. K. HORST¹,⁴, S. A. W. JACKSON¹,⁴, N. HORIGUCHI¹,⁴, R. N. CARDINAL²,⁴, T. W. ROBBINS¹,⁴, A. C. ROBERTS³,⁴;¹Dept. of Psychology, ²Dept. of Psychiatry, ³Dept. Physiology, Develop. and Neurosci., Univ. of Cambridge, Cambridge, United Kingdom; ⁴Behavioural and Clin. Neurosci. Institute, Univ. of Cambridge, Cambridge, United Kingdom

**Abstract:** Normal behaviour can either be goal-directed, when performing an action to obtain a specific goal, or they can become habitual, where a stimulus in the environment can trigger a response regardless of the outcome. An extreme form of this habitual responding may hypothetically be seen in obsessive-compulsive disorder (OCD) patients. Degrading contingencies between responses and their outcomes challenges beliefs about cognitive control and can be used to distinguish between behaviour that are goal-directed or habitual. Given that in OCD patients the prefrontal cortex (PFC) and prefronto-striatal circuits are dysfunctional, it is important to determine the causal role of these regions in controlling the balance between goal-directed and habitual behaviour. Thus, we compared the roles of the primate anterior cingulate cortex, BA32, 24 and 25, anterior orbitofrontal cortex (OFC, BA11), medial OFC (BA14) and the caudate nucleus (CN) in mediating effects of contingency degradation. We developed a touchscreen-based contingency degradation task for the common marmoset, a New World non-human primate that is a key link bridging rodent and human studies as it has a PFC more similar to humans than rodents. Trained marmosets were implanted in two brain regions with chronic, indwelling cannulae. We then examined the effects of reversible pharmacological inactivation (using GABA A/B agonists, muscimol/baclofen) or activation (using a glutamate transport blocker, dihydrokainic acid (DHK) to enhance extracellular glutamate levels) of each region on
the ability to adapt responding after degradation of action-outcome contingencies. Results showed that inactivation of either BA24 or CN impaired, while BA11 apparently enhanced, animals’ sensitivity to contingency degradation. In contrast, manipulations of BA14 or BA32 did not affect sensitivity. In summary, BA24 and CN are critical for the expression of action-outcome associations, while other frontal regions such as BA11, BA14 and BA32 are not required for their expression. The apparent facilitatory effect of BA11 inactivation may be understood in terms of a dysregulation of motivational arousal. Based on a careful analysis of the response patterns across degraded and non-degraded sessions the differential contribution of these distinct regions to goal directed actions will be discussed.


Poster

332. Decision Making: Medial Prefrontal Cortex

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 332.02/AA27

Topic: H.01. Animal Cognition and Behavior

Support:  Center for Neurotechnology, NSF EEC-1028725
         NIH Grant NS078127
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Title: Laminar profile of context-dependent computation in dorsomedial frontal cortex

Authors:  *H. SOHN*¹, M. JAZAYERI²;
         ¹McGovern Inst. for Brain Res., ²Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Laminar organization of neocortex is a key feature of cortical microcircuitry, providing a structured scaffold for neural computation. Recent technical developments have opened the possibility of simultaneous recording from multiple cortical layers allowing investigators to dissect the underlying circuitry. For example, laminar recordings in early sensory areas have revealed how bottom-up sensory inputs entering intermediate layers gradually propagate to the deep and superficial layers for further processing. In contrast, little is known about the role of laminar information processing in higher brain areas that are involved in cognitive computations.

We tackled this question by analyzing the laminar profile of neural activity in the dorsomedial
frontal cortex (DMFC) of monkeys during a context-dependent time reproduction task. In the task, animals measured a sample interval demarcated by two visual flashes ("Ready", "Set") and had to reproduce that interval immediately after the second flash using a motor response ("Go"). In each trial, the sample interval was randomly drawn from a uniform prior distribution. Animals performed the task in two contexts, a "Short" context where the interval varied between 480 and 800 ms, and a "Long" context where the interval was between 800 and 1200 ms. The context was cued at the beginning of each trial allowing animals to adjust their prior expectations. Animals learned to perform the task and their responses exhibited a characteristic regression to the mean for both contexts, indicating that they relied on their prior belief about the sample interval to perform the task.

In DMFC, the firing rate of a large proportion of neurons (>60%) was modulated by the prior context, and the strength of the modulation increased in anticipation of the Set flash. These dynamic contextual modulations could either reflect modulations of inputs to DMFC or result from recurrent interactions within DMFC. Given the laminar organization of inputs to DMFC, we reasoned that these modulations would exhibit laminar specificity if they were inherited from upstream areas. In contrast, a lack of laminar specificity would suggest that contextual modulations are supported by local recurrent interactions in DMFC. We analyzed how context-dependent modulations in DMFC change as a function of cortical depth. Although the magnitude and dynamics of contextual modulations were highly heterogeneous across the population, they did not seem to exhibit any laminar specificity. These preliminary results suggest that contextual modulation of neural activity are largely dependent on recurrent interactions within the frontal cortex.

**Disclosures:** H. Sohn: None. M. Jazayeri: None.

**Poster**

**332. Decision Making: Medial Prefrontal Cortex**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 332.03/AA28

**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIH Grant MH108643

**Title:** Effects of ketamine on prefrontal cortex activity during decision making in macaques

**Authors:** *M. OEMISCH*, A. F. ARNSTEN, D. LEE, H. SEO; Neurosci., Psychiatry, Yale Sch. of Med., New Haven, CT

**Abstract:** In everyday life, we make a plethora of decisions, in which we process multiple options and then choose an optimal course of action. Across many choices, we tend to repeat actions that have previously led to a positive outcome, and tend to refrain from repeating actions
that have previously led to negative outcomes. One psychiatric disorder in which decision-making behavior is often disrupted is depression. Individuals with depression show for instance a diminished tendency to base their decisions on the likelihood of obtaining rewards, and they show hypersensitive behavioral and electrophysiological responses to losing. How current antidepressants may alleviate this type of behavior is not fully understood. Here we set out to elucidate the effects of the rapid-acting antidepressant ketamine on neural activity in prefrontal cortex. We recorded single unit activity in dorsomedial prefrontal cortex (dmPFC) of macaque monkeys performing a biased matching pennies task. In each trial, monkeys chose one of two targets, which led to either the gain of a token, the loss of a token, or no change in token number. Any time the monkey had collected six tokens, these were exchanged for juice reward. We found that a low dose (0.5mg/kg) of ketamine affected the monkeys’ choices selectively following loss and neutral outcomes, but not following gain outcomes. Specifically, monkeys were less likely to switch away from a target that had led to a loss or neutral outcome under ketamine compared with control conditions. In line with this change in behavior, we found that many dmPFC neurons changed their spiking activity following ketamine injections. We found that 60% of task-modulated neurons changed their firing rate modulation with ketamine, with most showing a decrease in their modulation strength. These neurons were most prominently those that encoded, at the time of feedback, (a) whether the animal was gaining a token or not, or (b) the number of tokens to be collected until the next reward, suggesting that the disruption of these signals may relate to the change in behavior observed with ketamine. Overall, we found that ketamine may mitigate negative valuations of aversive outcomes, in line with its antidepressant actions and thereby lead to selective changes in choice behavior. We found evidence that neurons in dmPFC may contribute to mediating this change in behavior, allowing further insights into the neural basis of ketamine’s actions in the brain.

Disclosures:  M. Oemisch: None. A.F. Arnsten: None. D. Lee: None. H. Seo: None.

Poster

332. Decision Making: Medial Prefrontal Cortex

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 332.04/AA29

Topic: H.01. Animal Cognition and Behavior

Title: Exploration of the eligibility trace as a link between choice and temporally-delayed feedback

Authors: *S. K. MURRAY¹, D. LEE², H. SEO³;
¹Interdepartmental Neurosci. Program, Yale Univ., New Haven, CT; ²Neurosci., ³Psychiatry, Yale Sch. of Med., New Haven, CT
Abstract: Theoretical and empirical work has demonstrated that in uncertain environments, humans and other animals learn desirable actions through experience by iteratively updating the estimates of long-term reward from alternative actions (i.e. action values) with unpredicted outcomes; these estimates are used to select the action that maximizes expected reward. In the natural world, outcomes are often separated in time from their causative actions by long and variable delays. Reinforcement learning theory (RL) postulates that an exponentially decaying memory trace of chosen actions, called the eligibility trace (ET), can bridge temporal gaps for delayed outcomes. However, it remains unknown whether and how ET might be implemented in the brain. Previous work has shown that neurons in primate prefrontal cortex (PFC) exhibit mnemonic signals representing past choices and their outcomes. For example, signals related to previous actions and choices encoded by individual neurons tend to decay exponentially, with heterogenous time constants. In some contexts, these signals are dynamically reactivated around the time of choice on the subsequent trial. Although cortical memory traces for past choices may provide a neural ET, whether those mnemonic signals can bridge long and variable temporal delays between choice and its outcome has not been rigorously tested. It is also unclear how the temporal dynamics of mnemonic signals might contribute to the update of the values of actions with delayed outcomes. In order to understand the role of choice memory in reinforcement learning, we trained a rhesus monkey on a variant of the computer-simulated matching pennies task, in which the feedback for each choice was delivered after variable temporal delays. We independently manipulated the interval between each choice and its feedback (choice-feedback interval, CFI) and the interval between feedback and each subsequent choice (inter-trial interval, ITI). Each of these time intervals was either 1 or 5s, with both CFI and ITI randomly and independently drawn on each trial. Using logistic regression, we analyzed how choice and outcome affected the animal’s subsequent choice as a function of CFI and ITI. We found that both CFI and ITI independently affected the animal’s tendency to adopt a win-stay-lose-switch (WSLS) strategy. Specifically, the probability of WSLS decreased with both CFI and ITI length (t-test, p<.01 for both), suggesting that decaying ET might be involved during reinforcement learning from delayed outcomes. We will examine the dynamics of neural activity in PFC that mediate this effect of temporal delay in learning, a potential instantiation of ET.


Poster

332. Decision Making: Medial Prefrontal Cortex

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 332.05/AA30

Topic: H.01. Animal Cognition and Behavior

Support: Fondation pour la Recherche Médicale Grant #DEQ20160334905
labex CORTEX ANR-11-LABX-0042
Title: Functional signatures and intrinsic timescales of single unit types in the midcingulate and lateral prefrontal cortex

Authors: *E. PROCYK*¹, F. M. STOLL², V. FONTANIER¹;
¹Stem Cell and Brain Res. Inst., Inserm U1208, Lyon, France; ²Dept. of Neurosci., Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: Fluctuation in spiking activity is a characteristic of neurons across cortical areas. The timescales on which such fluctuation operates are likely shaped by multiple factors including membrane properties, local network and synaptic properties as well as large scale network architecture. Prefrontal regions such as the lateral prefrontal cortex (LPFC) and especially the midcingulate cortex (MCC) exhibit the longest timescales across primate cortical areas. Long timescales, indicative of a stable activity state across time, could contribute to integration over long time periods, a fundamental feature of functions like decision making and working memory. We recently showed different properties of activity during exploratory decisions and integration of information across trials in MCC vs LPFC (Stoll, Fontanier and Procyk 2016 Nat. Comm). Here we assess whether those functional differences relate to different single spike dynamic properties.

To describe intrinsic timescales we computed between-trials autocorrelograms of spiking activity as well as classical spike autocorrelograms of individual MCC and LPFC units (251 MCC units and 248 LPFC units) recorded in awake monkeys engaged in a cognitive task. Because area intrinsic timescales might reflect properties of local dynamics and excitation/inhibition balance in local networks, we tested whether single unit types - fast or regular spiking clustered based on spike shape- could reveal differences between MCC and LPFC.

We first replicated findings showing that MCC units have longer timescales than LPFC on average. Moreover we found that the MCC autocorrelogram temporal signatures differ from LPFC in particular for regular spiking units. However, cross-correlograms between single unit types did not reveal any particular functional difference between the two regions. Additionally, we found that populations of units with short or long average intrinsic timescales contribute differently to information processing at short (post-feedback adaptation) or long (across trial information integration) time scales.

In conclusion, different spiking dynamics in MCC and LPFC reveal intrinsic properties that contribute to functional difference between the two frontal regions.

Disclosures: E. Procyk: None. F.M. Stoll: None. V. Fontanier: None.

Poster

332. Decision Making: Medial Prefrontal Cortex

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 332.06/AA31
Title: The causal role of midcingulate cortex in the regulation of decisions to check

Authors: *V. FONTANIER¹, F. M. STOLL², E. PROCYK¹;
¹INSERM U1208 - Cell and Brain Res. Inst., Lyon, France; ²Dept. of Neurosci., Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: Recent theories argue that midcingulate cortex (MCC) dynamically tracks the value of options to guide behaviour away from default or routine actions, and explore alternatives. Yet MCC causal contribution to behaviour is still subject of intense debates. Interestingly the anterior part of MCC includes cingulate motor areas with somatomotor maps. The role of these maps to exploratory decisions remains to be uncovered.

We designed a so-called checking-task in which monkeys, while performing a default categorization task, can at will seek information (provided by a visual gauge increasing in relation to categorization performance) on an incoming reward bonus. In this task, monkeys’ checks are positively modulated by positive categorization outcome and increasing gauge. We performed single unit and LFP recordings in MCC and dLPFC in two monkeys doing the task (Stoll, Fontanier and Procyk 2016 Nat. Comm), and showed specific MCC neural dynamics for feedback processing, checking decisions, as well as encoding of gauge size. We here test the causal role of MCC in this task.

We performed pharmacological perturbations in the MCC where we observed neural activity related to checking using either a GABA agonist (multiple injections of 2 µL of muscimol at 10,15 or 20 µg/µL) or a GABA antagonist (multiple injections of 2µL of bicuculline methiodide at 15 µg/µL) (1 monkey, 16 muscimol sessions, 8 bicuculline sessions). We assessed effects on checking frequency, checking patterns, RTs, oculomotor patterns and saccade frequency, performance in categorization, etc.

MCC muscimol inactivation altered the frequency of checking but did not change the probability of checking after positive vs negative outcomes. Bicuculline in particular altered the pattern of checking in relation to gauge increase. Oculomotor patterns indicate that part of the deficit might be related to information gathering at the time of gauge observation.

In conclusion, combined electrophysiological and pharmacological data suggest a key role of the MCC in the maintenance and updating of information used to regulate exploratory decisions.

Disclosures: V. Fontanier: None. F.M. Stoll: None. E. Procyk: None.
Prospective goal encoding in monkey OFC-RSC circuit in a 3D virtual reality foraging task

Authors: *M. WANG¹, B. Y. HAYDEN²;
¹Neurosci., ²Univ. of Minnesota, Minneapolis, MN

Abstract: During natural foraging, animals exhibit goal-directed behavior to learn the structure of the environment and obtain reward. Prospective goal state representation and navigational planning are essential to this process. Although neural activities for route planning have been shown in hippocampus, the neural underpinnings for the learning and use of goal state encoding, which potentially informs hippocampal route-planning activities, is still unknown. We hypothesized that reward simulation, i.e. reactivation of reward state in neural populations, is the key to prospective goal state representation. We developed a 3D virtual reality foraging task to test this hypothesis. In this task, a jackpot reward is hidden at a random location in the virtual maze (goal location) for each session, while other maze configurations stay the same across all sessions. On each trial, the subject is teleported to another random location (start location) during the inter-trial interval. The subject then navigates through the virtual maze with a joystick to forage for the hidden jackpot reward. Subjects took significantly longer search time (from start to goal location) and less efficient travelled paths during pre-learning than during post-learning trials. We simultaneously recorded around 40 single-units in each of orbitofrontal (OFC) and retrosplenial (RSC) cortices across a minimum of 50 trials for each session. We defined goal state as population activity pattern containing averaged firing rates from each neuron across 1500 ms at goal locations during jackpot reward receipt. Representational similarity and state-space analyses reveal that: (1) goal state representation was prospectively reactivated at start of the navigation; (2) it was reactivated during navigation, after but not before, the learning of hidden jackpot location; (3) goal state representation across trials was stable in OFC but correlated with learning in RSC. We further investigated: (1) the correspondence between decision points during navigation and goal state reactivation; (2) the correlations among the behavioral indications of learning, the population dynamics in OFC, and the population dynamics in RSC.

Disclosures: M. Wang: None. B.Y. Hayden: None.
Abstract: Reward-based decisions are prevalent in our daily life. To choose optimally, we sometimes need to take into account multiple attributes of the prospective outcome and generate an estimate of the reward value over all attributes. Alternatively, sometimes we need to ignore certain attributes of the prospective outcome and heuristically and strategically use information from a selective set of attributes. This flexibility is crucial to successful decision-making and yet the neurocomputational basis for such flexibility in evaluation and decision-making, and the dynamics of information represented in reward circuits during such process remain largely unknown. To address this problem, we trained two rhesus macaques on a simple gambling task. Each trial started with a 1000ms inter-trial interval, followed by the visual presentation of gamble1 for 500ms and a delay with blank screen for 500ms. Gamble2 then was visually presented for 500ms followed by another delay of 500ms with blank screen. After a brief eye fixation, both gambles were visually presented and monkeys made their choice between gamble1 and gamble2 by directing a saccade toward the chosen gamble and maintaining fixation on the choice for 200ms. Following choice, the outcome of the chosen gamble was visually revealed for 800ms while monkeys experienced the gamble outcome: a winning gamble resulted in water reward delivery and a losing gamble resulted in no reward delivery. Each gamble was presented explicitly with two distinct attributes: reward magnitude and reward probability. Reward magnitude came in three sizes: 125, 165, 250 uL. Reward probability follows a uniform distribution ranging from 0-1 with 0.01 step size. During the gambling task, we recorded simultaneously in both area 13 of orbitofrontal cortex and area 23/31 of posterior cingulate cortex. To examine the neural population dynamics on a trial-by-trial basis, we simultaneously held ~50 single-units in each of OFC13 and PCC across each session with a minimum of 500 trials. We found that monkeys understood the task and showed consistent preference for gambles with larger expected values in over 80% of the trials. We found that OFC13 and PCC neurons dynamically encoded each attribute (reward magnitude and probability) and expected values (= magnitude x probability) simultaneously on both single-unit and population decoding level. With
representational similarity and state-space analyses, we examined the encoding and decoding dynamics for each attribute and the expected value in relation to trial-by-trial task/behavioral parameters. We also investigated the information flow and population dynamics correlations between OFC13 and PCC.

**Disclosures:** H. Lee: None. M. Wang: None. B.Y. Hayden: None.

**Poster**

332. Decision Making: Medial Prefrontal Cortex

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program#/Poster #:** 332.09/AA34

**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIH R01 DA037229

NIDA T32

**Title:** Dorsal anterior cingulate cortex encodes cognitive control signals in a value-based conflict task

**Authors:** *P. MEHTA, B. Y. HAYDEN;* Univ. of Minnesota, Minneapolis, MN

**Abstract:** A lot of a good thing, or a little of a great thing: the most difficult decisions often involve comparing two options that have both positive and negative attributes. Weighing the relative pros and cons of one option causes us to experience “conflict”: to hold two competing mental representations of the option. Numerous human functional magnetic resonance imaging (fMRI) studies report that the brain’s dorsal anterior cingulate cortex (dACC) encodes the presence of conflict during behavioral tasks. However, most electrophysiological studies of macaque dACC neurons report no such signatures of conflict. It remains unclear whether the incompatibility of these results is due to a difference in species (humans versus non-human primates), methodology (fMRI versus electrophysiology), or something different. Explaining this difference is crucial both for legitimizing the widespread application of macaque neurophysiological data to human neurophysiology, and for facilitating comparisons between imaging and electrophysiological data. We hypothesize that the differences between human fMRI and macaque electrophysiology results are at least partially due to a difference in the kinds of tasks used to test conflict encoding between species. To emulate the more abstract nature of human conflict tasks, we have designed a novel “value-conflict” task in which macaque subjects must select between large amounts of a small reward and small amounts of a large reward. Here, we find single neurons in dACC whose firing rates differ contingent upon the presence of conflict within a trial. We also find encoding of error monitoring, task difficulty, and other variables related to decision-making. We conclude that cognitive control signals are present
within macaque dACC. On a broader level, our conclusions will not only inform the conflict literature but also facilitate interpretation of translational research that is performed in macaques but applied to humans.

**Disclosures:** P. Mehta: None. B.Y. Hayden: None.

**Poster**

332. Decision Making: Medial Prefrontal Cortex

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 332.10/AA35

**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIH R01 DA037229

**Title:** Suboptimal decision making in macaques during low reward trials

**Authors:** *K. EDMONSTON*¹, P. MEHTA³, B. R. EISENREICH⁴, B. Y. HAYDEN²;
¹Neurosci., ²Univ. of Minnesota Twin Cities, Minneapolis, MN; ³Neurosci., ⁴Univ. of Minnesota, Minneapolis, MN

**Abstract:** Neuroeconomics seeks to better understand the mechanisms of decision-making processes in the brain, which have commonly been analyzed in contexts like gambling and delay discounting. Foraging theory emphasizes the importance of developing a decision making strategy to fit the environment, and being in a deprived environment may warrant searching for a new environment to forage in. Previous studies have used macaques (Macaca mulatta) as a model to examine decision making behaviors through tasks that involve choosing between two risky choices. In our task, macaques were presented with two possible reward values associated with a dimension of probability and stakes. Gambles were chosen randomly, so by chance some trials had two low values. We call these low reward trials. During low reward trials, macaques exhibit suboptimal behaviors such as time wasting and longer reaction times. Time wasting involved physical frustration and lack of focus. Furthermore, their ability to choose the larger reward drops close to chance. Interestingly, when compared to trials with at least one high reward, the macaques made significantly worse decisions. These behaviors imply that low reward trials significantly impair decision making in the task. According to foraging theory, tasks should reflect naturalistic environments and in naturalistic environments macaques can walk away from a decision. Low reward trials may represent a deprived environment, which is unfavorable for the macaques to be in. Therefore, we next examine macaques’ choices during this task with the option of completely skipping trials. Adding this flexibility in the decision-making process for macaques more accurately reflects natural situations. This would result in easier analysis of the brain in decision-making tasks as it was evolved to process these decisions in natural contexts.
**Disclosures:**  

**Poster**

**332. Decision Making: Medial Prefrontal Cortex**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 332.11/AA36

**Topic:** H.01. Animal Cognition and Behavior

**Title:** Encoding of spatial information in orbitofrontal cortex neurons

**Authors:** *H. AZAB*¹, S. M. YOO¹, V. B. MCGINTY², B. Y. HAYDEN¹;  
¹Univ. of Minnesota, Minneapolis, MN; ²Ctr. for Mol. and Behavioral Neurosci., Rutgers Univ. - Newark, Newark, NJ

**Abstract:** The orbitofrontal cortex (OFC) is a core element of the brain’s decision-making system whose contribution to cognition remains to be determined. Recent work supports the idea that OFC representations are not limited to economic parameters, but instead include a detailed representation of the structure of the task environment. Consistent with this idea, a recent study (McGinty, Rangel, and Newsome, Neuron, 2016) indicates that OFC activity is sensitive for the locus of gaze relative to the position of salient (reward-predicting) objects in the visual scene. This result suggests that OFC may have a richer and more complex representation of gaze information, and raises the question of whether this representation is tethered to salient items in the environment. Here we performed a new analysis on that previously collected data set to answer this question. In the task used in that study, visual cues predicting specific reward amounts were displayed on a computer monitor. Macaque subjects were free to direct their gaze anywhere on the monitor during the display period. Rewards were given regardless of the subject’s behavior. We used a recently-developed generalized linear modeling (GLM) approach that was designed to characterize spatial maps in rat entorhinal cortex (Hardcastle, Ganguli, Maheswaranathan, and Giocomo, Neuron, 2017). We find that a significant proportion of neurons (23%) show spatially-selective responding depending on gaze position, and a similar proportion of neurons (22%) were selective to reward. Receptive fields for gaze position generally included a single peak, located at different locations of the gaze field for different neurons. Information about these two variables was often encoded in the same neurons. The pattern of modulation for individual neurons was often non-linear, which may be indicative of mixed selectivity. These results indicate that reward and non-reward information are simultaneously encoded in OFC neurons, and may not be encoded independently.

**Disclosures:**  
H. Azab: None. S.M. Yoo: None. B.Y. Hayden: None. V.B. McGinty: None.
Poster

332. Decision Making: Medial Prefrontal Cortex

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 332.12/AA37

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant DA038615

Title: Single trial anterior cingulate population dynamics predict ensuing decision-related premotor cortex activity and behavior

Authors: *T. CASH-PADGETT1, B. Y. HAYDEN2;
1Univ. of Minnesota, Minneapolis, MN; 2Univ. of Minnesota Twin Cities, Minneapolis, MN

Abstract: The neural computations underlying cognition and behavior are carried out through ongoing, dynamic interactions between brain areas. A key challenge in understanding these interactions is determining which components of population activity play a causal role in information processing. To investigate how specific population dynamics contribute to the neural computations underlying value-based decision making, we recorded from dorsal anterior cingulate cortex (dACC) and dorsal premotor cortex (PMd) while subjects performed a risky decision-making task. Using non-linear recurrent neural networks, we trained sequential autoencoders to infer the latent factors underlying single-trial activity in each area. We then examined how these reduced-dimensionality population trajectories related to one another over time, using linear regression.

To examine potential signatures of inter-area communication, we regressed PMd activity against dACC population trajectory over a range of temporal offsets. Activity in dACC was most strongly predictive of PMd activity that followed it by approximately 60 - 100 ms. On trials in which the subject made an inaccurate choice (by choosing the less valuable of the two presented offers), PMd activity was significantly more dependent on dACC activity. DACC population trajectory displayed a transient, bidirectional relationship with PMd activity immediately preceding the choice epoch of inaccurate-choice trials, while there was no modulation on accurate-choice trials. Specifically, choice accuracy was well explained by the extent to which sharper deflections in the dACC trajectory predicted changes in subsequent PMd activity. This effect was only observed at temporal offsets from 60 to 100 ms.

The relationship between dACC and PMd population activity also predicted which offer would be chosen. Early in the offer-2 epoch, dACC trajectory magnitude was associated with an increase in PMd activity on trials in which offer 1 was chosen and a decrease in PMd activity on trials in which offer 2 was chosen. Interestingly, while this modulation was significantly predicted by offer 2 value, it was not significantly predicted by offer 1 value, suggesting an asymmetric decision process. The effect was only observed at temporal offsets of 60 and 80 ms.
These results are consistent with the idea of a hierarchical relationship in which dACC exerts control over PMd.

Disclosures:  T. Cash-Padgett: None. B.Y. Hayden: None.

Poster

332. Decision Making: Medial Prefrontal Cortex

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 332.13/AA38

Topic: H.01. Animal Cognition and Behavior

Support: NIH R01 DA037229

Title: Investigation of economic decisionmaking in macaques performing delaybased foraging task

Authors: *A. SURI1, B. R. EISENREICH2, T. CASH-PADGETT2, B. Y. HAYDEN1;  
1Univ. of Minnesota Twin Cities, Minneapolis, MN; 2Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Within the natural environment, animals face a critical challenge in balancing the costs and benefits of searching for and consuming different food sources, or more generally rewards. Foragers must decide between what is and what could be. Often this involves making sequential decisions to either accept what is currently available or to continue searching for better offers. By contrast, traditional studies of animal choice behavior often use simultaneous presentations and formats that fail to reflect the natural transitions from searching to consuming rewards. We designed a task based on the restaurant row paradigm (Steiner and Redish, 2014) adapted for non-human primates that allowed for the study of choice behavior as it evolves over transitions from searching to consuming. In brief, this task involves encountering sequential offers that involve a delay to reward and offer amount. Taking advantage of this paradigm we examine economic decision-making behavior as rhesus macaques make wait/skip decisions based on pseudo-randomly varied delay times required to receive the reward. In total, the subjects travel through a series of four different reward zones or ‘restaurants’, each of which contains an ‘offer’ and ‘wait zone’. When a subject approaches the entrance of each restaurant (the ‘offer zone’) it views a delay. This length of time is signaled on a screen and only made visible when the subjects are in close proximity to the feeder. At this point, the subject could choose whether to stay or go. When a subject chose to stay, it proceeded to enter the ‘wait zone’ where it must stay the entirety of the delay in order to receive a reward. Rewards were consistent within a session for each restaurant despite the variation in delay times and the total session time was a set duration. We trained macaque subjects (n=2) on the task. Our analysis of subject behavior revealed that subjects are more likely to accept a longer delay when they had previously
skipped a shorter one and traverse faster to the next restaurant based on whether or not they had previously skipped a smaller delay restaurant. Another result found that lingering time was significantly predicted by the delay. Higher lingering times were associated with longer delays. These results provided tentative evidence that monkeys utilize recent past experience when making new choices.

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Poster

332. Decision Making: Medial Prefrontal Cortex

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 332.14/AA39

Topic: H.01. Animal Cognition and Behavior

Title: Neurons in the pigeon nidopallium caudolaterale, but not the corticoidea dorsolateralis display value and effort discounting related activity

Authors: *M. DYKES, B. S. PORTER, M. COLOMBO;
Psychology, Univ. of Otago, Dunedin, New Zealand

Abstract: There is a growing body of literature finding that the pigeon nidopallium caudolaterale (NCL) is the anatomical and functional equivalent of the mammalian prefrontal cortex (PFC). We recorded from single neurons in two areas of the pigeon brain while birds were required to peck a stimulus indicating that either a high effort task or a low effort task would follow. Upon completion of the task, the birds received the same reward. We recorded from the NCL in four pigeons, and from the corticoidea dorsolateralis (CDL) in two pigeons. We found that activity in the NCL was modulated by the value of the reward that would be received based on how much effort was required to obtain it. The pattern of firing we saw was similar to that observed in the mammalian anterior cingulate cortex (ACC). Value coding was most prominent during the presentation of the stimulus indicating a high or low effort task, and in the delay period immediately prior to carrying out the effort task. In contrast, activity in the CDL was not modulated by value; however, population firing patterns suggest that it may be involved in associating actions with outcomes. Our findings support the view that activity in NCL reflects value of reward as a function of effort discounting and as such may serve functions similar to the mammalian anterior cingulate cortex.

Disclosures: M. Dykes: None. B.S. Porter: None. M. Colombo: None.
**Title:** Intracellular determinants of CA1 pyramidal cells activation or silencing during locomotion

**Authors:** F.-X. MICHON, G. MARTI, C. FILIPPI, R. BOURBOULOU, J. KOENIG, *J. EPSZTEIN; INSERM U1249/Aix-Marseille University/INMED, Marseille, France

**Abstract:** Spontaneous locomotion strongly influences the state of the hippocampal network and is critically important for spatial information coding. However, the intracellular determinants of CA1 pyramidal cells activation during locomotion are poorly understood. Here we recorded the membrane potential of CA1 pyramidal cells (PCs) while non-overtrained mice spontaneously alternated between periods of movement and immobility during a virtual spatial navigation task in a familiar environment. We found opposite membrane polarization between regular firing and bursting CA1 PCs during movement. Regular firing CA1 PCs were more depolarized and fired at higher frequency during movement compared to immobility while bursting CA1 PCs were hyperpolarized during movement in a speed dependent manner. We propose that the speed-dependent suppression of a subpopulation of CA1 PCs could enhance signal to noise ratio for efficient spatial coding during locomotion.

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Support: McKnight Foundation
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Title: The spatial localization of reward-related changes in hippocampal sharp-wave ripple rate requires normal dopamine signaling

Authors: *M. R. KLEINMAN, D. J. FOSTER;
Psychology, Univ. of California, Berkeley, Berkeley, CA

Abstract: The hippocampus is involved in goal-directed spatial navigation. Previous work has demonstrated that hippocampal replay sequences overrepresent remembered high value locations, and that increases in reward at a particular position increase the rate of sharp wave ripples (SWRs) and reverse replays there, providing a potential substrate for associating the location of a reward with the spatial locations, actions, and stimuli that temporally preceded it. We sought to determine whether this structuring of SWRs and replay to reflect reward contingencies in the environment requires normal signaling in the midbrain dopaminergic (DA) system. The inhibitory DREADD hM4Di was expressed in dopaminergic neurons in the ventral tegmental area (VTA) using targeted injection of a cre-dependent viral construct in transgenic TH-cre rats. Each animal was implanted to perform tetrode recordings from the dorsal CA1 field of the hippocampus. Animals then explored familiar and novel linear environments to collect chocolate fluid rewards, with different trial blocks incorporating spatially localized unexpected changes in reward. During control saline-injection sessions, locations in which the relative reward volume increased led to an increased rate of SWRs, in line with previous work. Intriguingly, suppression of DA VTA neurons by i.p. CNO injection led to increased SWR rates at all reward locations when the reward volume at only one was increased, despite the relative value of the unchanged locations actually decreasing from the equal reward block. Across three rats, there was a significant interaction between CNO condition, reward block and track end, suggesting that the suppression of normal dopamine signaling disrupted the spatial localization of reward-related changes to SWR rate, and thus possibly SWR-associated replay. These results provide evidence for a learning circuit in which hippocampal SWRs rely on DA signaling to properly link the spatial locations associated with new, behaviorally-relevant information. This poor localization of SWRs to positions in the environment with increased reward is expected to cause impaired learning in downstream structures relying on hippocampal output to relate positions with value.

Disclosures: M.R. Kleinman: None. D.J. Foster: None.

Poster

333. Hippocampus: Spatial Maps, Reward, and Replay

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 333.03/AA42
**Title:** Hippocampal replay rapidly and repeatedly adapts to reconfigurations of barrier wall structure in a changing complex maze

**Authors:** *J. WIDLOSKI¹, D. J. FOSTER²; ¹Univ. of California Berkeley, Berkeley, CA; ²Psychology, Univ. of California, Berkeley, Berkeley, CA

**Abstract:** Hippocampal replay has been proposed as an important substrate for path planning and learning because it encodes behaviorally relevant trajectories. However, there is little work on how replay responds to environments with barriers. Barriers change the topological structure of the environment and can have profound effects on how the environment is subsequently navigated. To address this, we developed a new task to elicit replay through barriers. We adapted a previous lab task (goal-directed navigation in unobstructed open field) to include transparent jail bar barriers that impeded the rat's path but minimized interruption of visual and olfactory cues. The 6 barriers could be fitted into 12 different positions, giving a total of 231 unique configurations (not including rotations); new configurations were chosen pseudorandomly for each session, and each rat received between 30-70 training sessions before recording. All other features of the environment remained fixed. The task consisted of goal directed trials to a fixed unmarked "home" well (which changed across sessions) alternating with cued trials to random wells backlit by a flashing light. These trials in particular were designed to lure the replay through the barriers. We recorded up to 300 place cells simultaneously in dCA1 using high tetrode-density hyperdrives. Remarkably, across rats and across barrier configurations, awake replays never crossed the barriers, instead exhibiting trajectories that strongly resembled the rat's own obstacle-avoiding paths. We developed a new quantitative method to measure and visualize replay-like representational transitions in all candidate replay events, to avoid being biased toward smooth trajectories that might not have been as readily detected because of sampling issues at the barriers. This method confirms the finding. Since multiple sessions were recorded each day, we were able to assess the relationship between place field remapping and barrier-respecting replay. We found that most place cells do not remap between configurations, suggesting that the same place cells with the same place fields rapidly reconfigure their relationships to one another to support awake replay that obeys the topological constraints of each environment.

**Disclosures:** J. Widloski: None. D.J. Foster: None.
Fast triggering of brain stimulation contingent on the trajectory content of online-detected hippocampal replay

Authors: W. D. CROUGHAN, *D. J. FOSTER;
Univ. of California, Berkeley, Berkeley, CA

Abstract: Place cells in the hippocampus exhibit during rest or sleep spontaneous activity patterns that depict behavioral trajectories. These patterns, known as replay events, compress long behavioral sequences into rapid bursts of activity on the order of 100ms, making them intriguing candidates for synaptic plasticity’s role in memory consolidation, as well as in the possible planning of future behavior. Numerous studies have shown correlations between the content of replay events and an animal’s goals during spatial navigation. However, causal testing of replay function requires the ability to disrupt or otherwise interact with replay events in realtime, with selectivity for replay content. For example, testing the hypothesis that the prospective replay of a planned trajectory is necessary for its subsequent execution requires the ability to disrupt replays of the trajectory while leaving other replays intact. However, the fast time scale of replay, and the need to decode information from hundred of cells simultaneously, have made such closed-loop experiments extremely challenging. The difficulties include both the time required to analyze recorded data by hand for accurate replay decoding, and the low latency required to decode the events in real time. Here we describe a computational package that enables fast and accurate decoding of replay for use in closed-loop experiments. First, recorded spiking data is analyzed and single units are isolated automatically. The place field of each unit is then determined and used to decode ongoing activity. Replay events are decoded in real-time, and events with specific content can be isolated to trigger arbitrary interventions. This enables, for instance, feedback to target only forward or reverse replays, or replays of a certain area in an environment. Classification accuracy was compared to offline decoding using hand-clustered units. Multi-core processing and continuous decoding allows content to analyzed with a processing latency on the order of 1ms. Latency was measured on a standard desktop computer with an 8-core processor. This tool sets the stage for a variety of closed loop experiments to uncover the function and mechanisms of hippocampal replay, and how replay events relate to wider brain function and behavior.
Disclosures: W.D. Croughan: None. D.J. Foster: None.

Poster

333. Hippocampus: Spatial Maps, Reward, and Replay

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 333.05/AA44

Topic: H.01. Animal Cognition and Behavior

Support: R01MH085823

Title: Prefrontal neurons are tuned to the spatial trajectory information content of hippocampal neurons during non-local hippocampal representation of future and past places, but not during local hippocampal representation of current place

Authors: *A. BERNERS-LEE*1,2, X. WU3, D. J. FOSTER2;
1Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD;
2Psychology, Univ. of California, Berkeley, Berkeley, CA; 3Comprehensive Epilepsy Ctr., NYU Langone Med. Ctr., New York, NY

Abstract: The use of experience to guide decision-making is critical for adaptive behavior. The hippocampus (HP) can represent experience locally, by signaling the current position in an environment, or non-locally, by representing future or past experiences, either in the form of short look-ahead sequences during theta or extended replay sequences. Activity in the medial prefrontal cortex (mPFC) is often coincident with hippocampal activity and local field potential events, and is implicated in decision-making. However, unlike HP neurons, mPFC neurons do not exhibit tightly spatially tuned place fields, prompting the questions of whether and how the information content in each area becomes integrated to promote decision-making. We recorded neurons in mPFC and HP simultaneously while rats navigated a novel Y-maze, during early learning. We found that individual mPFC neurons were differentially modulated to replay sequences depicting different arms of the maze. Indeed, the trajectory identity of the HP replay could be decoded from the coincident mPFC firing alone. Despite this trajectory selectivity during HP replay, mPFC neurons did not show spatially selective activity patterns during running on the maze, as has been reported. However, mPFC neurons exhibited trajectory selectivity during the non-local portions of HP theta sequences (also called theta sweeps). Furthermore, the pattern in which mPFC cells were modulated to theta sequences was consistent with how they responded to replay sequences. Thus, across different behavioral and brain states, mPFC neurons reflect the information content of HP specifically during non-local representation. This suggests that mPFC neurons are specifically tuned to the representations generated by the internal model of the world in HP, possibly to support model-based decision-making, and indeed mPFC firing temporally associated with non-local theta sequences was predictive of the rats’ future choice, while firing temporally associated with local representations was not. These data
shed light on the dialogue between HP and mPFC as they generate and explore an internal model to guide decision-making.

**Disclosures:** A. Berners-Lee: None. X. Wu: None. D.J. Foster: None.

**Poster**

**333. Hippocampus: Spatial Maps, Reward, and Replay**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 333.06/BB1

**Topic:** H.01. Animal Cognition and Behavior

**Support:** BRAIN Initiative Award, R01NS104897 (Xu, Nitz); R01MH105427 (Xu)

**Title:** Hippocampus neural ensemble representations of spatial mapping and learning in freely behaving mice

**Authors:** *L. CHEN*¹, X. LIN¹, S. JIN¹, N. QING², D. A. NITZ³, X. XU⁴;
¹Univ. of California Irvine, Irvine, CA; ²Dept. of Mathematic, Univ. of California, Irvine, Irvine, CA; ³Univ. of California San Diego, La Jolla, CA; ⁴Anat. and Neurobio., Univ. California, Irvine, Irvine, CA

**Abstract:** Population neuronal activity in the hippocampus underlies spatial navigation and memory formation processes in mammals, including mice and humans. However spatiotemporal dynamics of neuronal ensembles are not well understood in the context of spatial mapping, learning and memory. In the present study, we address spatiotemporal ensemble representations in hippocampal CA1 during behavior tasks through *in vivo* Ca++ imaging using head-mounted miniature microscopes (“miniscopes”). For imaging experiments, mouse brains express the genetically encoded calcium indicator GCaMP6 (after injection of AAV expressing GCaMP6) such that neural activities of large ensembles of neurons over extended periods of time can be recorded through microscopic imaging of calcium signal events. This approach allows us to simultaneously examine hundreds of the same neurons in hippocampal CA1 across different environments and different days, and correlate single-cell firing patterns with mouse spatial mapping and learning behaviors. We find that during the free exploration of open field arenas and running on a linear track, CA1 neurons form place specific firing fields, which is consistent with previous studies with electrical recordings. Interestingly, the neural ensembles can be divided into specific activation groups as they share similar calcium event patterns in the temporal domain. This “temporal clustering” of longitudinally tracked neurons exhibits dynamic changes and the same set of neurons form distinct clusters as the mouse explores in different environments. The “optimal” grouping was determined by a nonnegative matrix factorization (NMF) based method adapted from Brunet et al. (2004). Given a searching range of 1-50, the optimal number of groups ranges from 10 to 40 for each imaging session. Neurons belonging to
the same cluster tend to share place firing fields, while firing fields of neurons from different clusters are largely non-overlapping but cover different locations of the arena. Such temporally specific clustering and spatial tiling of CA1 neuronal ensembles also exists in the training and testing sessions of a hippocampus-dependent object-location memory task. Together, these new and important results lead to the notion that CA1 populations are organized into distinct groupings that share spatiotemporal patterns of activity.

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Poster

333. Hippocampus: Spatial Maps, Reward, and Replay

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 333.07/BB2

Topic: H.01. Animal Cognition and Behavior

Support: Howard Hughes Medical Institute

Title: Dendritic plateau potentials shape hippocampal place cell representations

Authors: *C. GRIENBERGER, J. C. MAGEE;
Baylor Col. of Med., Houston, TX

Abstract: A crucial function of the brain is to produce useful representations of the external world. The hippocampus contains neurons tuned to fire action potentials (APs) in particular spatial locations within an environment. Collectively, populations of these place cells encode abstract and concrete environmental features. Our previous results demonstrate that place cells are driven by a subset of inputs whose elevated synaptic weights provide excitation that exceeds a spatially uniform level of inhibition. A novel form of synaptic plasticity called behavioral timescale synaptic plasticity (BTSP) mediates the increase in synaptic weights. BTSP has several distinct characteristics, including that it depends on dendritic plateau potentials (plateaus) instead of APs. Here we sought to determine how plateaus and BTSP shape CA1 place cell representations. We used two-photon Ca\(^{2+}\) imaging in mice exploring a novel linear track for the first time. We observed the formation of an environment-specific representation during the first recording session that correlated with learning of reward position. Notably, the majority of place fields were not initially present but abruptly appeared later during the session, suggesting that completing the representation requires the animal’s experience. Finally, several pieces of evidence implicate BTSP: (1) the abrupt appearance itself; (2) the width of the new fields was correlated with the animal’s velocity during induction; (3) new fields were shifted back in space with respect to their induction; (4) the development of the representation was severely attenuated by the plasticity and Ca\(^{2+}\)-spike blockers, APV and SNX-482. Taken together, our results point
towards a fundamental role of BTSP in creating CA1 representations and, thus, identify plateaus as a key signal that instructs CA1 neurons in how to represent an environment.

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**Poster**

**333. Hippocampus: Spatial Maps, Reward, and Replay**

**Location:** Hall A

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**Topic:** H.01. Animal Cognition and Behavior

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NSF grants DMS-1821286

**Title:** Continuous reward-place coding properties of dorsal distal CA1 hippocampus cells

**Authors:** *Z. Xiao¹, S. Nagl², K. Lin¹, J.-M. Fellous²;
¹Program of Applied Mathematics, The Univ. of Arizona, Tucson, AZ; ²Psychology, Univ. of Arizona, Tucson, AZ

**Abstract:** Hippocampal place cells are involved in goal-directed spatial navigation and the consolidation of spatial memories. Recently, using calcium-imaging in mice navigating in a virtual reality environment, Gauthier and Tank found a subpopulation of hippocampal cell selectively activated in relation to rewarded goals. However, the relation between these cells’ spiking activity and goal-representation as well as goal-related-memory consolidation remains elusive.

In this study, we analyzed data from experiments in which rats were pre-trained to acquire sugar-water rewards from 8 equidistant feeders at the periphery of an open-field arena. The rats then underwent experiments made up of 5 different tasks and 10 flanking rest epochs. These tasks included separate cue-driven and memory-driven spatial navigation epochs in which the spatial context was manipulated.

Using tetrode recordings, we found CA1 populations with coding properties continuously ranging from place cells to reward cells. Specifically, we found regular place cells insensitive to reward locations, reward cells that only fired at correct rewarded feeders in each task regardless of context, and “hybrid cells” that responded to both fixed locations and change of locations of feeders. In addition, we observed transitions between place cells and reward cells within each session, i.e., a reward cell in previous tasks could encode a fixed location in the next few tasks, and vice versa. In this population, most of the reward sensitivity was due to obtaining the reward rather than expecting it.

With these observations, we hypothesized that some pyramidal cells (if not all) integrate both
spatial and reward inputs with various and plastic weights. Consistent with previous studies, some place cells exhibited excess firing around correct feeders, which was well explained by the hypothesis. To measure the place and reward components of a cell quantitatively, we defined “place” & “reward” scores for each neuron based on its firing rate map. Generally, place cells had larger place scores and smaller reward scores, and vice versa. Hybrid cells lay between the other 2 groups of cells.

We also studied the relationship between reward sensitivity of these cells and their contribution to replay-like rest population bursts. We found that the portion of reward-cell-dominated population bursts was much higher than expected by chance, suggesting an independent reactivation pathway from reward-related areas to CA1.

Overall, this study provides insights into the integrative coding properties of CA1 pyramidal cells, focusing on their abilities to carry spatial and reward information in a mixed and plastic manner.

maze. During training, rats obtained the same type of reward (sunflower seeds, or SS) in both arms (SS session) as a baseline. Afterward, in the main task, both arms were unexpectedly baited with less desired reward (Cheerios, or CC) in certain blocks interleaved with the baseline conditions. We analyzed only place cells (mean firing rate > 0.5Hz, spatial information > 0.25 bits/spike) of the dHP and vHP. When the reward was changed from SS to CC, the proportion of units maintaining their location-associated firing rates was higher in the dHP (72%, n = 169/234) than in the vHP (59%, n = 196/332) (p < 0.01). On the other hand, place cells in the vHP (28%, n = 94/332) shifted their firing locations more within a session than those in the dHP (16%, n = 38/234) (p < 0.01). The spatial rate maps of the place cells recorded from the vHP in the SS session were significantly changed as reward was changed to less desirable one (i.e., CC) (p < 0.001), whereas this was not the case in the dHP (p > 0.05 between the rate maps of the SS and CC sessions). Approximately 40% (n=15/37) of the vHP place cells that remapped in the CC session formed their place fields immediately after the reward type was changed from SS to CC. Next, we tested whether such differences between the dHP and vHP would also show similarly in a mnemonic task. For this purpose, rats were trained in a spatial food-preference task in which two arms of a T-maze were baited with different types of reward (SS and CC). Rats naturally tried to visit the SS-baited arm more. After the rat chose the SS-baited arm for 12 of the last 15 trials, the reward types associated with the two arms were reversed. Our preliminary results show that the place fields of the vHP cells shifted forward toward the location where the more preferred reward was found, whereas no such reward-related dynamic location encoding occurred in the dHP place cells (p < 0.05). Our results suggest that reward value and its associated locations make dynamic changes in the vHP, but not in the dHP.


Poster

333. Hippocampus: Spatial Maps, Reward, and Replay

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 333.10/BB5

Topic: H.01. Animal Cognition and Behavior

Support:  Wellcome Fellowship 202810/Z/16/Z

Title: Extending evidence for REM-associated replay in hippocampal CA1 place cells

Authors: T. HOWE1, M. A. WILSON2, D. JI3, *M. W. JONES1;  
1Physiology, Pharmacol. & Neurosci., Univ. of Bristol, Bristol, United Kingdom; 2Picower Inst. Learn/Memory, MIT, Cambridge, MA; 3Baylor Col. of Med., Houston, TX

Abstract: During periods of inactivity, hippocampal CA1 neurons with spatial receptive fields (“place cells”) reactivate in patterns that recapitulate previously experienced spatial sequences, a
phenomenon known as replay. CA1 replay is most prominently associated with sharp-wave ripple (SWR) events during non-REM sleep or quiet wake, and has been implicated in the consolidation of episodic memory.

Replay has also been reported during REM sleep [1], however evidence for this phenomenon is substantially less extensive than for replay during non-REM. Non-REM replay occurs on a temporally compressed timescale (approximately 8 times faster than during active behaviour) around brief, discrete SWR events. REM-replay appeared less temporally compressed and occurred during extended periods of elevated theta power, necessitating alternative detection methods to those established for SWR-associated replay.

Using tetrode recordings from adult rat dorsal CA1, we present data that corroborate the existence of REM-replay. The activity of multiple place cells was recorded simultaneously while rats performed simple goal-directed maze tasks, and during subsequent extended rest periods in a sleep box. Replay was detected using a moving-window correlation algorithm (from [1]), and confirmed with complementary approaches including hidden Markov model (HMM) and Bayesian trajectory decoding.

Extending evidence for REM replay paves the way for analyses exploring its experience-dependence, extra-hippocampal correlates and functional contributions.

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Poster

333. Hippocampus: Spatial Maps, Reward, and Replay

Location:  Hall A

Time:  Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #:  333.11/BB6

Topic:  H.01. Animal Cognition and Behavior

Support:  NIH520335
          Sloan635616
          Searle635296
          whitehall694677

Title:  The dynamics and mechanisms of place field formation in the hippocampal CA3

Authors:  *C. DONG, M. SHEFFIELD;
          Neurobio., Univ. of Chicago, Chicago, IL

Abstract:  Hippocampal place cells are a subset of pyramidal neurons in the hippocampus that fire at specific locations in an environment. They are a critical component of the brain’s
cognitive map of space, and are thought to represent the spatial component of episodic memories (memories that occur at a particular time and space). The development of 2-photon calcium imaging techniques enables neuroscientists to measure the activity of large populations of neurons, and also fine dendritic structures, in the hippocampus. This approach has been used to study the dynamics and mechanisms of place field formation in the CA1 region of the hippocampus during novel environment exposure, providing new insights into how place fields form in the CA1 at the population and dendritic level. One of the insights gained from this work is that across the population CA1 pyramidal neurons form many place fields instantaneously (instant place fields) in novel environments, with other place fields forming slowly with experience. The delayed forming place fields form through a dendritic spike induced synaptic plasticity mechanism that takes time/experience to occur. However, the major input source to CA1, the CA3 region, which forms its own place fields and integrates information from dentate gyrus (DG), entorhinal cortex (EC), and its own population in the CA3, has not been systemically investigated to the same level. Here, we studied the dynamics and mechanisms of place field formation in CA3 at both the population and dendritic level. We have found that compared with CA1 place cells, CA3 place cells form place fields slowly during novel environment exposure, and form a very limited number of instant place fields. Our data suggest that cognitive maps in the CA1 and CA3 form differently in response to novel environments, and that the CA1 encodes these new environments quickly, whereas the CA3 requires experience to form new maps. This also suggests that CA1 place fields that form instantly in novel environments are not simply driven by instant place fields in CA3. We are also measuring dendritic activity across CA3 dendrites during place field formation to try to understand the underlying mechanisms of place field formation in individual neurons.

Disclosures:  C. Dong: None. M. Sheffield: None.

Poster

333. Hippocampus: Spatial Maps, Reward, and Replay

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 333.12/BB7

Topic: H.01. Animal Cognition and Behavior


Title: Characterising stability and fluctuation of hippocampal place cell activity using calcium imaging

Authors: *C. P. KOH, A. TASHIRO;
Nanyang Technological Univ. (NTU), Singapore, Singapore
Abstract: Hippocampal place cells exhibit stable location-specific activity in a relatively fixed place. However, fluctuations in their activity were also commonly observed. For example, it has been reported that their firing rate may vary or the cells may turn on/off between multiple sessions of exploring a constant environment. To characterise the stability and fluctuation, male C57/BL6 mice were injected with adeno-associated viral vector expressing GCaMP6s under the control of the CaMKIIα promoter and implanted with a GRIN lens, and calcium imaging was conducted using a head-mounted microendoscope while each animal traversed a one-metre long linear track in two sessions one-week apart. Subsequently, we selected neurons which were active in both sessions, and CNMF-e (Constrained Non-negative Matrix Factorisation for Endoscopic data) was used to detect Ca²⁺ signal and approximate spike information from individual neurons. The event output from CNMF-e for each cell was compared visually against the corresponding calcium imaging movies. We found that there were 57.9% and 6.3% false positive and false negative events respectively. After eliminating the false positive events, the remaining events and the animal’s positional information in the track were used to generate rate maps. Of all the active cells analysed from the two sessions one-week apart, three groups of active cells emerged. The first group was only active at the ends of the track in both sessions (46.15%) while the other two groups were active when the animal was in motion and exhibited direction-specific activity (53.85%). The second group (30.77%) is place cells with stable location-specific activity between the two sessions, showing statistically significant, strong, positive spatial correlation between the sessions (r = 0.729 to 0.965; p < 0.01); shift in place field centre of mass was 4.59 ± 3.73 cm (mean ± S.D). The third group (23.08%) showed location-specific activity with variable locations, and did not display statistically significant spatial correlation (r = 0.130 to 0.199; p > 0.05); shift in place field centre of mass was 23.06 ± 17.34 cm (mean ± S.D). From a previous study, we expected to find a major population of cells which were active while the animal was in motion in one session but not the other. Although the number of cells examined at present is limited, none of the cells showed this pattern of activity. Our findings from calcium imaging followed by CNMF-e support the idea that there are two distinct groups of active cells when an animal is in motion, one with stable, and the other with variable location-specific activity.

Disclosures: C.P. Koh: None. A. Tashiro: None.

Poster

333. Hippocampus: Spatial Maps, Reward, and Replay

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 333.13/DP13/BB8

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster

Topic: H.01. Animal Cognition and Behavior
Support: Royal Society TA\R\170047
The Michael Uren Foundation
Universities UK Rutherford Fund RF-2018-27
BBSRC BB/K001817/1
EPSRC EP/L016737/1

Title: Place cells in head-fixed mice during navigation in a floating real-world environment

Authors: *M. GO¹, J. ROGERS¹, C. DAVEY², S. V. PRADO¹, G. GAVA¹, L. KHIROUG³,⁴, S. R. SCHULTZ¹;
¹Imperial Col., London, United Kingdom; ²The Univ. of Melbourne, Melbourne, Australia; ³Univ. of Helsinki, Helsinki, Finland; ⁴Neurotar Ltd, Helsinki, Finland

Abstract: “Place cells” in the hippocampus encode spatial information, and have been observed in both rodents moving freely, and in head-fixed mice navigating in virtual reality environments. It has been shown that hippocampal patterns of activity in rodents differ between virtual and real environments (Aghajan et al 2015, Nature Neurosci 18:121-8). Recently, a real-world environment system for head-fixed mice which allows for sensory feedback has been developed, consisting of an air-lifted track (Kislin et al 2014, J Vis Exp 88:e51869). Until now, the presence of place cells in such a system has not been shown. Methods: We injected mice with hSyn1-GCaMP6s-mRuby into the hippocampal CA1 region and aspirated the cortex above the injection site until the corpus callosum was exposed. After three weeks, animals were put under water restriction and trained to move on a track, either a circular linear track or an open circular arena, lined with phosphorescent visual cues. The track floats on an air table (Neurotar Ltd) under a two-photon resonant scanning microscope (Scientifica Ltd) and is fitted with a magnet-based position tracking system. Animals were trained in 45 min sessions twice daily with water rewards randomised by location (8 for circular linear track and 3 for open arena). After a training period of 6 days and 13 days, for the circular linear track and open arena, respectively, two-photon calcium imaging was performed during navigation. Calcium imaging videos were corrected for movement artefacts and segmented for cell regions using the ABLE algorithm (Reynolds et al 2017, eNeuro 4:ENEURO.0012-17.2017). Place field analysis was performed to monitor the changes in neural activity across the different locations across several imaging sessions. To identify cells with spatial selectivity, we computed the mutual information between neuronal activity and track position. Place field maps above the mutual information threshold were sorted in order of vector average place preference. To verify that the increase in firing rate in place fields was statistically significant, we performed a bootstrap shuffle test. Results and conclusions: Free exploration behaviour was induced in the mice as a result of cued learning. Consistent place tuning was observed in approximately 60% of the cells in the circular linear track. Moreover, place cells remapped when the animal was in a novel environment, with additional cells recruited. Two-dimensional place cell tuning was also observed in the open arena. To our knowledge, this is the first demonstration of place cells in head-fixed mice navigating an air-lifted track.
Disclosures: M. Go: None. J. Rogers: None. C. Davey: None. S.V. Prado: None. G. Gava: None. L. Khiroug: A. Employment/Salary (full or part-time); Neurotar Ltd. S.R. Schultz: None.

Poster

333. Hippocampus: Spatial Maps, Reward, and Replay

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 333.14/BB9

Topic: H.01. Animal Cognition and Behavior

Support: MH083809

Title: Spatial firing patterns of hippocampal CA1 neurons are not sensitive to changes in the social context

Authors: *W.-Y. WU, E. YIU, A. G. OPHIR, D. M. SMITH; Psychology, Cornell Univ., Ithaca, NY

Abstract: The hippocampus is thought to play a key role in social memory, particularly the ventral region which is closely interconnected with the social behavior network. For example, inactivation of ventral CA1 neurons impairs social memory (Okuyama et al, 2016, Science, 353:6307). Hippocampal neurons exhibit spatial firing patterns (place fields) which undergo remapping in response to changes in the environmental context, suggesting that these representations might also be sensitive to changes in the social context. However, recent results indicate that dorsal CA1 neurons do not remap when a conspecific is added to the environment (Alexander et al, 2015, Nat. Comm. 7:10300). Interestingly, dorsal CA2 neurons did show remapping and projections from dorsal CA2 to ventral CA1 have been implicated in social memory (Miera et al, 2018, Nat. Comm. 9:4163). In the present study, we manipulated the social context while rats foraged in an open field and we recorded neurons in dorsal and ventral CA1. In order to maximize the salience of the social manipulation, two conspecifics were present for each recording trial (in small cages) and their cage bedding was scattered around the environment. Recordings were obtained during four 15 min trials in an ABAB pattern with alternating sets of conspecifics, including different sets of familiar and unfamiliar same- or opposite sex rats. We also included a control manipulation of the visual environment for a subset of subjects in which the conspecifics were constant but the wall color was changed from black to white. We found little evidence of remapping in either dorsal or ventral CA1 in response to the social context manipulations. Dorsal CA1 neurons exhibited similar spatial firing patterns across trials, regardless of whether the social context was the same or different (same r=0.57, different r=0.60). Preliminary data indicate that ventral CA1 neurons exhibited lower spatial correlations than dorsal CA1, but they were unaffected by the social context (same r=0.24, different r=0.26). Importantly, the same neurons exhibited significant remapping in response to manipulation of
the visual context, as indicated by reduced spatial correlation scores for black and white trials (dorsal CA1: t(25) = 4.08, p < .001; ventral CA1: t(72) = 3.77, p < .001). Interestingly, some neurons exhibited selective firing near the conspecifics and this appeared to be more common in ventral CA1. Overall, these results suggest that hippocampal neurons do not encode social contexts in the same way they encode other kinds of environmental context and social memory may be supported by mechanisms other than hippocampal remapping.


Poster

333. Hippocampus: Spatial Maps, Reward, and Replay

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 333.15/BB10

Topic: H.01. Animal Cognition and Behavior

Support: NIH-5-20335
Sloan-6-35616
Searle-6-35296
Whitehall-6-94677

Title: Role of hippocampal CA1 place cells in fear memory encoding and recall

Authors: *S. KRISHNAN, C. CHERIAN, M. SHEFFIELD;
Neurobio., Univ. of Chicago, Chicago, IL

Abstract: Episodic memories—memories of experiences placed in time and space—form through associations made in the hippocampus between distinct stimuli within specific contexts. The spatial components of these memories are thought to be represented in place cells that encode animal position. Consistently, in a contextual fear conditioning (CFC) paradigm, which causes the formation of an episodic-like memory, optogenetic activation of hippocampal neurons “tagged” during induction of contextual fear can sufficiently trigger retrieval of the fear memory. These neurons termed “engram-cells” are thought to contain the physical trace of the fear-memory. However, because direct neurophysiological data collected from engram-cells during memory formation is lacking, it is unclear if the fear-memory engram-cells are also place-cells or are they distinct cells located in distinct layers or regions. What are the firing characteristics of engram cells during memory formation that allows them to be tagged, and how do they fire during natural memory recall? To answer these questions, we designed a device that provides tail shocks to head-fixed mice during navigation of virtual reality (VR) environments. In these mice, we implemented a novel behavioral paradigm for CFC where simultaneous functional two-photon imaging of large populations of hippocampus neurons at cellular resolution can be performed prior to and during fear conditioning as well as during fear memory recall. During
memory recall, mice showed substantial increase in freezing and backward running in the VR environment associated with the tail shock when compared to a control VR environment, allowing our paradigm to be comparable with CFC performed in freely-behaving mice in which engram tagging studies have been performed. Thus, this paradigm provides us with access to the activity of hippocampal neurons during fear memory formation and recall allowing us to identify and quantify the characteristics of fear engram-cells and place cells within the same animal. By precisely probing memory circuits in the hippocampus on a large scale during behavior in this way, we aim to reveal the mechanisms and population dynamics involved in the formation and recall of a specific memories and how distinct memory representations interact at the population level.

Disclosures: S. Krishnan: None. C. Cherian: None. M. Sheffield: None.

Poster

334. Genetic and Molecular Mechanisms of Memory Formation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 334.01/BB11

Topic: H.01. Animal Cognition and Behavior

Support: MH 114990
       DA039650
       DA034681
       startup funds from UAB, the Evelyn F. McKnight Brain Research Foundation

Title: Transcriptomic analysis to identify brain region specific enhancers

Authors: *R. A. PHILLIPS, III, N. CARULLO, K. SAVELL, J. J. DAY;
Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Genomic enhancers are cis-acting regulatory elements that undergo bidirectional transcription to form long non-coding RNA molecules, termed enhancer RNAs. In the brain, enhancers control the precise gene expression programs needed to regulate cellular function in response to stimuli. Furthermore, SNPs discovered in GWAS studies for brain diseases often map to regulatory regions within the genome that contain enhancers. While evidence for the importance of enhancers in cellular function and brain disease is steadily increasing, enhancer discovery often neglects the presence of transcription at genomic enhancers. Here we show that transcriptomic analysis from total RNA identifies bidirectional transcription from putative enhancers across the genome and can be used to predict potential enhancer-gene pairs based on co-regulated basal and stimulus-dependent activity states. Using this approach in multiple neuronal subtypes, we demonstrate comprehensive mapping of cell-type and stimulus-dependent putative enhancers and show that these enhancers differ based on brain region of origin and
activity-regulated neuronal responses. We are currently using this transcriptome-assisted discovery pipeline to identify candidate enhancers for CRISPR-based functional validation of enhancer-gene pairs. Our results suggest that the presence of enhancer RNAs can assist in enhancer discovery, providing an additional tool for the characterization of regulatory elements within the genome.


Poster

334. Genetic and Molecular Mechanisms of Memory Formation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 334.02/BB12

Topic: H.01. Animal Cognition and Behavior

Support: MH 114990

DA039650

DA034681

startup funds from UAB, the Evelyn F. McKnight Brain Research Foundation

Title: Interactions between Creb binding protein (CBP) and enhancer RNAs regulate enhancer activity and gene expression

Authors: *N. CARULLO¹, S. ROMAN², R. A. PHILLIPS, III³, J. S. REVANNA⁴, J. HINDS¹, J. J. DAY⁵;

¹Univ. of Alabama at Birmingham, Birmingham, AL; ²Univ. of Alabama at Birmingham, Birmingham, AL; ³Univ. of Alabama At Birmingham, Birmingham, AL; ⁴Neurobio., Univ. of Alabama At Birmingham, Vestavia, AL; ⁵Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Epigenetic mechanisms are central regulators of neuronal function, experience-dependent gene expression, and adaptive behavior. Histone acetylation is a well studied epigenetic modification that is commonly used to define enhancer elements in the genome, as these sites are marked by H3K27 acetylation and binding of histone acetyltransferases such as Creb binding protein (CBP). However, although histone acetylation patterns at enhancers are actively modified by neuronal activity and behavioral experience, it is presently unclear how these site-specific changes are induced and maintained over time. Here, we aimed to elucidate the role of CBP on enhancer activity and gene expression with particular focus on the intersection of non-coding enhancer RNAs (eRNAs) and CBP. RNA mobility shift assays (REMSA) revealed that two distinct eRNAs arising from separate enhancers upstream and downstream of the Fos gene bind the CBP HAT domain but not the bromodomain, suggestive of direct physical interactions between CBP and eRNAs. HAT domain recruitment to Fos
enhancers with a CRISPR-dCas9 approach increases local eRNA transcription and distal mRNA expression from the Fos gene, supporting the role of histone acetylation in gene regulation and enhancer dynamics. Finally, we used another CRISPR-dCas9 system (CRISPR-Display) to recruit CBP-interacting eRNAs to specific sites in the genome. This approach revealed that tethering of Fos eRNA sequences to their respective enhancer induces similar increases in Fos mRNA expression as HAT recruitment, while recruitment of a control RNA (based on a non-regulatory region between the Fos enhancers) does not reproduce these effects. Intriguingly, targeting of the eRNAs to other sites (other enhancer or control regions) did not affect Fos mRNA levels. Along with our data showing that non-targeting overexpression doesn’t affect Fos mRNA expression, this suggests location specificity as major regulatory factor in eRNA function. In ongoing work, we investigate the role of eRNAs on HAT activity, and modulate endogenous CBP expression to examine the effects on CBP target enhancers and genes. Overall, our results suggest that CBP interacts with eRNAs to regulate enhancer and gene induction. The location and sequence specificity of eRNA-mediated effects provide a novel mechanism for spatiotemporal control of CBP function.


**Poster**

**334. Genetic and Molecular Mechanisms of Memory Formation**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 334.03/BB13

**Topic:** H.01. Animal Cognition and Behavior

**Support:** BBSRC Grant BB/L00139X/1
BBSRC Doctoral Support BB/M01116X/
WPH Charitable Trust
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**Title:** Combined electrophysiological, behavioural and bioinformatic assessment of the role of MSK1 in the molecular mechanisms of experience-dependent synaptic plasticity

**Authors:** *D. D. Cooper, P. Richardson, E. Condon, S. Pinder, B. G. Freングuelli; Sch. of Life Sci., Univ. of Warwick, Coventry, United Kingdom

**Abstract:** Exposure of experimental animals to an enriched environment (EE) has profound effects on neuronal morphology, synaptic activity and cognitive function. Brain-derived neurotrophic factor (BDNF) has been repeatedly implicated in initiating the molecular cascade and genomic response that leads to enduring changes in the nervous system that underpin the
benefits of EE. However, the identity of these molecular and genomic events have remained elusive. We have previously shown that a nuclear enzyme activated by BDNF, mitogen- and stress-activated protein kinase 1 (MSK1), and which regulates transcription, notably via the phosphorylation of cAMP response element binding protein (CREB), is necessary for the EE-induced enhancement of synaptic transmission. (Corrêa et al., 2012; Lalo, et al., 2018). Using a mutant mouse expressing a kinase-inactive form of MSK1 (MSK1 KD), we have been investigating the role MSK1 plays in modulating the cognitive, synaptic, anatomical and genomic responses to environmental enrichment. 3D neuronal reconstruction, RNA sequencing and single-cell patch-clamp electrophysiological recordings from the hippocampal region of male mice (3-5 months of age) reared in standard housing and EE housing were used to characterise the spectrum of changes from the transcriptomic to the cellular after various durations of enrichment. In addition, tests of cognitive function (object location, sociability) were performed to relate these cellular and molecular changes to behavioural performance. Currently these studies indicate that the majority of genomic changes induced by EE require the kinase activity of MSK1. This suggests that MSK1 is responsible for initiating a genomic homeostasis in response to enrichment that may both stabilise EE-induced neuronal changes, and make the brain better able to respond to new challenges. Corrêa, S.A.L., et al. (2012). “MSK1 Regulates Homeostatic and Experience-Dependent Synaptic Plasticity”. The Journal of Neuroscience, 32, 13039-13051. Lalo, U. et al. (2018) “Role for Astroglia-Derived BDNF and MSK1 in Homeostatic Synaptic Plasticity”, Neuroglia, 1, 381-394

Disclosures:  D.D. Cooper: None. P. Richardson: None. E. Condon: None. S. Pinder: None. B.G. Frenguelli: None.

Poster

334. Genetic and Molecular Mechanisms of Memory Formation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 334.04/BB14

Topic: H.01. Animal Cognition and Behavior

Support: BBSRC Grant BB/L00139X/1
        BBSRC Doctoral Support BB/M01116X/1
        WPH Charitable Trust
        Slyvia Waddilove Foundation

Title: The role of MSK1 in the experience-dependent regulation of AMPA receptors and plasticity-related proteins

Authors: *P. RICHARDSON, D. D. COOPER, B. G. FRENGUELLI; Sch. of Life Sci., Univ. of Warwick, Coventry, United Kingdom
Abstract: The benefits of environmental enrichment (EE) for experimental animals include increased spine density, enhanced synaptic transmission and plasticity, and improved cognition. A prime molecular candidate underlying these positive effects is the enrichment-induced release of brain-derived neurotrophic factor (BDNF). We have shown that mitogen- and stress-activated protein kinase 1 (MSK1), which is activated by BDNF and which regulates transcription through CREB phosphorylation, is not only necessary for the regulation of the strength of basal synaptic transmission in hippocampal area CA1 (Daumas et al., 2017), but also necessary for the enhancement of mEPSCs by EE (Correa et al., 2012; Lalo et al., 2018). Since changes in cell-surface expression of glutamate AMPA receptors (AMPARs) is a likely explanation for the MSK1- and EE-dependent effects on synaptic transmission, we have been investigating the influence of MSK1 on AMPAR GluA subunits and key plasticity-related proteins using wild-type (WT) mice and mice harbouring a knock-in mutation of the MSK1 gene that inactivates the kinase activity of MSK1 (MSK1 KD mice).

We have compared AMPAR GluA subunit expression across four groups: WT and MSK1 KD mice raised under either standard or enriched conditions for 1 week, 5 weeks or 3 months. In addition, we have investigated the expression of CREB and arc/Arg3.1 after various durations of enrichment in WT and MSK1 KD mice, and have performed parallel behavioural studies (novel object recognition, spontaneous alternation) to equate any expression changes with cognitive function.

These studies will shed new light on the influence of MSK1 in the experience-dependent regulation of key proteins regulating synaptic activity and will illuminate the molecular pathways linking BDNF to enduring cellular and molecular changes underpinning the enhancement of cognition by enrichment.

References:
Lalo, U. et al. (2018) Role for Astroglia-Derived BDNF and MSK1 in Homeostatic Synaptic Plasticity, Neuroglia, 1, 381-394.
Daumus, S. et al. (2017) The Kinase Function of MSK1 Regulates BDNF Signaling to CREB and Basal Synaptic Transmission, But Is Not Required for Hippocampal Long-Term Potentiation or Spatial Memory, eNeuro, 4.

Disclosures: P. Richardson: None. D.D. Cooper: None. B.G. Frenguelli: None.

Poster
334. Genetic and Molecular Mechanisms of Memory Formation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 334.05/BB15

Topic: H.01. Animal Cognition and Behavior
Title: Large-scale, in vivo functional characterization of molecularly-defined gabaergic interneurons in CA1 by 3D random-access imaging during spatial navigation

Authors: *T. GEILLER¹, B. VANCURA¹, S. TERADA¹, B. RÓZSA², A. LOSONCZY¹; ¹Columbia Univ., New York, NY; ²Hungary Acad. of Sci., Budapest, Hungary

Abstract: In the hippocampal region CA1, GABAergic interneurons account for nearly 15% of the neuronal population and are comprised of heterogeneous subtypes. This diverse group of inhibitory cells varies with respect to developmental origin, anatomical location, connectivity patterns, gene expression profiles, and electrophysiological properties. Despite their relatively small number, hippocampal interneurons have been shown to play crucial roles in controlling network oscillations, as well as input integration in and selectivity of pyramidal cells. So far, most in-vivo studies aiming at characterizing their functions during behavior were performed using low-throughput electrophysiological techniques that allowed relatively limited sampling from this very heterogeneous population. Here, we combine a novel high-throughput, random-access imaging technique with post-hoc immunochemistry to perform an unbiased sampling of nearly 300 interneurons in CA1 during spatial navigation and goal-directed learning. VGAT-Cre mice are injected with the calcium indicator GCaMP6f and trained to run on a belt for either randomly delivered water rewards (random foraging) or hidden rewards kept at a fix location (goal-directed navigation). Interneurons activity is recorded by 3D random-access two-photon imaging using acousto-optical deflection (AOD) in a nearly cubic-millimeter volume from Stratum Oriens (S.O) to Stratum Lacunosum Moleculare (S.L.M) at a fast rate (~5Hz). Post-hoc immunochemistry is then performed in fixed brain slices and the molecular identity of imaged interneurons is determined by registering confocal images to two-photon stacks. We aim at assessing the spatial tuning of molecularly-defined interneurons, a characterization currently missing from the literature, notably from cells residing in distal dendritic layers. We also examine the extent to which different subclasses of interneurons exert collective inhibition during navigation, as such analysis is not possible with classic, low-throughput recording techniques. Thus, our strategy will allow us to perform an unbiased, functional characterization of hundreds of neurochemically-identified CA1 interneurons in a given animal, ultimately providing a more complete picture of interneuronal networks during behavior.

Disclosures: T. Geiller: None. B. Vancura: None. A. Losonczy: None.

Poster

334. Genetic and Molecular Mechanisms of Memory Formation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 334.06/BB16

Topic: H.01. Animal Cognition and Behavior

Support: NIMH Grant 8K00MH121382-03
Title: Correlated physiology and transcription amid learning in the hippocampus

Authors: *S. A. HERRLINGER, J. GOGOS, A. LOSONCZY; Columbia Univ., New York, NY

Abstract: Episodic memory (EM) encoding is dictated in part by place cell activity and tuning in the CA1 region of the hippocampus. However, it remains largely unknown what the biological mechanisms in place cells are that facilitate this learning capacity. Notably, there is a marked heterogeneity of in vivo physiological properties (e.g., in vivo firing rate, place cell identity, and place cell properties) within pyramidal neurons in the hippocampus, including the CA1PCs (Soltesz and Losonczy, 2018). For example, in any given environment, only a subset (~20-40%) of CA1PCs exhibit place cell activity. While it has been generally assumed that this active coding subset is selected from and allocated randomly onto the CA1PC population, multiple pieces of evidence suggest that differences in intrinsic, genetic profiles among CA1PCs also determine their in vivo physiological properties. These properties 1) do not follow a normal distribution (Buzsáki and Mizuseki, 2014; Grosmark and Buzsáki, 2016), 2) are assumed to be organized along major anatomical axes of the hippocampus (Geiller, 2017; Soltesz and Losonczy, 2018), and 3) exhibit a spatial code, suggesting developmental or transcriptional origins (Lee 2019). Gradients of gene expression have been identified across the hippocampus (Cembrowski et al., 2016a, 2016b; Shah et al., 2017) but not with the single cell resolution required to parse out the full heterogeneity of the hippocampus. Despite these intriguing observations, there is no available information on how gene expression patterns correlate with in vivo physiological properties at the level of individual hippocampal cells, nor how these patterns are affected by mutations (such as 22q11.2 deletions) associated with cognitive dysfunction and known to affect episodic memory as well as the pattern of activity and plasticity of place cell ensembles in the hippocampus.

To this end, we have 1) sought out to correlate physiological changes in place cell activity with their transcriptomic changes to better understand the molecular biology underlying place cell tuning in the hippocampus, and 2) compared changes in the transcriptome in wildtype and Df(16)A+/- mice in vivo to uncover biological processes disrupted in a model for 22q11.2 deletion syndrome during EM encoding. To achieve this, we are utilizing single-cell sequencing in the hippocampus to define transcriptional hippocampal cell type identifiers and adapted an in vivo Patch-seq technique to correlate physiology and transcriptomics in behaving mice.

Disclosures: S.A. Herrlinger: None. J. Gogos: None. A. Losonczy: None.
**Title:** Heterogeneous activity of parvalbumin synaptic boutons surrounding CA1 pyramidal cell bodies during trace fear learning and memory

**Authors:** *S. TERADA, M. AHMED, E. BALOUGH, J. ZHANG, A. SOLIS CANALES, A. LOSONCZY;*
The Mortimer Zuckerman Mind Brain and Behavior Inst., Columbia Univ., New York, NY

**Abstract:** The hippocampus mediates the association between discontinuous events during episodic memory. While circuit ensemble activity patterns of CA1 pyramidal cells, such as theta sequences or activity motifs during replay, are studied extensively, little is known about how interneuron activity is recruited during memory formation. This is a critical gap in knowledge, especially given that various interneuron types are believed to be essential to control the spike timing of pyramidal cells and shape their ensemble recruitment. Here, we combined two photon Ca^{2+} imaging of synaptic boutons of parvalbumin (PV) basket cells in stratum pyramadale with LFP recording in hippocampal CA1 to chronically track the activity from the same boutons during several days of a head-fixed auditory trace fear learning paradigm. We found subpopulations of PV boutons showing activity responses to task related events and stimuli, such as the tone (CS), trace period, and airpuff (US). Notably, the activity patterns of these subpopulation ensembles of boutons became gradually structured, paralleling the animals’ fear learning behavior. We observed that the occurrence of sharp wave ripples (SWRs) increased during the behavioral course of fear leaning and that SWR events evoked rapid and strong activation of PV boutons. These results suggest that PV basket cells input task-related information to CA1 pyramidal cells during the learning of associated events across time delays. We hypothesize these inputs could contribute to structure the circuit ensembles of CA1 pyramidal cells mediating learning and supporting episodic memory.

**Disclosures:** S. Terada: None. M. Ahmed: None. E. Balough: None. J. Zhang: None. A. Solis Canales: None. A. Losonczy: None.
Title: Role of ER-mitochondria contacts in Ca\(^{2+}\)-dependent processes in dendrites

Authors: *J. O'HARE\(^{1,2}\), A. VILLEGAS\(^{1,2}\), S. ROLOTTI\(^{1,2}\), A. LOSONCZY\(^{1,2,3}\), F. POLLEUX\(^{1,2,3}\);
\(^{1}\)Zuckerman Mind Brain Behavior Inst., \(^{2}\)Dept. of Neurosci., \(^{3}\)Kavli Inst. for Brain Sci., Columbia Univ., New York, NY

Abstract: In dendrites, Ca\(^{2+}\) is critical in determining how neurons respond to incoming excitation. In non-neuronal cells, mitochondria can act as sinks for Ca\(^{2+}\) released from the endoplasmic reticulum (ER) by forming direct contacts with these concentrated intracellular Ca\(^{2+}\) stores. Recently we identified a tethering protein, PDZD8, that is required for these contacts and, consequently, for mitochondrial buffering of synaptically-evoked Ca\(^{2+}\) release from ER in dendrites. Using a variety of in vivo and ex vivo approaches in hippocampal area CA1, we are currently investigating how ER-mitochondria tethering (ERMT) contributes to dendritic morphology during development as well as ongoing dendritic function in adult mice. In addition to understanding the biological role of ERMT in neurons, we are interested in leveraging these interorganellar contacts as a means to specifically manipulate dendritic Ca\(^{2+}\) dynamics in vivo.

Disclosures: J. O'Hare: None. A. Villegas: None. S. Rolotti: None. A. Losonczy: None. F. Polleux: None.
Title: A role for the locus coeruleus in hippocampal CA1 place cell reorganization during spatial reward learning

Authors: *A. KAUFMAN¹, T. GEILLER¹, A. LOSONCZY²;
²Neurosci., ¹Columbia Univ., New York, NY

Abstract: During goal-directed navigation, place cells in the hippocampal area CA1 exhibit strong overrepresentation of rewarded locations, which is correlated with behavioral performance. Although previous studies have implicated canonical cellular mechanisms underlying the maintenance of this enrichment, the circuit mechanisms at its origin are still largely unknown. Neuromodulation via locus coeruleus (LC)-hippocampal projections regulates hippocampal circuit functions, notably spatial memory and learning. The co-release of both dopamine and norepinephrine in CA1 from LC-fibers makes this structure a strong candidate to promote the encoding and retention of reward locations through reconfiguration of hippocampal place cells. Here, we combine two-photon calcium imaging, together with a head-fixed goal-directed paradigm, to record the activity of LC axons and pyramidal neurons in CA1 of the hippocampus. We report that the LC-hippocampal projection signals the translocation of a reward in a familiar environment, predicting behavioral performance. Optogenetic stimulation mimicking this change in LC activity, performed simultaneously with CA1 pyramidal cell imaging, is sufficient to induce goal-directed remapping, primarily through a shift and stabilization of place cells near the reward. Stimulation in a task outside the reward zone, or that did not require the animal to learn the location of a reward, did not cause place field reorganization. Our results implicate the LC in a conjunctive system underlying spatially-guided reward learning.

Poster

334. Genetic and Molecular Mechanisms of Memory Formation

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 334.10/BB20

Topic: H.01. Animal Cognition and Behavior

Support: Revson Foundation Fellowship
Brain Initiative 1U01NS090583-02

Title: Network mechanisms of long-term spatial memory consolidation

Authors: *A. D. GROSMARK¹, F. T. SPARKS², M. J. DAVIS⁴, A. LOSONCZY³;
¹Dept. of Neurosci., ²Neurosci., ³Columbia Univ., New York, NY; ⁴Inst. for Neurosci., The Univ. of Texas at Austin, Austin, TX

Abstract: The mechanisms by which SWR-related activity leads to stable or unstable spatial-coding outcomes remains little understood. Moreover, limits on the sensitivity and speed of Ca²⁺-imaging techniques, as well as the absence of well-defined Ca²⁺-analogues of established biomarkers of ‘offline’ activity such the LFP-detected SWRs, have restricted the accessibility of ‘offline’ activity to Ca²⁺-imaging. Here we employ combined chronic CA1 LFP recordings with simultaneous fast (60 Hz) two-photon Ca²⁺ imaging to stably track the activity of genetically identified mouse CA1 principal neurons over weeks of ‘online’ spatial behavior and ‘offline’ resting. This project aims to predict the diverging cross-day spatial coding stability outcomes of place cells by their recruitment to and replay in SWR events - to link long-term spatial memory to the ‘offline’ SWR-related replay activity thought to underlie memory consolidation. On each recording/imaging day head-fixed animals ran on one of two cued-rich treadmill belts for a stably placed but ‘hidden’ water reward (RUN-epoch), thus facilitating the assessment of long-term spatial coding and its specificity to a given treadmill belt. On each day immediately ‘PRE’ and ‘POST’ the RUN epoch two additional recording/imaging sessions were carried out on an uncued, unrewarded treadmill belt facilitating the analysis of ‘offline’ content. Our preliminary results recorded under this paradigm demonstrate: 1) that joint LFP recording and Ca²⁺-imaging is an effective tool for studying fast neural dynamics, such as ‘offline’ SWR-related responses and ‘online’ theta-phase preferences, 2) the presence of both stable and unstable long-term (cross-day) spatial coding in CA1 place cells, 3) the first reported Ca²⁺-imaging of SWR-associated place cell reactivation of a recent but non-local spatial experiences, 4) that POST epoch SWR-recruitment, but not overall firing rate, predicts long-term spatial stability outcomes, and 5) that individual cells’ increases in PRE to POST contributions to SWR-associated reactivation are predictive of their long-term, multi-day spatial coding stability. These results, for the first time, directly implicate ‘offline’ SWR-related mnemonic activity as serving as a privileged window for the long-term consolidation of specific spatial memory traces in the
hippocampus. Ongoing work will test this hypothesis mechanistically through interventions triggered through the online detection of individual SWR events.

**Disclosures:**  
A.D. Grosmark: None.  
F.T. Sparks: None.  
M.J. Davis: None.  
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**Poster**

334. Genetic and Molecular Mechanisms of Memory Formation

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**Topic:** H.01. Animal Cognition and Behavior

**Support:**  
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NIH/NINDS 1U19NS104590

**Title:** The relationship of CA1 place cell dendritic activity during SWRs to online spatial tuning

**Authors:** *S. V. Rolotti, H. Blockus, F. T. Sparks, A. Losonczy; Neurosci., Columbia Univ., New York, NY

**Abstract:** Recent findings have connected active dendritic integration mechanisms to CA1 place cells’ (PCs) apparent role in spatial memory, suggesting that the generation of dendritic spikes might be important for the long-term maintenance of spatial tuning in single neurons. While previous work has focused on the role of dendritic activity during online navigation, far less is known about dendritic activity during ‘offline’ immobility states. In particular, sharp-wave ripples (SWRs) - network events that predominate during immobility - are thought to drive synaptic potentiation that may strengthen CA1 PC ensembles previously co-active during navigation and, by extension, stabilize the place map.

It remains unknown, however, how SWRs alter dendritic activity in vivo, or how dendritic activity during offline states affects online PC spatial tuning dynamics. We therefore conducted experiments in which we pair multi-plane, two-photon calcium imaging of sparsely labeled CA1 PCs with extracellular electrophysiological recordings in a head-fixed, behaving mouse over the course of many days. Using these methods, we longitudinally observed dendritic, somatic, and network activity of CA1 PCs as mice passively explore a novel environment or actively learn the location of a hidden reward.

We have found clear evidence for behavior- and network-state differences in somatic-dendritic coupling. The amplitude and frequency of somatic-related activity across the basal dendritic arbor is significantly increased during awake immobility, and in particular during SWRs, suggesting that SWRs may indeed modulate dendritic excitability and spiking. Further analysis of the specific subsets of basal dendrites active during exploration suggests that reactivation of
these patterns during SWRs may be indicative of a SWR-dependent dendritic stabilization process. We will share results on the impact of these changes on the stability of spatial tuning of CA1 PCs during exploration and learning behaviors. Using this unique intersection of approaches, we directly examine the impact of network-level events on CA1 PC dendritic activity \textit{in vivo} and in turn explore how these changes in dendritic activity might persistently modify PC spatial tuning.

**Disclosures:** S.V. Rolotti: None. H. Blockus: None. F.T. Sparks: None. A. Losonczy: None.

**Poster**

334. Genetic and Molecular Mechanisms of Memory Formation

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 334.12/BB22

**Topic:** H.01. Animal Cognition and Behavior

**Support:**
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R01 NS094668
U19 NS104590
R01 MH100631

**Title:** Characterizing direct cortical influences on hippocampal region CA1 in behaving mice

**Authors:** *J. BOWLER, A. LOSONCZY; Neurosci., Columbia Univ., New York, NY

**Abstract:** The hippocampus plays critical roles in navigation and episodic memory, with the most prominent neural correlate of these behaviors being “place cell” activity. It is widely assumed that afferent excitatory inputs to the hippocampus carry critical information required for these navigational and memory-related changes in hippocampal network dynamics. Two subregions of the Entorhinal Cortex (EC) have been particularly implicated in this process by providing distinct input streams to the hippocampus. Specifically, the medial EC (MEC) is thought to be involved in primarily processing spatial information related to global contextual reference frame, while the lateral EC (LEC) is thought to primarily process information related to individual items and locations based on a local reference frame. Despite these activity patterns having been well studied in the EC, a detailed understanding of the circuit level mechanisms of entorhinal cortical influences on hippocampal population dynamics is still lacking. The direct projections from MEC and LEC to CA1 pyramidal cells (CA1PCs) offer a tractable circuit to bridge this knowledge gap. Chronic, subcellular-resolution two-photon functional imaging of identified EC afferents and CA1PCs during head-fixed virtual reality navigation and learning provides an opportunity for direct observation and characterization of the information transferred between the EC and CA1. These experiments test the hypothesis that CA1PCs
receive spatial and sensory information from identified EC inputs which instruct CA1PCs population dynamics. Results indicate that it is possible to decode navigational variables from activity observed in the direct EC projections to CA1 such as position, cue identity and speed which could be critical to spatial representations in the hippocampus.

**Disclosures:** J. Bowler: None. A. Losonczy: None.

**Poster**

**334. Genetic and Molecular Mechanisms of Memory Formation**

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**Title:** Eph-Ephrin signaling is essential for formation of the cell clusters in layer II of medial entorhinal cortex

**Authors:** *N. YAMAMOTO*¹, H. OSANAI¹, W. D. MARKS¹, S. K. OGAWA¹, M. HENKEMEYER², T. KITAMURA¹;  
¹Psychiatry, ²Neurosci., Univ. of Texas Southwestern Med. Ctr., Dallas, TX

**Abstract:** The medial entorhinal cortex (MEC) has characteristic hexagonally arranged cell clusters in the layer II (MECII). These cell clusters are composed of Wolfram syndrome1 (Wfs1)+ pyramidal cells and surrounded by Reelin+ stellate cells in the MECII. The MEC integrates multimodal sensory inputs for memory formation, spatial navigation and time perception. Accumulating evidence from neural recording and imaging suggest that cells coding similar spatial/temporal information tend to be clustered in MEC, implicating that the cell clusters in the MECII may generate the functional modules. However, it remains unknown the molecular basis for the formation of anatomical cell clusters in MEC and how the anatomical modules affect functional modules in the MEC. Here, we hypothesized that the cell clusters may be generated by cell-cell-mediated interaction/repulsion. As the Eph receptors B1-3 and their ligands, the Ephrin-Bs, are well known as initiators of cell-contact-mediated neumomigratory signals, we first comprehensively examined their expression patterns in the MEC of adult mouse by fluorescent *in situ* hybridization (FISH). Among EphB/Ephrin-B family genes, we found EphB1 and EphrinB2 were selectively expressed in MECII. Double-FISH analysis revealed that EphB1 was expressed in Reelin+ cells, while Ephrin-B2 was expressed in Wfs1+ cells. Next, we
examined their expression pattern in the MEC during developmental stage. At postnatal day 0 (P0), EphB1 was already expressed in Reelin+ cells in MECII. At that time, no cell clusters were identified in MECII by nuclear staining. Rather, Ephrin-B2+ cells were located in the layer V of MEC at P0. At P2, Ephrin-B2+ cells have migrated to layer II and formed cell clusters in the MECII. At P4, the Ephrin-B2+ cells have expressed Wfs1. Finally, we examined the effect of EphB/Ephrin-Bs knockout (KO) on the formation of cell clusters. We found that cell clusters in the MECII of Ephrin-B2 mutant mice were severely impaired. We also found that the cell clusters were smaller in EphB1/EphB2 double knockout mice. These results suggest that Eph/Ephrin signaling between Reelin+ and Wfs1+ cells is critical for formation of cell clusters in the MECII during early postnatal development.


Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

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Title: Disrupting medial prefrontal cortex with DREADDs alters hippocampal place cell firing in sharp wave ripples in rats

Authors: *B. SCHMIDT, A. D. REDISH;
Dept. Neurosci, Univ. of Minnesota, Minneapolis, MN

Abstract: Current theories suggest that place cell firing sequences during hippocampal (HC) sharp wave ripple complexes (SWR, 180-220 Hz) play multiple roles in learning, memory, and decision-making. These functional differences can vary contingent upon the animal’s behavioral state. The medial prefrontal cortex (mPFC) may interact differently with HC in these different behavioral states. To examine the role of the mPFC in HC SWRs, rats were transfected with inhibitory DREADDs (h4MDi) targeting the prelimbic cortex. Behavior was compared under CNO or vehicle (VEH) control from 7 rats while recording neural ensembles from HC and mPFC. Rats ran the Restaurant Row task for 1 hr each day. On this task, rats encounter a series of stay or skip
decisions for flavored food pellets. Upon restaurant entry, the pitch of a tone signals the wait needed to receive the food reward (random delay 1-30s). At each choice, the rat could wait out the delay to receive the reward or skip and leave for the next “restaurant”. On this task, rats show thresholds for each flavor, below which they accept the offer and above which they reject the offer. The Restaurant Row is a neuroeconomic task because of the limited time on this task (time was a scarce resource).

To measure the effects of mPFC disruption on consolidation, we compared SWR events during pre- and post-maze rest. Disrupting mPFC with CNO reduced the number of SWRs expressed during post-maze rest. Under VEH, SWRs increased from pre- to post-maze rest, consistent with previous studies, a phenomenon believed to support consolidation. However, we did not observe this increase under CNO. However, we also found that sequence scores (Gupta et al 2010 Neuron) were greater under CNO than VEH. These data suggest that while mPFC disruption reduced the number of SWRs in post-maze rest, the SWR sequences that were expressed remained coherent. On the maze, during the time waiting for the reward, SWRs were expressed at a higher rate in the most-preferred restaurants (highest threshold). In contrast, during time lingering after receiving reward, SWRs were expressed at a higher rate in the least preferred restaurants (lowest threshold). During both waiting and lingering, CNO reduced the number of SWRs expressed in the most-preferred restaurants more than in the less-preferred restaurants. The different relationships on and off the maze suggest that on-maze and post-maze SWRs likely play different roles. Consistent with this, we also found that the relationship between mPFC cell firing and HC SWRs changed on and off the maze, and that on-maze mPFC cell firing relationships to SWRs were changed more than off-maze relationships.

Disclosures: B. Schmidt: None. A.D. Redish: None.

Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

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Title: Representation of choice and value in the ventral striatum during the restaurant row task
**Authors:** *G. W. DIEHL, B. SCHMIDT, Y. A. BRETON, A. D. REDISH;
Dept. Neurosci, Univ. of Minnesota, Minneapolis, MN

**Abstract:** A central component of decision making is the ability to weigh the subjective value of a choice and determine if it is worthwhile. The Restaurant Row task (RRow) provides an effective means of investigating such subjective decisions. On this task, rats circle an octagonal track earning different flavored food rewards at each of four “restaurants”. Upon entering each restaurant, rats are “offered” a random 1-30 sec delay, cued via an auditory tone, that they must wait out before earning the reward. Rats must decide to either stay and wait out the delay to earn the reward, or skip the offer and advance to the next restaurant where a new offer will be presented. Because rats are limited to 1hr on RRow, waiting out long delays has inherent cost and reduces the number of subsequent rewards that can be earned.

We sought to evaluate how the ventral striatum (predominantly Nucleus Accumbens Core; NAcC), a structure strongly implicated in the assessment of value, may represent the value of offers presented during RRow. We recorded 384 phasically spiking neurons (putative MSNs) from NAcC of 10 rats while they performed RRow and examined their firing properties when offers were presented and rats made stay/skip decisions. As rats approached each restaurant, aware of which flavor they were approaching but not yet informed of the delay they must wait to earn a reward, 23% of vStr cells exhibited significant firing rate changes with about half increasing and half decreasing firing rate. Following offer presentation, 31% of vStr cells exhibited additional changes in firing rate, again with about even proportions of cells increasing and decreasing firing rates. Interestingly, we found 13% of NAcC cells that exhibited rhythmic increases in firing rate every second, likely corresponding to the auditory tones that were played each second counting down the delay period. Collectively about 50% of NAcC cells exhibited significant firing rate changes around the initial offer period in the RRow task during which rats must make a stay/skip decision.

To determine how these firing rate changes may encode information necessary for a rat to make its decision we used Bayesian decoding to decode stay/skip decisions or offer value at each restaurant visit. NAcC ensembles did not represent offer value along a continuum but rather as a binary designation of “Good Offers” and “Bad Offers”. Furthermore, stay/skip encoding was initially consistent with skip decisions on most trials, but on trials with good offers encoding shifted to stay decisions after 0.5 - 1 sec. Thus, on this version of RRow, skip decisions appear to be the default cognitive state, with stay decisions emerging over time and only for high value offers.

**Disclosures:** G.W. Diehl: None. B. Schmidt: None. Y.A. Breton: None. A.D. Redish: None.

**Poster**

335. Learning and Memory: Cortical-Hippocampal Interactions II

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 335.03/BB26
**Title:** Goal encoding in prelimbic cortex and CA1 on a contingency-switching task for rats

**Authors:** *B. HASZ*¹, A. D. REDISH²;
¹Grad. Program in Neurosci., ²Dept. Neurosci, Univ. of Minnesota, Minneapolis, MN

**Abstract:** Hippocampus (HC) and prelimbic cortex (PL) are thought to play roles in a deliberative neural system which uses information about reward, state, and context to make goal-directed decisions. Previous work has shown that during vicarious trial and error (VTE, a behavioral marker of deliberation), HC ensembles sometimes represent paths to the goal. While PL is known to represent contextual information, it remains unclear if PL participates more actively in deliberative processes, and what relationship exists between the information encoded in PL and HC during deliberation.

To investigate this relationship, we designed a contingency-switching task for rats, in which rats alternated between procedural modes (as they repeatedly made choices under a single contingency) and deliberative modes (after intermittent contingency changes). The task consisted of a spatial maze with a single choice point between left or right. The contingency on each lap was either “left” (go left at the choice point to receive reward), “right”, or “alternate” (make the opposite choice as the previous lap to receive reward), and this contingency changed every 30 +/- 5 laps. Consistent with previous results, VTE increased after contingency changes and decreased as paths became regularized with repeated laps under a given contingency.

6 rats (4M, 2F) were run on this switch task while recording simultaneously from HC dorsal CA1 (using 24-tetrode drives) and PL (using 32-site silicon probes). Position was decoded from HC and PL ensemble firing rates using Bayesian decoding. Replicating previous results, we found that HC ensembles encoded positions further ahead of the rat during passes through the choice point where VTE occurred. We also found that PL ensembles encoded positions further ahead of the rat during VTE than during non-VTE choice point passes, though the effect was smaller than in HC. Additionally, the distance ahead of the animal encoded by HC ensembles was correlated with the distance ahead of the animal encoded by ensembles in PL. This correlation may have been driven by nonlocal representation of the goal: we also observed a correlation between the encoding of the reward zone in PL and forward representations in HC at the choice point. Our results suggest that in addition to HC, PL also represents future goals during deliberation, and that these goals are often encoded in both structures simultaneously.

Future work will need to determine whether the goal encoding in one structure is driven by the other, or whether this representation is due to some common input from a separate source.

**Disclosures:** B. Hasz: None. A.D. Redish: None.
**Title:** Human cocaine users, like cocaine-treated mice, display unique disruptions on a neuroeconomic task

**Authors:** *B. M. SWEIS*¹, J. CAMCHONG², S. V. ABRAM³, S. SPECKER², A. W. MACDONALD, III³, M. J. THOMAS⁴, A. D. REDISH⁴, K. O. LIM²;
²Dept. Psychiatry, ³Dept. Psychology, ⁴Dept. Neurosci, ⁴Univ. of Minnesota, Minneapolis, MN; ⁵Mental and Behavioral Hlth., San Francisco VA Med. Ctr., San Francisco, CA

**Abstract:** Individuals who desire to recover from addiction often make poor decisions and relapse despite this desire. Impairments in decision conflicts that result in relapse could arise from dysfunctions in distinct neural systems. Recently, we used a novel neuroeconomic task translated across species to dissociate separable conflict algorithms through complex behavioral analyses equally applicable to both rodents and humans. We previously found that conflict when deliberating about the future was uniquely disrupted in cocaine-treated mice distinct from conflict when re-evaluating past actions, the latter of which was uniquely disrupted in morphine-treated mice and sensitive to infralimbic-accumbens shell circuit manipulations in mice. To date, only healthy humans have been tested in this novel paradigm. How human cocaine dependent users perform within this neuroeconomic framework is unknown.

We recruited 12 cocaine users (mean age: 39.7 [sem 3.0], 16.6% female, 58.3% white) and 9 healthy matched controls (mean age: 35.9 [sem 2.9], 22.2% female, 55.6% white) from online advertisements. Participants were tested on the computer-based Web-Surf task. Participants spent time from a limited budget (20 min) to earn natural rewards, i.e., entertaining video clips (4s in playback length) of different genres (cued by an icon), available after varying costs (delays ranging 1-30s, cued by a download bar and text). Participants were tasked to accept or reject serial offers and then rate consumed videos on a scale of 1-4 stars. Participants filled out drug use history questionnaires and were compensated $40.
All participants performed the Web-Surf task, reliably responding to randomly presented cued offers differently in each video gallery as a stable function of cost and subjective preferences. Only when encountering unique conflict scenarios (offers with a delay above one’s willingness to wait), cocaine users were less likely to appropriately reject economically disadvantageous offers compared to healthy controls. Furthermore, cocaine users did so despite spending more time deliberating. These data match cocaine-treated mice tested on the translated Restaurant Row task. Interestingly, for post-consumption hedonic valuation responses, human cocaine users and cocaine-treated mice each differ in a dissimilar way from their respective controls. These data help elucidate the pathophysiological mechanisms of substance use disorder underlying impairments in distinct aspects of decision making that are both shared and different between humans and non-human animals, particularly in the context of the conflict between wanting versus knowing better.


Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 335.05/BB28

Topic: H.01. Animal Cognition and Behavior

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Title: Neuroeconomic decision-making in J20 mice

Authors: *R. M. ANDERSON*1, B. M. SWEIS2, D. HART1, M. SHERMAN1, S. E. LESNE1, M. J. THOMAS1, A. D. REDISH1;
1Dept. Neurosci, 2Univ. of Minnesota, Minneapolis, MN

Abstract: Current theories suggest that decisions arise from interacting systems accessing distinct neural networks. Disruptions (such as in Alzheimer’s Disease) can change the integrity of these networks and thus change neuroeconomic behavior. 15 6-month old mice were run behaviorally on the Restaurant Row task, in which mice forage for food under a time constraint. The task consists of four restaurants, each providing a different flavor of food. On encountering a
restaurant, the mouse is informed of the delay before food arrives (1-30s) by pitch of a tone. The mouse can then accept the deal, waiting out the delay, or reject it, proceeding on to the next restaurant. Each restaurant consisted of two zones: an OFFER zone in which the mouse was informed of the delay, but the delay did not count down, and a WAIT zone in which the delay counted down until reward was delivered. On this task, mice reveal unique delay thresholds for each flavor below which they tend to accept and above which they tend to reject offers. Deliberation can be observed in the OFFER zone as vicarious trial and error behavior, while quitting out of the WAIT zone engenders regret.

Mice (8 wild type, 7 J20 transgenic) were run for 70 consecutive days. The environment began reward-rich and transitioned to reward-scarce as the range of delays increased (1s for 1 wk, 1-5s for 5d, 1-15s for 5d, 1-30s for the rest of the experiment). At this age, J20 mice display spatial reference memory retention deficits in the Barnes maze. All mice altered their strategy after reaching the reward-scarce scenario, increasing deliberation and planning behaviors over time. However, J20 mice altered their behavior much more quickly compared to their control counterparts. As a result of this quick change in their behavior, J20 mice became more efficient more quickly as evident by their ability to earn significantly more rewards in the same time span when compared to control animals.

J20 mice deliberated significantly more than controls in the OFFER zone. Upon further examination, J20 mice were found to deliberate for both bad deals (delays above threshold) and good deals (delays below threshold) unlike control mice who only deliberated for bad deals. J20 mice showed fewer enters into restaurants and fewer quits for bad deals. These results suggest that rather than showing a problem with deliberation, J20 mice deliberate more, potentially due to disruptions in other neural circuits.

This study highlights the importance of behavioral tasks that allow for dissection of multiple decision-making systems that may be at play and independently change in neurodegenerative diseases that might otherwise manifest as gross “memory deficits” using simpler paradigms.

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Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 335.06/BB29

Topic: H.01. Animal Cognition and Behavior

Title: A spoonful of sugar helps theta-gamma coupling go down

1Psychology, 2Psychiatry, Univ. of Nevada Las Vegas, Las Vegas, NV

Abstract: Working memory impairments are among the many debilitating symptoms of Alzheimer’s disease (AD) and other neurodegenerative disorders. Working memory is supported by synchronization of theta and gamma frequencies in the anterior cingulate cortex (ACC) and hippocampus (HC). In fact, decreased theta-gamma coupling is the most significant predictor of WM performance in human AD patients, and alterations in this cross-frequency relationship precedes histological hallmarks in a rodent model of AD. Although the etiology of sporadic AD is unknown, several risk factors have been identified and prominent among these is diabetes mellitus (DM). Patients with DM are 4 times more likely to develop AD and also exhibit similar working memory impairments as those found in AD. Chronic neuroinflammation is a pathological symptom of both DM and AD, and it is possible that neuroinflammation itself impacts cognitive performance. Given the links between DM and AD, an examination of ACC and HC cross-frequency synchronization in a diabetic rodent model could potentially help elucidate the neural processes underlying AD-related working memory impairments. In this study, we hypothesize that neuroinflammation disrupts network oscillatory activity within the ACC and HC, as well as the interactions between these areas, these combined effects lead to impaired cognitive function. To test this, we trained rats to perform a delayed alternation task, which is dependent upon both the ACC and HC, as well as interactions between these areas. Following training, we induced chronic hyperglycemia by injecting experimental animals with multiple low doses of streptozotocin. We found delay dependent spatial working memory impairments in hyperglycemic animals compared to healthy controls. Electrophysiological data revealed decoupling between the theta (5-12 Hz) and gamma (30-50 Hz) bands within both areas. We also found significant disruptions in theta coherence between brain regions in the hyperglycemic group. These animals also exhibited increased theta band oscillations and decreased delta (1-3 Hz) band oscillations in the ACC and HC. Together, these results suggest that the hyperglycemia-driven neuroinflammatory state that is associated with the physiological and cognitive pathologies of AD, also induces alterations in ACC and HC oscillatory activity and interactions.


Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 335.07/BB30

Topic: H.01. Animal Cognition and Behavior
Title: Isolating orthogonal learning signals from ACC ensembles

Authors: *R. M. FRANCIS¹, R. A. WIRT¹, J. M. HYMAN²;
¹Psychology, ¹Univ. of Nevada Las Vegas, Las Vegas, NV

Abstract: Basic principles of learning suggest that associations are preserved until new, contradictory information is encountered. When expectations are not met, one must quickly adapt to the newly encountered information. This can be observed in both behavioral and physiological responses, but the mechanisms involved have yet to be fully characterized. When outcomes change, neurons in multiple brain areas adjust their responses to the new information which is thought to lead to behavioral shifts. How neurons track these outcomes, or more generally, how they track the entire learning experience, is still a matter of intense study. In recent years, neuronal discharge patterns have been successfully fit to Q-learning algorithms, suggesting that neuronal activity states follow simple learning rules. To investigate such responses further, we evaluated the role of the rodent anterior cingulate cortex (ACC) during a reversal learning task with three reward ports, each with a different reward probability. We performed a principal component analysis on ACC ensemble signals during this task to create base parent signals for further examination. We then isolated various signals within the top 3 principal components using support vector machines tuned to decode categorical state homologues to specific learning strategies. This method revealed nine clearly orthogonal signals derived from specific learning assumptions nested within the same parent signal. These included a signal that was sensitive to the mid-session probability reversal, a signal that was reversal-agnostic and sensitive to the outcome of only the previous trial, and another that updated based on the entire sample of trials akin to Q-learning models. Thus, we demonstrate how the ACC can simultaneously process multiple changing reward probabilities while tracking time-sensitive representations of the most recent outcomes and time-invariant signals such as reversal occurrence. The ability to maintain real-time parallel computations potentially explains why the ACC is so strongly involved in this type of learning. These types of embedded multiplexed representations are likely integral to cognitive flexibility by offering alternative streams of information to exploit based upon specific contextual criteria.


Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 335.08/BB31

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH108729
Title: Interactions in the local field potentials of the perirhinal and postrhinal cortices during memory and attention tasks

Authors: *S. G. TRETTEL, R. D. BURWELL;
Dept. of Cognitive, Linguistic, and Psychological Sci., Brown Univ., Providence, RI

Abstract: The perirhinal and postrhinal cortices each are involved in both memory and attention. The perirhinal cortex is primarily involved in object recognition memory whereas the postrhinal cortex is primarily involved in visual-spatial memory and attention. Though these two regions appear to have differing roles, there are reciprocal connections between the two regions. How these two regions interact, and the roles these interactions play in supporting the functions of the other region during memory and attention tasks are not well understood. In this study, our objective was to use field potential recordings during tasks that engage attention and memory to investigate how these two regions interact. We recorded simultaneously from both regions while rats performed either a visual-spatial attention task or a novel-object-recognition task. Oscillations in the local field potential are thought to coordinate the activity of neuronal ensembles across brain regions. Previous studies have shown that in the hippocampus and its connected cortical regions, theta (6-10 Hz), slow gamma (25-55 Hz), and fast gamma (65-100Hz) oscillations all appear to play a role in temporarily synchronizing cell ensembles to perform different computations. In previous work from the Burwell lab, 10-15 Hz stimulation of the perirhinal cortex caused rats to explore novel objects as if they were familiar during a novel-object recognition task. We used local field potential data recorded simultaneously from the perirhinal and postrhinal cortices of Long-Evans rats as they performed a visual-spatial attention task in addition to a novel-object-recognition task. We compared the oscillations between these two regions, and we investigated how these oscillations changed across cortical layers. Preliminary analyses from animals exploring an open field suggest that the strongest oscillatory coupling happens in the fast gamma band, but there is some evidence that theta and beta (11-20 Hz) oscillations in the postrhinal cortex synchronize with weak theta and beta-band activity, respectively, in the perirhinal cortex.

Disclosures: S.G. Trettel: None. R.D. Burwell: None.

Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 335.09/BB32

Topic: H.01. Animal Cognition and Behavior

Support: NIMH 1R01MH108729
NSF IOS-1656488
Carney Institute for Brain Science at Brown University
Title: Local field potential and single cell encoding of the retrosplenial cortex during performance of a visuospatial attention task

Authors: *E. HWANG¹, F.-C. YANG³, R. D. BURWELL²;
²Dept. of Cognitive, Linguistic, and Psychological Sci., ¹Brown Univ., Providence, RI; ³Dept. of Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: The retrosplenial cortex (RSC) is defined in primates and non-primate mammals. The anatomical location and neuronal connections of the RSC make this brain area an important putative intermediary region for a variety of cognitive and behavioral functions. Recently, the RSC has become an important focus of human cognition research, but there is considerable disagreement about the precise role of the RSC in cognition. Current studies implicate the RSC in navigation, spatial memory, processing of contextual information, sensory integration, cross-modal information processing, and the representation of stability or reliability. Although the preponderance of the literature addresses hypotheses about the spatial functions of the RSC, there is evidence to suggest that the core function of the region is not limited to the processing of spatial information. We hypothesize that the core function of the RSC contributes to non-spatial domains. Here we report a study where we examine single cell recordings and local field potential (LFP) of the RSC in a visuospatial attention task. The VSA task is a purely visual task that was adapted from the five choice serial reaction time task (Bari, Dalley, Robbins, 2008) for a double-sided, bowtie shaped enclosure atop the Floor Projection Maze (Jacobson et al., 2014). The VSA task involves top-down and bottom-up visuospatial attention, multiple spatial reference frames, and the processing of multiple task epochs and reward. In this study, we specifically investigate if the single cell recordings and LFP power changes show correlates to stimulus onset and prediction error. Detailed analyses of neuronal correlates and LFP for RSC will be presented.


Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: 335.10/BB33

Topic: H.01. Animal Cognition and Behavior

Support: R01MH057414
         R01MH101209
         R01NS024760

Title: Hippocampal projections target excitatory and distinct inhibitory neurons in anterior cingulate cortex in primates
Authors: *J. WANG, M. D. FEINBERG, I. N. ONOCHIE, H. BARBAS;
Boston Univ., Boston, MA

Abstract: Direct pathways from hippocampus to medial prefrontal areas in the anterior cingulate cortex (ACC) carry information about current context and past experiences and play key roles in decision making and emotional regulation. However, our knowledge about the features of these pathways in primates is limited. We thus investigated hippocampal terminations in ACC, including A25, A32 and A24a, with anterograde tracers in rhesus monkeys. We found that hippocampal pathways innervate both upper (I-III) and deep (V-VI) layers of ACC. Stereologic analysis showed that subgenual A25 contained the highest proportion and density of hippocampal boutons among the three areas, suggesting that it is a major target of the hippocampus. Using brightfield microscopy, we found that the major bouton diameters were similar in A25, A32 and A24a, and between the upper and deep layers, a pattern confirmed for hippocampal terminations in A25 using electron microscopy (EM). We then studied the postsynaptic targets of the hippocampal terminations in the upper and deep layers of A25 using EM. Interestingly, most (~90%) hippocampal terminations formed synapses on spines of presumed excitatory neurons in all layers of A25, while a small proportion (~7%) formed synapses on dendritic shafts of presumed inhibitory neurons. To further investigate the type of inhibitory neurons innervated by hippocampal afferents in A25, we used calcium binding proteins calbindin (CB), calretinin (CR) and parvalbumin (PV), which label non-overlapping populations of functionally distinct cortical inhibitory neurons in primates. Using fluorescent confocal microscopy, we investigated appositions between hippocampal terminals and labeled inhibitory neurons. More hippocampal axon boutons were apposed with CR+ neurons than CB+ or PV+ neurons, consistent with EM analyses. These findings suggest that the hippocampus has a stronger effect on CR+ neurons, which are thought to inhibit other inhibitory neurons, and thus disinhibit nearby pyramidal neurons. This evidence suggests that primate hippocampal pathways can enhance activity in A25 via innervation of mostly excitatory neurons and CR+ (disinhibitory) neurons, facilitating mnemonic and cognitive processes.


Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 335.11/BB34

Topic: H.01. Animal Cognition and Behavior

Support: NIH R01MH057414
NIH R01MH101209
NIH R01NS024760
Title: Context and motivation: A computational model of interactions between hippocampus and medial prefrontal cortex, and their roles in depression

Authors: *Y. J. JOHN*, D. H. BULLOCK, H. BARBAS;  
1Neural Systems Lab., 2Psychological & Brain Sci., Boston Univ., Boston, MA

Abstract: Depression is a multifaceted disorder that affects emotion, cognition, and motivation for goal-oriented behavior. Despite the existence of several types of treatment, treatment-resistant forms of depression are still widespread, as are post-treatment relapses. Understanding the neural bases of depression subtypes will enable insight into the origins of depression as well as the diversity of patient responses to treatments. Several lines of evidence implicate limbic circuitry in depression, particularly limbic prefrontal area A25, ventral striatum, and the hippocampus. Here we focus on possible neural substrates of the loss of motivation in depression. Evidence suggests this aspect of depression may involve abnormal signaling from medial prefrontal cortices to the ventral striatum, which has long been linked with motivational vigor. Specifically, abnormal firing in medial prefrontal cortices appears to preferentially suppress reward-seeking behaviors mediated by ventral striatal activity. Normally, contextual representations activated in the anterior hippocampus inform processes in key limbic areas that enable an organism to flexibly switch among behaviors, by invigorating those with net-positive prospects and disengaging from those with net-negative prospects, which vary by context. In depression, disordered processing in hippocampus and A25 may lead to dis-invigoration of behaviors with net-positive prospects. In our analysis, projections from the primate anterior hippocampus to medial prefrontal cortices, and particularly A25, allow abnormal processing of context to contribute to the emergence of low motivational states. Our simulations show how perturbations to the circuit linking hippocampus and A25 can disrupt context processing, cause abnormal signaling to ventral striatum, and disrupt its ability to generate invigoration signals. The proposed mechanisms suggest experimental tests to probe the origins of depression-related symptoms, and may lead to novel strategies for treatment.


Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 335.12/BB35

Topic: H.01. Animal Cognition and Behavior

Support: Branco Weiss Fellowship - Society in Science

Title: Behaviour matters learning influences hippocampal ripple occurrence and cross brain responses during sleep
**Authors:** A. ALEMAN¹, F. P. BATTAGLIA², *L. GENZEL¹;
¹Donders Inst., Nijmegen, Netherlands; ²Donders Inst. for Brain, Cognition and Behavior, Radboud Univ., Nijmegen, Netherlands

**Abstract:** Many studies have shown that sleep benefits memory. We are starting to understand the mechanisms occurring during sleep, with sleep-related memory replay leading to systems consolidation and strengthening cortical memory networks. Memory replay in the hippocampus and prefrontal cortex occurs during sharp-wave-ripples, a very fast oscillation measurable in the hippocampus. However, most replay studies focus use over-trained tasks such as track-running or non-informative behaviors such as random foraging. Under such circumstance one would not expect memory consolidation mechanisms to be as present as after a significant learning event. Here we record from electrodes in the hippocampus, prefrontal and parietal cortex during sleep after different behaviors: a novelty event, a track-running foraging task and learning a new goal location in a Plusmaz e task. Surprisingly, learning led to a decrease in hippocampal ripple power, however the ripples that did occur showed stronger responses in both the prefrontal and parietal cortex within the ripple frequency range. The opposite was seen after track-running. Further, granger causality indicated stronger connectivity between the three brain areas selectively after learning. We went on to show that learning the new goal location in the Plusmaze is dependent on sleep after learning and using a closed-loop, electrical ripple disruption paradigm we could mimic the sleep-deprivation effect on memory. In sum, we could show that only after significant learning experiences increased hippocampal-cortical connectivity could be seen during NonREM sleep ripples, which was necessary for the beneficial effect of sleep on memory.

**Disclosures:** A. Aleman: None. F.P. Battaglia: None. L. Genzel: None.

**Poster**

335. Learning and Memory: Cortical-Hippocampal Interactions II

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #: Poster #:** 335.13/BB36

**Topic:** H.01. Animal Cognition and Behavior

**Support:** Branco Weiss Fellowship

**Title:** Allocentric vs egocentric memory in rats and humans: Effects of sleep

**Authors:** *A. SAMANTA¹, J. JACOBSE², J. ROSSATO², R. G. MORRIS³, L. GENZEL⁴;
¹Radboud Univ., Nijmegen, Netherlands; ²Univ. of Edinburgh, Edinburgh, United Kingdom; ³Ctr. for Cognitive and Neural Systems, The Univ. of Edinburgh, Edinburgh, United Kingdom; ⁴Donders Inst., Nijmegen, Netherlands
Abstract: AIM: To study the effect of sleep on egocentric vs allocentric spatial memory training and the underlying neural mechanisms in rats and humans
It has been shown that hippocampal-led replay during sleep leads to subsequent improvements in memory and is thus interesting to see its differential effects on allocentric (hippocampal) vs egocentric (striatal) memory conditions.
Rats were first trained on the water maze under either allocentric or egocentric condition. In the allocentric condition, they had a different starting location to find the platform, whereas in the egocentric condition, the starting location remained the same throughout. Post training they either had 6h sleep or sleep deprivation (SD) followed by a probe trial after 24 hours. After probe trial, retrieval induced immediate early gene expression were measured in the hippocampus, PFC and striatum. Analogously a virtual water maze was developed to train humans under both conditions. They were initially trained on the task in the MRI scanner under either allocentric or egocentric condition, followed by a short nap or movie and later tested with a probe trial in the scanner.
In rats, the improvement in memory was observed for those who slept only in the allocentric condition. Performance in the egocentric condition was independent of sleep effects. Following sleep they showed increased levels of c-fos, ARC and zif in all three brain areas for both training conditions. SD rats, however, showed increased expression only in the hippocampus and striatum under allocentric and egocentric conditions respectively. In humans, however, the improved memory effect was observed for those who took a nap independent of training condition. Analogously, MRI findings in humans show an activation of regions in the Default Mode Network during training and a shift to activation of cortical regions during test session, only in the sleep condition.
Overall, we show sleep to have an improved memory effect across both models. IEG expression analyses and MRI findings indicate that sleep leads to a whole brain systems consolidation, which potentially is the underlying reason behind the improved memory effects.

Authors: *I. NAVARRO-LOBATO*¹², F. P. BATTAGLIA³, L. GENZEL⁴;
¹Neuroinformatics, Radboud Unviversity, Nijmegen, Netherlands; ²Neuroinformatics, Donders Inst. for Brain, Cognition and Behaviour / Radboud Unviversity, Nijmegen, Netherlands; ³Donders Inst. for Brain, Cognition and Behavior, Radboud Univ., Nijmegen, Netherlands; ⁴Donders Inst., Nijmegen, Netherlands

Abstract: RGS14 is an endogenous multidomain protein implicated in the control of several intracellular signalling pathways which facilitate memory consolidation. Insertion of this protein leads to an increased structural plasticity and memory consolidation of simple tasks. Here we want to test the effect of increased plasticity in the cortex on semantic memory updating. How are semantic memories created and stored? These questions have been under theoretical inquiry for decades. Marr proposed the need for a two-step storage system: 1) a fast learner rapidly recording for intermediate storage - represented by the hippocampus - and 2) a slow learner with compressed, long-term storage - the cortex. These two storage systems are thought to differ in the amount of plasticity and how easily memory traces are updated, however concrete evidence is missing due to the lack of appropriate methods. The Object Space Task is a newly developed multi-trial behavior task to test for memory abstraction in rodents. The rats are exposed to multiple sample trials where they are exposed to novel objects in specific spatial arrangements in a box. In the key condition of the task (overlapping), one location is stable across all trials while the other keeps changing. In the test trial, rats show a preference for the object in the changing location, indicating that they are capable of abstracting memory across several episodes. Interestingly, by rendering the prefrontal cortex more plastic via the overexpression of RGS14 made the usually robust abstracted memory become vulnerable to an interference trial at 24 h, which spatial configuration was incongruent to the previous trained pattern. In contrast, simple episodic memory was strengthened. Interestingly, in control animals the interference event led the original abstracted memory to be more long lasting (72 h instead of only 24 h).


Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 335.15/BB38

Topic: H.01. Animal Cognition and Behavior

Support: Phd Scholarship Studienstiftung des Deutschen Volkes

Title: A hippocampus prefrontal cortex network for reward prediction

Authors: *J. L. KLEE, F. P. BATTAGLIA*
Donders Inst. for Brain, Cognition and Behavior, Radboud Univ., Nijmegen, Netherlands
Abstract: Learning to predict future rewards constitutes a central element of intelligent behavior but also poses a complex problem for neural networks. While a multitude of studies have implicated both the hippocampus and the prefrontal cortex in learning as well as in responses to reward predicting cues, relatively little is known about the underlying interactions between the two structures. In this context, we combined an auditory trace conditioning paradigm in head-fixed mice with simultaneous silicon probe recordings from the hippocampus CA1 region and the medial prefrontal cortex. We find that CS+ presentations result in a learning dependent increase in HPC PFC Gamma phase coherence (80-120 Hz) and simultaneously elevated CA1 and mPFC Gamma power. Furthermore, on the single cell level, subpopulations of both CA1 as well as mPFC neurons exhibit learning dependent sustained activity during the trace period following CS+ presentations. Interestingly, by introducing a second CS+ sound to the conditioning task, we show that CA1 as well as mPFC population activity maintains a working memory representation of stimulus identity rather than of response type. Finally, we find that patterns of evoked responses after CS presentations are replayed during Sharp-Wave Ripples in CA1 and PFC and that this replay is stronger for patterns derived from CS+ stimuli. In combination, these results suggest that the hippocampus and medial prefrontal cortex interact in multiple ways to support learning of and correct responses to reward predicting cues.

Disclosures: J.L. Klee: None. F.P. Battaglia: None.

Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 335.16/BB39

Topic: H.01. Animal Cognition and Behavior

Support: Predoctoral fellowship 2018fi_B_00112
MINECO SAF2013-49129-C2-2-R (AEI/FEDER, UE)
MINECO SAF2016-80726-R (AEI/FEDER, UE)

Title: Neural substrates of memory impairment and rescue in a mouse model of schizophrenia

Authors: *C. DELGADO-SALLENT¹, T. GENER¹, A. B. FATH¹, P. NEBOT¹, M. BRY², M. V. PUIG¹;
Barcelona, Spain; ²Univ. of Bordeaux, Bordeaux, France

Abstract: Cognitive deficits are a core feature of schizophrenia (SCZ) but respond poorly to the available medication. Better understanding of the neural basis of these deficits is essential for the development of new treatments. Higher-order cognitive processes depend on a precise synchronization between the prefrontal cortex (PFC) and the hippocampus (HPC) that is
disrupted in SCZ. The subchronic phencyclidine (sPCP) mouse model induces SCZ-like symptoms including cognitive impairment. We recorded neural activity in the PFC and HPC of C57BL/6J mice before and after administration of sPCP during quiet wakefulness, open field exploration and the novel object recognition test that assesses working memory and long-term memory abilities. During rest, sPCP mice showed increased delta (2-5 Hz) power in PFC, increased prefronto-hippocampal phase synchronization (wPLI) at theta (8-12 Hz), beta (15-25 Hz) and low gamma (30-50 Hz) and desynchronization at high gamma (50-100 Hz). During active exploration, sPCP mice showed increased PFC theta, beta and high gamma and reduced HPC low and high gamma oscillations. Prefronto-hippocampal synchronization was also disrupted during active exploration in sPCP-treated mice. Theta and beta wPLI increased, as it also did in resting states, while low gamma wPLI decreased. Importantly, working memory and long term memory were profoundly impaired in sPCP-treated mice and partially rescued by a 14-day treatment with the atypical antipsychotic drug risperidone. Risperidone is a broadly used drug that targets the dopaminergic and serotonergic systems. Our findings suggest that the alterations on prefronto-hippocampal networks may underlie the neural mechanisms of memory impairment in sPCP-treated mice that can be corrected by risperidone.


Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 335.17/BB40

Topic: H.01. Animal Cognition and Behavior


Title: Serotonin 5-HT1a, 5-HT2a and dopamine D2 receptors strongly influence prefronto-hippocampal neural networks in alert mice: Contribution to the actions of risperidone

Authors: *T. A. GENER1, A. TAUSTE CAMPO2, M. ALEMANY1, P. NEBOT1, C. DELGADO-SALLEN1, J. CHANOVAS3,1, M. V. PUIG1; 1IMIM -Hospital Del Mar Med. Res. Inst., Barcelona, Spain; 2Barcelonañeta Brain Res. Ctr., Barcelona, Spain; 3Dept. of Ophthalmology, SUNY Downstate Med. Ctr., Brooklyn, NY
Abstract: Atypical antipsychotic drugs (APDs) useful to treat positive and negative symptoms in schizophrenia block serotonin receptors 5-HT2AR and dopamine receptors D2R and stimulate 5-HT1AR directly or indirectly. However, the cellular mechanisms mediating their therapeutic actions remain unresolved. We recorded neural activity in the prefrontal cortex (PFC) and hippocampus (HPC) of freely-moving mice before and after acute administration of 5-HT1AR, 5-HT2AR and D2R selective agonists and antagonists and atypical APD risperidone. We investigated the contribution of the three receptors to the actions of risperidone on brain activity via statistical modeling and pharmacological reversals (risperidone + 5-HT1AR antagonist WAY-100635, risperidone + 5- HT2A/2CR agonist DOI, risperidone + D2R agonist quinpirole). Risperidone, 5-HT1AR agonism with 8-OH-DPAT, 5-HT2AR antagonism with M100907, and D2R antagonism with haloperidol caused varying degrees of sedation in mice that correlated with a suppression of neural spiking, power of theta and gamma oscillations in PFC and HPC, and a reduction of PFC-HPC theta phase synchronization. By contrast, activation of 5-HT2AR with DOI enhanced high-gamma oscillations in PFC and PFC-HPC high gamma connectivity likely mediating its hallucinogenic effects. Together, power changes, regression modeling and pharmacological reversals suggest an important role of 5-HT1AR and 5-HT2AR in risperidone-induced effects on delta, beta and gamma oscillations, while D2R may contribute to risperidone-mediated changes in delta oscillations. This study provides novel insight into the neural mechanisms of risperidone and may help elucidate the neural substrates of widely prescribed psychiatric medication targeting the serotonin and dopamine systems in two brain regions involved in the pathophysiology of schizophrenia.


Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 335.18/BB41

Topic: H.01. Animal Cognition and Behavior

Support: MINECO SAF2016-80726-R (AEI/FEDER, UE)
Fulbright Grant

Title: Neurophysiological effects of the selective 5-HT7 antagonist, SB-269970, in a mouse model of schizophrenia

Authors: *A. B. FATH\textsuperscript{1}, T. GENER\textsuperscript{1}, C. DELGADO-SALLENT\textsuperscript{1}, P. NEBOT\textsuperscript{1}, M. BRY\textsuperscript{2}, M. PUIG\textsuperscript{1};
\textsuperscript{1}Integrative Pharmacol. and Systems Neurosci., IMIM -Hospital Del Mar Med. Res. Inst., Barcelona, Spain; \textsuperscript{2}Univ. of Bordeaux, Bordeaux, France
Abstract: A wide body of evidence has extensively demonstrated that normal executive function depends on a specific pattern of spatio-temporal communication between the prefrontal cortex (PFC) and the hippocampus (HPC). It has been found that this type of PFC-HPC communication is abnormal in schizophrenia (SCZ). 5-HT7 receptors have been implicated in learning and memory processes and the blockade of these receptors may be a potential target for cognitive amelioration in SCZ. In order to elucidate the cellular mechanisms of the 5-HT7R we administered the 5-HT7R agonist, AS19 (10 mg/kg), and subsequently the 5-HT7R antagonist, SB-269970 (8.0 mg/kg) while recording neural activity in the PFC and HPC of freely-moving C57BL/6J mice. After injection of AS19 we find a decrease in low gamma (30-50 Hz) and high gamma (50-100 Hz) power in the PFC and HPC that is recovered by SB-269970. In order to elucidate the pro-cognitive actions of 5-HT7R antagonism, we assessed the behavioral and neurophysiological effects of the 5-HT7R antagonist before and after subchronic phencyclidine (sPCP)-treated mice while assessing memory performance in the Novel Object Recognition task (NOR). The NOR was performed one hour after acute injection of the 5HT7R antagonist (4.0 mg/kg). The 5HT7R antagonist increases memory performance in healthy mice and ameliorates sPCP-induced memory impairment in the NOR task. In normal and sPCP-treated mice, the 5HT7R antagonist induces a robust decrease in the PFC low gamma and high gamma power and increases delta (2-5 Hz) power. In the HPC it decreases beta and high gamma power, while increasing delta. Following sPCP, there is also a hypersynchronization of prefronto-hippocampal connectivity at high gamma which is rescued by the 5HT7R antagonist. Our findings provide new insights into neural mechanisms of the 5HT7R actions and potential new treatments for cognitive impairment in SCZ.


Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 335.19/BB42

Topic: H.01. Animal Cognition and Behavior

Support: Predoctoral fellowship BES-2014-070429
MINECO SAF2013-49129-C2-2-R (AEI/FEDER, UE)
MINECO SAF2016-80726-R (AEI/FEDER, UE)
Foundation Jerome Lejeune project #1419
RyC-2012-10042

Title: Neural substrates of memory impairment and rescue in a mouse model of Down syndrome
Authors: M. ALEMANY-GONZÁLEZ¹, T. GENER¹, P. NEBOT¹, M. VILADEMUNT¹, M. DIERSSEN², *M. V. PUIG¹;
¹IMIM -Hospital del Mar Med. Res. Inst., Barcelona, Spain; ²CRG - Ctr. For Genomic Regulation, Barcelona, Spain

Abstract: Down syndrome (DS) is the most common form of intellectual disability. The cognitive alterations in DS are thought to depend on abnormal neuro-architecture, deficient synaptic plasticity, and excitation-inhibition imbalance in brain regions critical for learning and memory such as the prefrontal cortex (PFC) and the hippocampus (HPC). Previous studies have proposed that immature development of connectivity in DS would lead to impaired ability to integrate information from distant brain regions. Here we recorded neural activity simultaneously in the PFC and HPC of trisomic Ts65Dn mouse model of DS and their non-trisomic littermates during quiet wakefulness, natural sleep and memory acquisition and retrieval. During rest, trisomic mice consistently showed pathological theta oscillations, increased slow-to-fast phase-amplitude coupling in both PFC and HPC and hypersynchronous prefrontal-hippocampal phase synchronization. During sleep, slow waves were reduced and gamma oscillations amplified in Ts65Dn, likely reflecting sustained superficial sleep states. In addition, hippocampal sharp-wave ripples were disrupted, probably contributing to deficient memory consolidation. In WT mice, memory acquisition was encoded by PFC-to-HPC theta connectivity, whereas memory retrieval was encoded by HPC-to-PFC low gamma connectivity. These measures correlated strongly with memory performance and were disrupted in trisomic mice. Importantly, both behavioral and neurophysiological alterations were rescued by chronic oral administration of a green tea extract containing epigallocatequin-3-gallate (EGCG), previously shown to improve executive function in young adults with DS. Our findings suggest hyper-synchronization of prefrontal-hippocampal circuits as a major neural mechanism underlying intellectual disability in DS and could explain EGCG´s cognitive improvement.


Poster 335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 335.20/BB43

Topic: H.01. Animal Cognition and Behavior

Support: R01MH112661
Sloan Research Fellowship in Neuroscience (Alfred P. Sloan Foundation)
Whitehall Foundation award to SPJ
Title: Hippocampal theta sequences and associated prefrontal activity during memory guided behavior

Authors: *M. C. ZIELINSKI¹, J. D. SHIN¹, S. P. JADHAV²;
¹Grad. Program in Neurosci., ²Neuroscience, Psychology & Volen Ctr. for Complex Systems, Brandeis Univ., Waltham, MA

Abstract: Rhythmic coordination of network activity between the rodent hippocampus (CA1) and medial prefrontal cortex (PFC) supports working memory guided decision-making. Continuous theta (6-12 Hz) oscillations are a prominent mode of rhythmic hippocampal activity in the local field potential (LFP), seen during goal driven behavior, active sensory sampling of the environment, and rapid eye movement (REM) sleep. Theta oscillations pattern the firing of hippocampal place cells and mediate temporal organization of PFC activity. A majority of PFC cells exhibit phase-locked spiking to hippocampal theta during spatial behavior (Siapas et al., 2005; Jones & Wilson, 2005; Jadhav et al., 2016), and we have shown that theta phase sharpens coherent spatial representations in CA1 and PFC (Zielinski, Shin, and Jadhav, 2019). Within theta cycles, short and fast sequences of hippocampal spikes continuously recapitulate the slower behavioral timescale and place field order. These “theta sequences” are thought to underlie cognitive chunks of information, with their timing implying a possible means of continuous synaptic strengthening between units (Lisman and Redish, 2009; Mizuseki et al., 2009; Foster and Wilson, 2007). Theta sequences are hypothesized to support cognitive processes such as planning and evaluation of paths in space and time (Wikenheiser and Redish 2015; Colgin, 2013), with theta-paced PFC responses likely facilitating these processes (Zielinski, Tang, and Jadhav, 2017), although the nature of PFC activity during theta sequences remains unexplored. To address these unresolved questions, we simultaneously recorded neural ensembles in PFC and CA1 throughout learning and performance during a W-track spatial alternation task, known to require spatial working memory and dependent on interactions between these two areas (Maharjan et al., 2018). We then used a simultaneous Bayesian decoding approach to determine spatial representations of ensemble activity during periods of high theta power. We then identified putative hippocampal theta sequences and concomitant prefrontal activity within theta cycles in different states. We show the evolution of theta sequences over behavior and their corresponding prefrontal representations throughout learning. These findings establish and expand a role for theta sequences and associated prefrontal activity in memory guided decision-making behavior.

Disclosures:  M.C. Zielinski: None. J.D. Shin: None. S.P. Jadhav: None.

Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 335.21/BB44
Title: CA1 neurons encode non-spatial cues as a conjunction of location and cue identity

Authors: *W. HOCKEIMER\textsuperscript{1,2}, J. J. KNIERIM\textsuperscript{1,2};
\textsuperscript{1}Solomon Snyder Dept. of Neurosci., \textsuperscript{2}Zanvyl Krieger Mind/Brain Inst., Johns Hopkins Univ., Baltimore, MD

Abstract: The hippocampus is believed to subserve episodic memory by integrating spatial and non-spatial information into a cognitive map (O’Keefe and Nadel, 1979). This integration is believed to occur through a form of multiplexing wherein a population of hippocampal neurons with stable spatial firing fields encodes the animal’s current position and the distribution of their firing rates encodes the constellation of non-spatial cues present (Leutgeb et al., 2005). To provide a direct test of this hypothesis, populations of hippocampal CA1 neurons were recorded via tetrodes as rats randomly foraged through an arena consisting of a series of interlocking passageways arranged in a city-block format. Removable textured plates were placed on the floor of each segment and were interchanged during the course of a recording session such that each location harbored each stimulus multiple times. Pilot data from this design revealed a subset of cells (15/74) that exhibited texture-dependent firing rate modulations within a mostly stable place field, according to a permutation test. However, given the complicated nature of the design, a number of possible confounding variables were present. Thus, the design was simplified such that a second animal was restricted to traversing unidirectional laps around the outer perimeter of the arena. New data were collected from 107 isolated units across four days of recording. To determine what information this population encoded, a series of random forest classifiers was trained to decode different types of information using linearized rate maps of each stimulus site visit. These classifiers revealed a conjunctive coding of texture identity and spatial location. On an example day of recording, a random-forests decoder was able to decode what segment of the track the rat occupied at 30% accuracy, a level approximately three times above chance levels (11%). In contrast, the ability to decode the textured stimulus identity by itself was around chance levels. However, the decoder was able to extract information about texture identity when information about the location of that texture was incorporated. The random forest classifier was trained on categorical labels corresponding to every combination of texture identity and stimulus location and achieved a classification performance of 15%, or four times above chance (3.7%). The fact that the conjunction of spatial and non-spatial information can be accurately decoded, but not the stimulus identity by itself, supports the cognitive map theory’s assertion that information about the items and events of experience is encoded in terms of a conjunction of spatial and non-spatial information.

Disclosures: W. Hockeimer: None. J.J. Knierim: None.
Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 335.22/BB45

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant PO1 AG009973

Title: Age-related hyperactivity in the hippocampal CA3 region

Authors: *H. LEE¹, A. TILLEKERATNE¹, K. NNAH¹, M. GALLAGHER², J. J. KNIERIM¹,³; ¹Mind/Brain Inst., ²Dept Psych & Brain Sci., ³Solomon H Snyder Dept. of Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: Elevated hippocampal CA3/dentate activation is observed in individuals with amnestic mild cognitive impairment (Yassa et al., 2010; Bakker et al., 2012). Studies in an animal model of age-related memory loss have demonstrated subregion-specific hyperactivity in the CA3 region of the hippocampus (Wilson et al., 2005, Robitsek et al., 2015, Haberman et al., 2017). To address how the age-related hyperactivity in CA3 contributes to memory impairment, we recorded neurons from the CA3 region in 5 young (Y), 5 aged-impaired (AI), and 5 aged-unimpaired (AU) male, Long-Evans rats. Aged rats were pretested in the Morris water maze and categorized as learning impaired or learning unimpaired. Young rats were 5-7 months old and aged rats were 25-26 months old at the time of recording. All rats were trained to run around a circular track containing salient local texture cues in a curtained room containing salient global landmark cues. Three standard sessions were interleaved with two mismatch sessions, in which the local cues were rotated counterclockwise and the global cues were rotated clockwise to result in 45°, 90°, 135°, or 180° separations. We first examined the proportion of active cells with place fields. Active cells were classified as cells with cluster quality fair or better, total running spikes > 20, and mean firing rate < 10 Hz. An active cell was considered to have a place field if it passed the spatial criteria: spatial information > 0.5 with p < 0.01, and on track spikes > 20. Each session was counted independently so the same cell may have been repeated over sessions. Approximately 69% of active CA3 neurons (889/1292) in Y rats and 63% of active CA3 neurons (662/1048) in AU rats had place fields, while only 53% of active CA3 neurons (503/951) in AI rats had place fields ($X^2$=59.53, p < 0.0001). The on track (i.e. spikes that fired when the rat’s head position was on the track) mean firing rates of the place cells among the three groups were not different (Y: 1.09 Hz, AU: 1.17 Hz, AI: 1.16 Hz; One-way ANOVA, F = 1.1, p = 0.333). However, the off track (i.e. spikes that fired when the rat’s head position was off the track) mean firing rates of the place cells were significantly higher in the AI group (Y: 0.42 Hz, AU: 0.74 Hz, AI: 0.96 Hz; One-way ANOVA, F=92.91, p < 0.0001). The spatial information content of the place cells was significantly lower in the AI group (Y: 2.29 bits/spike, AU: 2.18 bits/spike, AI:
1.84 bits/spike; One-way ANOVA, $F = 59.17$, $p < 0.0001$). These results suggest that the memory impairment associated with age-related hyperactivity in CA3 may be due to spikes that fire outside the place field, reducing the spatial information in the AI rats.

**Disclosures:** H. Lee: None. A. Tillekeratne: None. K. Nnah: None. M. Gallagher: None. J.J. Knierim: None.

**Poster**

335. Learning and Memory: Cortical-Hippocampal Interactions II

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 335.23/BB46

**Topic:** H.01. Animal Cognition and Behavior

**Support:** PO1 AG009973

**Title:** Head scanning behavior and CA3 place field potentiation in aging rats

**Authors:** *G. RAO*, H. LEE, A. TILLEKERATNE, K. NNAH, C.-H. WANG, M. GALLAGHER, J. J. KNIERIM;

1Mind Brain Inst., 2Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

**Abstract:** Scanning occurs in rats when the animal pauses to investigate the environment with head movements (Whishaw et al., 1994). The significance of this behavior to hippocampal function has been previously demonstrated (Monaco et al., 2014). In that study a small but significant number of place fields appeared or potentiated at the site where the animal had engaged in a head-scan on the previous lap. We ask here whether this phenomenon is present in aged animals. Scan-induced place cell potentiation was analyzed in hippocampal cells from 14 rats: young (YG, n=5), aged-unimpaired (AU, n=4), and aged-impaired (AI, n=5) as behaviorally assessed in the Morris water maze. The animals were trained to run on a textured circular track, located in a curtained room containing peripheral objects. They were subsequently implanted with hyperdrives targeting CA3. Recordings consisted of 3 sessions with a standard cue configuration identical to training, interleaved with 2 mismatch sessions in which global cues were rotated CW and local cues CCW. The exposure to unique mismatch angles (45, 90, 135, 180 degrees) in this protocol evokes scanning behavior in all age groups. A total of 168 place fields demonstrated significant potentiation of their firing rates from one lap to the next. Forty-eight of these place fields were designated scan-activated, as the firing during a scan on the lap prior to potentiation was higher than expected. Scan-potentiated fields were detected at approximately equal proportions in all age groups: YG, 25/93; AU, 10/41; AI, 13/34 (chi-square =2.0372, $p =0.36$). Firing rates (spikes/sec) for the scan-activated place cells were not significantly different across the three age groups (mean± se, YG, $1.47 ± .23$; AU, $1.98 ± .56$; AI, $1.04 ± .18$; $F (2,45) =1.68$, $p =.20$). Information scores (bits/spike) for these place fields were
also not significantly altered with age (mean±se, YG, 2.98±.14; AU, 2.81±.26; AI, 3.36±.19; F(2,45) = 1.82, p = .17). Whether there are age-related differences in other characteristics of these scan-activated fields, such as persistence and proximity to local and global cues, will be investigated further. These initial analyses suggest no major differences among the age groups in the overall prevalence of scan-related place field potentiation.


**Poster**

335. Learning and Memory: Cortical-Hippocampal Interactions II

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 335.24/BB47

**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIH Grant R01 MH079511
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ARO MURI 72929-EG-MUR-01
JHU Kavli NDI Postdoctoral Distinguished Fellowship
JHU Mechanical Engineering Departmental Fellowship

**Title:** An open-source 3D pose tracking system for neuroscience research

**Authors:** B. P. VAGVOLGYI¹, R. P. JAYAKUMAR², M. S. MADHAV³, M. K. FERREYROS⁴, F. SAVELLI³, J. J. KNIERIM³,⁵, *N. J. COWAN²,¹;
¹Lab. for Computat. Sensing & Robotics, ²Dept. of Mechanical Engin., ³Zanvyl Krieger Mind/Brain Inst., ⁴The Solomon H Snyder Dept. of Neurosci., ⁵Johns Hopkins Univ., Baltimore, MD

**Abstract:** We present a robust high-precision single-camera system for tracking the pose - position (x,y,z) and orientation (roll, pitch, yaw) - of a custom visual target attached to a test subject. Our system builds upon existing monocular tracking algorithms, referred to as Perspective from n Points (PnP), that use a visual target comprising point-like fiducials (passive retroreflective markers in our implementation) arranged in known configuration (Faessler et al. ICRA 2014, Savkin et al. VRST 2017). Such methods are constrained by the tradeoff between fiducial count and range of detectable orientations. A low fiducial count limits the range of angles from which enough fiducials are visible due to self-occlusion, leading to loss of tracking. However, time needed for video processing and recapture of target pose upon loss of tracking scales poorly with the number of fiducials due the combinatorial complexity of possible point
correspondences. We introduce geometric constraints and multiple fiducial sizes, drastically reducing the number of possible correspondences facilitating a higher fiducial count. Our improvements enable real time operation (15-30 ms per frame at 4MP resolution using 11 fiducials) in a host of new applications where a wide range of orientations need to be tracked. The system consists of custom software, a Near-IR camera, an IR LED light source mounted near the camera, and the custom visual target described above. The software can process a video file input, but is also capable of real time operation on live camera images with a supported camera model. The 3D printable design of the visual target, target assembly instructions, calibration and tracking software will be open source. Cost of implementation is a fraction of commercially available solutions.

The tracker’s real time mode is being used to manipulate the visual scene in the augmented reality dome described in Jayakumar*, Madhav* et al., Nature 2019. Previously, the dome system relied on the animal being tethered to a boom arm instrumented with an angular optical encoder that measured the position of the animal as it ran on a circular track. The new real time tracking system allows for a freely behaving, untethered animal. We also present results from an experiment where the system, post-processing a video file, was used to track the head of a rat. In the experiment, the surrounding visual scene of virtual landmarks was either static or rotated coherently as a function of the rat’s running speed. Simultaneous neural recordings were taken from CA1 place cells and ADN head direction cells. We find that the place cell and head direction cell map respond coherently to the visual cue manipulation.


Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 335.25/BB48

Topic: H.01. Animal Cognition and Behavior

Support: Sloan Research Fellowship in Neuroscience (Alfred P. Sloan Foundation)
Whitehall Foundation award to SPJ

Title: Odor-place associative memory in the hippocampal-prefrontal network

Authors: *C. A. SYMANSKI, E. KULLBERG, S. P. JADHAV;
Brandeis Univ., Waltham, MA

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 behavior. The physiological mechanisms by which these odor-place
associations are formed and retrieved are thought to involve a brain-wide network that includes
the hippocampus and the prefrontal cortex (PFC) (Tse 2007; Fujisawa 2011; Igarashi 2016). Recent evidence suggests that coordinated activity between the hippocampus and PFC is important for formation and for recall of associative memories and memory-guided decision making (Maharjan 2018; Zielinski 2017; 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 = 7) 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 in all regions, as well as spiking activity in CA1 (n = 678 neurons) and PFC (n = 286) to investigate the physiological basis of the recall of odor-place associations. During the period of acute olfactory sampling and recall, respiratory rhythms and 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 exhibited choice-selective responses that were reversed on incorrect trials, implicating their involvement in memory-guided behavior; furthermore, ensemble responses in CA1 and PFC evolved during the recall period to indicate correct choice with CA1 leading PFC. These results point to an olfactory-hippocampal-prefrontal network underlying the recall of olfactory-place associations. Future analyses will investigate dynamic phase relationships between the beta rhythm and choice-related ensemble activity during the recall period, as well as predictive coding properties of ensembles during recall and maintenance of odor-place associations.

Disclosures: C.A. Symanski: None. E. Kullberg: None. S.P. Jadhav: None.

Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 335.26/BB49

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01 MH112661
Sloan Research Fellowship in Neuroscience (Alfred P. Sloan Foundation)
Whitehall Foundation award

Title: Awake hippocampal-prefrontal replay mediates spatial learning and decision making
Authors: *W. TANG*¹, J. D. SHIN¹, S. P. JADHAV²,³,¹;
¹Neurosci. Program, ²Psychology, ³Volen Natl. Ctr. for Complex Systems, Brandeis Univ.,
Waltham, MA

Abstract: Goal-directed spatial learning requires remembering and choosing critical paths to
desired goals, a process that requires hippocampal-prefrontal circuits (*Jadhav et al., 2012;
Maharjan et al., 2018; Tang and Jadhav, 2019*). Hippocampal place cells replay spatial paths in
reverse and forward order during sharp-wave ripples (SWRs) in immobility periods, and we have
shown that this awake hippocampal replay is coordinated with prefrontal cortical (PFC) activity
for memory reactivation (*Jadhav et al., 2016; Tang et al., 2017*). Although it is known that
awake replay is necessary for spatial learning (*Jadhav et al., 2012*), how replay activity mediates
both goal-directed learning and memory-guided decision making is unclear. We therefore
continuously tracked replay in the same hippocampal-prefrontal ensembles in rats (*n* = 6)
throughout learning of a W-track spatial alternation task across multiple behavioral sessions in a
single day. We found that during pauses between behavioral trajectories, awake reverse and
forward hippocampal replay consistently mediated an internal cognitive search of all available
past and future possibilities throughout learning and performance of the task. Further, reverse
and forward replay exhibited opposing learning gradients for prediction of behaviorally
actualized past and future paths, respectively. Coordinated hippocampal-prefrontal replay
distinguished correct past and future paths leading to reward from alternative choices based on
the hippocampal cognitive search, thus supporting recall of past path to guide selection of future
choice for execution of spatial working memory rules. Our findings reveal a learning shift from
hippocampal reverse-replay-based retrospective evaluation to forward-replay-based prospective
planning, with prefrontal filtering of memory-guided paths for learning and decision-making.
*WT and JDS have contributed equally to this work.

Disclosures: W. Tang: None. J.D. Shin: None. S.P. Jadhav: None.

Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 335.27/BB50

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01 MH112661
Sloan Research Fellowship in Neuroscience (Alfred P. Sloan Foundation)
Whitehall Foundation Award

Title: Ontogeny of coordinated representations in the hippocampal-prefrontal network during
spatial learning
Abstract: Spatial learning and memory-guided behavior involves interaction of hippocampal spatial maps with prefrontal cortical (PFC) representations for evaluation and selection of task-relevant information (Shin and Jadhav, 2016; Maharjan et al., 2018; Zielinski et al., 2019). However, the specific nature of these task-relevant representations, the relative timescales at which they emerge in the hippocampus and PFC in novel environments, and how they support learning and subsequent memory performance is still unclear. To address these questions, we continuously and simultaneously recorded the same neuronal ensembles in PFC and hippocampal CA1 area in rats (n = 8) throughout learning of a novel W-track spatial alternation task across multiple behavioral sessions in a single day. We found that behavioral learning was correlated with the co-stabilization and refinement of spatial representations, which emerged in parallel in both CA1 and PFC. Additionally, we observed the co-development on similar timescales for two key task-dependent representations in CA1 and PFC during learning. First, activity patterns became more distinctive in different running directions occurring in the same spatial location, i.e., directional selectivity. Second, neurons developed retrospective and prospective trajectory-dependent activity, representing not only instantaneous position but also past and future choices, i.e. choice selectivity, which is thought to mediate memory-guided trajectories (Wood et al., 2000; Baeg et al., 2003, Fujisawa et al., 2008). Our results suggest that spatial learning elicits coordinated changes in context-selective representations in both the hippocampus and PFC. The emergence of these stable representations from a naïve state underlies the establishment of hippocampal-prefrontal associations that are important for learning rules in novel environments, and can therefore provide the neural basis to support memory guided behavior.

* JDS and WT have contributed equally to this work.


Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 335.28/BB51

Topic: H.01. Animal Cognition and Behavior

Support: NIMH Grant R01MH118926

Title: Development of a computational theory of gain recalibration in the brain's path integration system
Authors: *K. R. HEDRICK*, B. GEIGER; Mathematics, Southern Methodist Univ., Dallas, TX

Abstract: Path integration is a navigational strategy that, in combination with landmark navigation, supports the creation of “cognitive maps” and allows an organism to remain oriented even in the transient absence of stable landmarks (Savelli & Knierim, J Exp Biol, 2019). With the use of a novel, augmented reality system and recordings from hippocampal place cells, the Knierim and Cowan Labs recently reported that the gain of the path integration system—i.e., the relationship between the distance traveled by an organism in the world and the corresponding updating of the organism’s location on its cognitive map—is a plastic variable that can be recalibrated by a continual mismatch between path integration and landmarks (Jayakumar et al., Nature, 2019). We are working to develop computational theories to understand the network dynamics underlying the interaction between path integration and landmarks in this recalibration phenomenon. In particular, we are incorporating a plastic gain into existing models of path integration using various hypotheses for the source of error correction. Through numerical simulations and mathematical analysis, we then use the models to predict the response of grid cell modules in the upstream MEC, which provide the primary spatial inputs to hippocampal place cells.

Disclosures: K.R. Hedrick: None. B. Geiger: None.

Poster

335. Learning and Memory: Cortical-Hippocampal Interactions II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 335.29/BB52

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01 NS102537
        NIH Grant R21 NS095075

Title: Quantifying the influence of grid-cell input scale relative to environment size in a Hebbian model of hippocampal place field formation

Authors: *F. SAVELLI*¹, J. J. KNIERIM²;
        ¹Zanvyl Krieger Mind/Brain Inst., ²Zanvyl Krieger Mind/Brain Inst. and Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: Entorhinal grid cells provide a prominent spatial input to hippocampal place cells. When this input is compromised, place cells can fail to develop place fields in novel and large environments, but not in familiar ones, or their stable fields tend to concentrate in the periphery of the environment, where other types of spatial inputs are available. Moreover, most existing
studies target CA1/3 place cells, which receive both direct and processed grid inputs. What exactly do grid cells contribute to hippocampal place fields? In a model based on a fast, postsynaptically-gated, Hebbian rule, place fields can be generated by spiking models of grid-cell inputs (Savelli & Knierim, J Neurophys, 2010). This model is compatible with the modular organization of grid scale and orientation, and it is robust against grid distortions when tested with real behavioral recordings in typical appurteni of various shapes and sizes (cylinder/square box/platforms, 0.36-1.87m²; Savelli & Knierim, SfN, 2018). In this model, however, ‘conventional’ place cells are prevalently found among cells that receive inputs from grids at scales that are commensurate with the environment. For example, rate maps of cells receiving inputs from a subset of grid modules at the lower end of the scale range show many place fields in the larger environments, similar to some experimental observations. Do these fields coexist throughout the session or are they expressed at different times? We quantified their intra-session stability in two ways: (1, cell-based) by the similarity of two partial rate maps from the first and second halves of the session; and (2, field-based) by the similarity of the temporal profiles of the cell’s activity and of the animal’s visits to the field over the course of the entire session. Spatial stability by both measures was observed across the whole population of simulated place cells in all environments. In contrast, instability was far more prevalent in place cells receiving grid inputs that had a low scale-to-environment-size ratio and in place fields that had a low area-to-environment-size ratio. These results suggest the possibility that experimental properties of place cells such as the spontaneous relocation of place fields and the ‘overdispersion’ of in-field firing may in part reflect continuing plasticity of grid inputs. It remains experimentally under-investigated, however, if actual place cells involved in the initial and/or most septal stage of hippocampal processing conform to our results, and if other inputs or a regulation of grid input plasticity in these cells promote greater field stability than observed in our current model.

**Disclosures:** F. Savelli: None. J.J. Knierim: None.

**Poster**

**335. Learning and Memory: Cortical-Hippocampal Interactions II**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 335.30/BB53

**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIH R37 NS047344

NIH R01 NS039456

**Title:** Object-related firing of cells in the dentate gyrus

**Authors:** *D. GOODSMITH*¹³, S. KIM³, K. M. CHRISTIAN³, H. SONG³, J. J. KNIERIM¹²;

¹Krieger Mind/Brain Inst., ²The Solomon H Snyder Dept. of Neurosci., Johns Hopkins Univ.,
Abstract: Granule cells in the dentate gyrus (DG) receive egocentric sensory and object-related information from the lateral entorhinal cortex (LEC) as well as an allocentric spatial signal from the medial entorhinal cortex (MEC). One function of the DG may be the conjunctive encoding of spatial and non-spatial information into a single representation of both the context and content of an experience. While there is behavioral evidence for conjunctive encoding in the DG (Kesner, 2013), how the firing of DG cells contributes to this function is unclear. We recorded from DG cells as mice foraged for food in an environment that contained two discrete objects. We alternated sessions in which the objects were in a standard location (STD) with sessions in which an object was moved (MOVE), to examine how manipulation of the objects affected the spatial firing of DG cells.

We recorded 381 DG cells (191 active during behavior) from 13 mice. In a subset of DG cells with place fields that were affected by object movement, we observed six main response categories: 1) move: distance to object is maintained when the object is moved, 2) trace: field appears at previous object location, 3) appear: new field appears near object, 4) disappear: loss of firing near object, 5) rotate: change in direction of firing relative to object, 6) Capture: field shifts to moved object location. Many cells (likely mossy cells) had multiple place fields, and individual fields of these cells appeared to respond to object manipulations, while other fields of the same cell remained stable. We did not find any significant clustering of place fields at the object locations, or any population differences in the number of place fields, place field size, or firing rates between STD and MOVE sessions. Rate map correlations between the STD sessions before and after a MOVE session were significantly higher than the correlation between a STD and MOVE session for 12% of DG cells. We next restricted our analysis to a portion of the environment centered at the location of the object or centered at a stable spatial location. 26% of DG cells had a significantly higher correlation between a STD and MOVE session at the object location than at the spatial location. Our results indicate that cells in the DG respond to the manipulation of objects in space, and support the proposed role for DG in conjunctive encoding.


Poster

336. Learning and Memory: Physiology II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 336.01/BB54

Topic: H.01. Animal Cognition and Behavior
Title: Effect of medial septal selective and non selective lesions on exploratory behavior and recognition memory

Authors: *L. KRUASHVILI, G. BESELIA, N. CHKHIKVISHVILI;
Lab. of Behavior and Cognitive Function, I.Beritashvili center of Exptl. Biomedicine, Tbilisi, Georgia

Abstract: Investigation of cholinergic system and memory interaction has especially become the object of scientific attention due to the clinical and experimental data, in which the severity of dementia in Alzheimer's disease (AD) was found to have a positive correlation with the extent of the cholinergic loss. The septum is connected to the hippocampus via the fimbria-fomix, which carries projections from the medial septum (MS), and the vertical limb of the diagonal band of Broca. These projections are predominantly cholinergic and GABAergic. Lesions of the fimbria-fomix, or electrolytic lesions of the MS, impair hippocampal-dependent learning and memory. The purpose of this study was to investigate ability to acquire and use spatial (or non-spatial) information as well as to habituate exploratory activity over time in sham-operated, electrolytic, neuro or immunotoxic MS lesioned rats.

Methods: A total of 39 male rats were used. For electrolytic lesions a stainless steel was inserted in the MS. All injections were performed stereotaxically. Rats were individually given five 3-min sessions in the open field. All experiments were approved by the Animal Care and Use Committee of the Center and were in accordance with the principles of laboratory animal care. Results: Examination of the AChE stained sections showed that after injections of 192 IgG saporin into the MS, animals exhibited significantly less AChE staining in MS and hippocampus as compared to sections obtained from control animals. The MS electrolytic and ibotenic acid lesioned rats showed an increase in their exploratory activity to the objects and were impaired in habituating to the objects in the repeated spatial environment, rats with immunolesions of the MS did not differ from control rats. Electrolytic lesions of the MS disrupt spatial recognition memory, rats with immuno- or neurotoxic lesions of the MS were normal in detecting spatial novelty. The MS lesioned and control rats clearly reacted to the object novelty by exploring the new object more than familiar ones Conclusions: MS is sufficient for spatial recognition, but is not sufficient for object recognition memory, the selective loss of septohippocampal cholinergic or noncholinergic projections does not disrupt the function of the hippocampus to a sufficient extent to impair spatial recognition memory. Therefore, the present study demonstrates dissociation between the two major components (cholinergic and noncholinergic) of the septohippocampal pathway in exploratory behavior assessed in the open field.

Disclosures: L. Kruashvili: None. G. Beselia: None. N. Chkhikvishvili: None.
Title: Sleep deprivation impairs learning and memory through downregulation of O-GlcNAcylation in the brain of adult zebrafish

Authors: *S. KIM, Y. LEE, J. PARK, J.-W. LEE, I.-O. HAN;
Dept. of Physiol. and Biophysics, Inha Univ., Incheon, Korea, Republic of

Abstract: Sleep is an evolutionarily conserved physiological process implicated in the consolidation of learning and memory (L/M). Here, we hypothesize that sleep deprivation (SD)-induced cognitive deficits in zebrafish are mediated through reduction in O-GlcNAcylation in the brain. At the molecular level, SD downregulated mRNA of key enzymes of HBP metabolism and O-GlcNAc transferase (OGT) along with increased O-GlcNAcase (OGA) expression. Fear conditioning (FC) induced an increase in the expression or activities of proteins associated with long term memory (LTM), and at the same time, OGT protein and O-GlcNAcylation were significantly increased in the normal- but not in the SD-group of zebrafish. Suppression of HBP by the GFAT inhibitor, diazo-oxo-norleucine (DON), impaired L/M function and FC-mediated activation of PKA/CREB signaling. Conversely, enhancement of HBP by glucosamine significantly restored cognitive deficits and increased PKA/CREB signaling in the SD group of zebrafish. To our knowledge, the current study has provided valuable insights into the molecular and biochemical changes associated with L/M at the whole-brain level using the zebrafish system for the first time. Our findings highlight the role of the HBP during the L/M process and provide potential therapeutic targets for cognitive defects.

Disclosures: S. Kim: None. Y. Lee: None. J. Park: None. J. Lee: None. I. Han: None.

Support: NSERC PDF516867-2018
Title: Mental navigation of mnemonic space in primates

Authors: *S. NEUPANE, M. JAZAYERI; Dept. of Brain and Cognitive Sci., McGovern Inst. for Brain Research, Massachusetts Inst. of Technol., Cambridge, MA

Abstract: A ‘cognitive map’ (Tolman, 1948) is thought to organize the underlying structure of a contingent task in a unitary space (O’keefe and Nadal, 1978). During spatial foraging, neural correlates of allocentric place code have been discovered in the hippocampal formation of rodents pointing towards a spatial map that utilizes Euclidean geometry (O’Keefe and Dostrovsky, 1971; Hafting et al 2005). More recently, signatures of such place code were found in rodents and humans along non-spatial dimensions of sound and arbitrary abstract concepts (Aronov et al 2017; Constantinescu et al 2016) supporting the possibility that the hippocampal formation functions as a generalized cognitive map. However, how the nervous system interrogates such a map to make cognitive inferences in the absence of sensory input is not understood.

To address this question, we designed a novel mental navigation task for humans and monkeys in which subjects had to make inferences about the relative direction and distance of arbitrary points on a cognitive map, without sensory feedback. On each trial, subjects were presented with two images chosen randomly from a previously learned ordered sequence of images. One image represented the subjects’ current position along the sequence and the other, the target position. Subjects could use two buttons to drag the sequence of images rightward or leftward at a fixed speed. To complete the trial, subjects had to use their memory of the image sequence to infer the position of the target relative to the current image, and press the appropriate button for the appropriate length of time. Importantly, button presses advanced the image sequence covertly forcing subjects to navigate the sequence mentally. Upon button release, the new position along the sequence was revealed.

Preliminary behavioural results exhibited three features consistent with the use of a cognitive map. (1) After training on a subset of image pairs from a given sequence, humans and monkeys were able to learn the remaining unseen pairs faster. The overall learning curve for a new sequence was also faster suggesting that the structure of the cognitive map was learned. (2) Probability of direction error decreased when either the current or target image were at the extrema of the sequence suggesting an inference mechanism that relies on the representation of borders as an integral part of the cognitive map (3) Reaction time distribution varied with image distance suggesting an inference strategy based on mental simulations of the sequence. Electrophysiology experiments are currently underway to examine the neural basis of learning and use of cognitive maps in the hippocampal formation of monkeys.
Disclosures: S. Neupane: None. M. Jazayeri: None.

Poster

336. Learning and Memory: Physiology II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 336.04/BB57

Topic: H.01. Animal Cognition and Behavior

Title: A short term relief learning paradigm to discriminate safety in *Drosophila*

Authors: *J. HE*¹, K. WANG², Y. ZHONG¹;
¹Tsinghua Univ., Beijing City, China; ²China Agr. Univ., Beijing City, China

Abstract: Posttraumatic Stress Disorder, PTSD, as a neurological disease afflicting thousands of people who experienced psychological trauma has been drawing widespread concern. Extensive efforts have been devoted to identifying causal paths leading to danger learning and memory. Alternatively, the low discrimination ability upon safety signal in PTSD remains neglected. Relief learning is an ideal paradigm to study safety signal discrimination. However, current relief learning paradigm needs long training cycles and has low behavior performance index. In this study, we introduce a new relief learning paradigm with one cycle training and high behavior performance index to study quick safety signal discrimination ability. Further studies about the mechanisms of how PTSD patients discriminate safety using this new paradigm may help to discover new treatments.

Disclosures: J. He: None. K. Wang: None. Y. Zhong: None.

Poster

336. Learning and Memory: Physiology II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 336.05/BB58

Topic: H.01. Animal Cognition and Behavior

Support: Institute for Basic Science (IBS-R002-A1)

Title: Exploring value-related signals in the rodent and primate striatum

Authors: *E. SHIN*¹², Y. JANG¹², S. KIM³, H. KIM⁴, X. CAI⁵, D. LEE⁶, M. JUNG¹²;
¹Dept. of Biol. Sci., Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of;
Abstract: Studies in rats, monkeys and humans have found multiple types of value-related neural signals in the striatum, suggesting that the striatum might play an important role in reinforcement learning and decision making. For example, action-value signals in the striatum might contribute to biasing actions toward more valuable options. Recently, concerns were raised regarding the statistical methods used to identify action-value signals (Elber-Dorozko and Loewenstein, 2018). First, the statistical significance of temporal correlation between neural activity and action value might be inflated by the serial correlation in both variables. Second, striatal activity seemingly related to action values might code other decision variables correlated with action value, such as a policy or relative action values. To address these concerns, we reanalyzed the neural data previously recorded from the striatum of rats performing a dynamic foraging task and monkeys performing an intertemporal choice task. We found that our previous analysis procedures, such as a within-block permutation test or the inclusion of autoregressive terms, effectively dealt with the problems related to serial correlation in neural and behavioral signals. Moreover, we also found significant action-value signals using a more stringent cross-session permutation test proposed recently. These results show that striatal action-value signals cannot be accounted for by serial correlation in neural activity and action value. Next, we analyzed neural activity in the rodent and primate striatum related to multiple types of decision-related variables, such as action value, policy, and state value. We found that in both species, action-value signals cannot be fully accounted for by activity related to policy or state-values. Together, these results indicate a robust coding of action values in the rodent and primate striatum.

Authors: *C.-H. CHIEN, C.-C. HUANG, P.-Y. WANG, S.-K. CHEN;
Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Since the light/dark cycle in the world is about 24 hours, in order to act normally throughout every single day, almost all organisms have developed the circadian clock system to sensor outside environment and collaborate the physiological function of different organs. Except for light, daily fed calorie is one of the external cues, which affecting body weight, immune system as well as gut microbiota, of an organism. Here, using wild-type mice as model animals and combining next generation sequencing and several behavior tests, we found that calorie restriction, i.e. only 60% energy intake compared to control groups, would modulate the composition and oscillation of gut microbiota and improve memory ability of mice.

Disclosures:  C. Chien: None. C. Huang: None. P. Wang: None. S. Chen: None.

Poster

336. Learning and Memory: Physiology II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 336.07/BB60

Topic: H.01. Animal Cognition and Behavior

Support: Centre of Excellence scheme of the Research Council of Norway – Centre for Neural Computation, grant number 223262, the National Infrastructure scheme of the Research Council of Norway – NORBRAIN, grant number 197467, and The Kavli Foundation.

Title: Diversity of molecularly defined GABAergic interneurons in the mouse perirhinal cortex

Authors: *M. J. NIGRO, R. NAIR RAVEENDRAN, C. KENTROS, M. P. WITTER;
Kavli Inst. for Systems Neuroscience, Ctr. for Neural Computation, Egil and Pauline Braathen and Fred Kavli Ctr. for Cortical Microcircuits, NTNU Norwegian Univ. of Sci. and Technol., Trondheim, Norway

Abstract: Cortical inhibitory interneurons powerfully shape information processing in cortical networks. Their wide diversity is believed to endow cortical microcircuits with a variety of computational capabilities. Recently the distribution of GABAergic interneurons has been shown to vary among cortical areas, particularly in the rhinal cortices (Kim et al., 2017). This is of particular relevance given that neocortical information traveling to the entorhinal-hippocampal system is subject to an inhibitory control in perirhinal cortex (PER). Here we used a combination of mouse genetics, immunofluorescence and intersectional genetics to describe the diversity of GABAergic classes in the mouse PER. We used immunofluorescence for parvalbumin (PV), somatostatin (SST) and vasoactive intestinal peptide (VIP) in the GAD67-GFP transgenic mouse
line to quantify these non-overlapping populations as well as the total GABAergic population in PER and barrel cortex. Our data show that PV cells represent a minority of interneurons in PER as compared to barrel cortex and their numbers decrease along the rostro-caudal axis. On the other hand, a population negative for all three markers represents ~50% of GABAergic interneurons across the layers of perirhinal cortex, but less than 30% in barrel cortex. By injecting interneuron specific viruses (Dimidschtein et al., 2016) in the CCK-cre mouse line we identified a population of GABAergic interneurons that do not express SST and partially overlaps with VIP (~6%) and PV (30-50%). These results suggest that in deep layers of PER 50-70% of intersectionally labelled cells are negative for PV, SST, and VIP. Current clamp recordings show that intersectionally labelled cells express an irregular type of firing pattern. This peculiar ratio of different molecularly defined GABAergic interneurons might endow PER circuits with specialized computational properties.

**Disclosures:** M.J. Nigro: None. R. Nair Raveendran: None. C. Kentros: None. M.P. Witter: None.

**Poster**

336. Learning and Memory: Physiology II

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 336.08/BB61

**Topic:** H.01. Animal Cognition and Behavior

**Support:** Grant-in-Aid for Scientific Reserch (KAKENHI Grant Number 17K09761)

**Title:** Reduced cholinergic activity in the hippocampus of phosphatidylethanolamine-binding protein 1 knockout mice

**Authors:** *Y. MADOKORO*¹, Y. YOSHINO², D. KATO¹, T. SATO¹, M. MIZUNO¹, T. KANAMORI¹, M. SHIMAZAWA², H. HIDA³, H. HARA², K. OJIKA¹, N. MATSUKAWA¹; ¹Dept. of Neurol., Nagoya City Univ., Nagoya, Japan; ²Dept. of Biofunctional Evaluation, Mol. Pharmacol., Gifu Pharmaceut. Univ., Gifu, Japan; ³Dept. of Neurophysiol. and Brain Sci., Nagoya City Univ. Grad Sch. Med. Sci., Nagoya, Japan

**Abstract:** The cholinergic efferent network from the medial septal nucleus to the hippocampus has an important role in learning and memory processes. This cholinergic projection can generate theta oscillations in the hippocampus to efficiently encode novel information. Hippocampal cholinergic neurostimulating peptide (HCNP) induces acetylcholine synthesis in medial septal nuclei. HCNP is processed from the N-terminal region of a 186 amino acid, 21 kD HCNP precursor protein (HCNP-pp), also known as Raf kinase inhibitory protein (RKIP) and phosphatidylethanolamine-binding protein 1 (PEBP1). In this study, we generated HCNP-pp knockout (KO) mice and assessed their cholinergic septo-hippocampal projection, local field
potentials in CA1, and their behavioural phenotype. No significant behavioural phenotype was observed; however, theta power in the CA1 of HCNP-pp KO mice was significantly reduced because of fewer ChAT-positive axons in the CA1 striatum oriens, indicating disrupted cholinergic activity in the septo-hippocampal network. These results indicate that HCNP may be a cholinergic regulator in the septo-hippocampal network.

Disclosures: Y. Madokoro: None. Y. Yoshino: None. D. Kato: None. T. Sato: None. M. Mizuno: None. T. Kanamori: None. M. Shimazawa: None. H. Hida: None. H. Hara: None. K. Ojika: None. N. Matsukawa: 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.; Grant-in-Aid for Scientific Reserch (KAKENHI Grant Number 17K09761) from Japan Society for the Promotion of Science.

Poster

336. Learning and Memory: Physiology II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 336.09/BB62

Topic: H.01. Animal Cognition and Behavior

Support: NIH R01 NS054281

Title: Mechanisms of inhibitory interneuronal network synchrony for type 1 versus type 2 excitability


Abstract: PV+ fast spiking (FS) basket interneurons are often implicated in gamma rhythms, which in the hippocampus are thought to organize information for memory encoding and retrieval. Here we focus on mechanisms present in purely inhibitory networks. Neurons with type 1 excitability are able to spike arbitrarily slowly, whereas those with type 2 excitability have an abrupt onset of repetitive firing that cannot be maintained below a threshold frequency. FS neurons likely exhibit type 2 excitability in the entorhinal cortex, striatum and cortex. We systematically examine how excitability type affects synchronization of individual spikes to a population rhythm in the presence of heterogeneity and noise. Model neurons of each type were calibrated to have very similar F/I curve, input resistance, time constant and action potential shape. In a coupled oscillator models with no noise or heterogeneity, all neurons fire on every cycle. During pharmacologically induced gamma in vitro putative fast spiking basket cells fire on average every other cycle, with a narrow variance in the spikes emitted per gamma cycle but with no
particular pattern with regard to which cycles are skipped. Recently, we showed that networks of type 2 neurons coupled by hyperpolarizing inhibition support cycle skipping due to their intrinsic dynamic properties. However, inhibitory networks comprised of type 1 neurons and networks with shunting inhibition also exhibit cycle skipping; we show the cycle skipping mechanism for type 1 neurons or type 2 neurons with shunting inhibition is synaptic and not intrinsic.

Type 2 networks with hyperpolarizing inhibition homogenize the firing rate across noisy, heterogeneous networks due to a strongly repelling quasithreshold that separates neurons that fire from those that skip. In contrast, in a type 1 network, the neurons that escape first through a bottleneck near the SNIC close the bottleneck for the remaining neurons. Since the rate of change of membrane potential is very slow in the bottleneck, the resultant firing times are easily scattered by noise and heterogeneity. Moreover, shunting inhibition is more robust than hyperpolarizing for type 1 because it moves the population closer to spike threshold, so that neurons spend less time in the bottleneck. Low amplitude theta modulation synchronizes type 2 networks with hyperpolarizing inhibition more quickly and tightly than type 1 because of the intrinsic mechanism for cycle skipping that is not as dependent on accumulation of inhibition. A more mechanistic understanding of oscillatory mechanisms may help elucidate the functional roles of oscillations.

Disclosures: R.A. Tikidji-Hamburyan: None. C.C. Canavier: None.

Poster

336. Learning and Memory: Physiology II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 336.10/BB63

Topic: H.01. Animal Cognition and Behavior

Support: JSPS KAKENHI Grant Number JP20650094

Title: Circadian rhythm modulate the frequency of carbachol-induced beta oscillations in rat hippocampal slices

Authors: *K. NATSUME, M. SHIGEMOTO; Kyushu Inst. Technol., Kitakyushu city, Japan

Abstract: Diurnal rhythm is 1-day biological rhythm of almost animals. The rhythm is affected by light stimulation and memory is also regulated by the rhythm. In behavioral experiments, rodents show different memory performance depending on the times of a day. The brain waves such as theta (4-8 Hz) and beta (13-30 Hz) waves relate to memory process, and the relationship between the occurrence of brain wave and the diurnal rhythm has not yet been clarified. A cholinergic agent carbachol can induce oscillations in the frequency similar to that of theta and beta waves with the application to hippocampal slices. Here we analyzed the effect of the diurnal rhythm on the carbachol-induced oscillations. Thirty two rats were kept on 12:12 Light / Dark
cycle for one week or more before the preparation of hippocampal slices. The activity of rats was measured using an infrared sensor to confirm that they were adapted to the LD cycle. The oscillation in the beta frequency band (called beta oscillation) was induced with the application of 30μM carbachol to hippocampal slices. We classified and compared the mean of frequency of beta oscillation based on zeitgeber time (ZT; from ZT0 to 11 indicates light phase and from ZT12 to 23 indicates dark phase). The frequencies were grouped every 4 ZT hours. In results, the frequency at ZT16-19 was 15.5 ± 0.7 (mean ± standard error of the mean) Hz and significantly lower than the others (17-20 Hz). The frequency of beta oscillations induced in the slices at other than ZT16-19 was significantly decreased with the application of 10μM bicuculline, a GABA_A receptor antagonist. The decreased frequency was close to that at ZT16-19. The results suggest that the GABA_A receptor transmission will cause to decrease the frequency of beta oscillations. Nakatsuka and Natsume (2014) have proposed that the long-term potentiation (LTP) increased in slices at night, that is, ZT 15-21 and the increase was also induced with application a GABA_A receptor antagonist at the day ZT5-11. The result also suggests that disinhibition occurs in hippocampus at night in the nocturnal rat. Therefore, inhibition will be modulated in the diurnal rhythm and the modulation will lead to the modulation of LTP and frequency change in beta rhythm of a rat.

Disclosures:  K. Natsume: None. M. Shigemoto: None.

Poster

336. Learning and Memory: Physiology II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 336.11/BB64

Topic: H.01. Animal Cognition and Behavior

Support: MOST107-2628-B-010-001
MOST105-2628-B-010-005-MY3
MOST106-2320-B-010-012-MY3
NHRI-EX108-10509NC

Title: In vivo imaging of membrane potential dynamics in populations of hippocampal interneurons during network oscillation

Authors: *Y.-C. HUANG\textsuperscript{1}, L. D. LAVIS\textsuperscript{2}, E. R. SCHREITER\textsuperscript{2}, B.-J. LIN\textsuperscript{1}, T.-W. CHEN\textsuperscript{1};\textsuperscript{1}\textsuperscript{\textsuperscript{1}}\textsuperscript{Inst. of Neurosci., Natl. Yang-Ming Univ., Taipei City, Taiwan; \textsuperscript{2}Howard Hughes Med. Institute, Janelia Farm Res. Campus, Ashburn, VA

Abstract: Interneurons play a critical role in coordinating network oscillation in many brain regions. In hippocampal CA1 area, distinct subtypes of interneurons participate in network oscillations in highly specific ways. For example, the firing of parvalbumin positive (PV+)
interneurons are strongly related to the timing of theta oscillation and sharp wave ripple (SWR) events. Manipulating the activity of PV+ interneurons disrupts these oscillations, suggesting their important roles. However, little is known about how subtypes of interneurons coordinate among themselves to control the dynamics of hippocampal network. Here we simultaneously imaged the membrane potential dynamics of multiple hippocampal PV+ neurons in awake, head-fixed mice. A novel protein voltage indicator ‘Voltron’ was expressed in CA1 PV+ neurons through virus injection. A tungsten electrode was implanted at the edge of the imaging window for field potential recording. JF525-Halotag was delivered by retro-orbital injection before imaging. Labeled neurons were locally illuminated by a pattern of excitation light tailored to optimize image contrast. Voltage dependent fluorescence signals were imaged at ~1-8 kHz using high speed camera. Pupil diameter and whisker movement were monitored using a video camera. Fluorescence signals related to supra- and subthreshold membrane potential dynamics were readily observable at single cell level. Action potentials of individual PV+ interneurons were modulated by both SWR events and arousal states. Simultaneously imaged PV+ interneurons display highly coordinated membrane potential dynamics. We are in the process of investigating other CA1 neuron subtypes. These data provide insights into the membrane potential mechanisms underlying the coordination among specific interneurons important for hippocampal circuit dynamics.


Poster

336. Learning and Memory: Physiology II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 336.12/BB65

Topic: H.01. Animal Cognition and Behavior

Support:  NIH Brain Initiative U19

Title: Optical measurement of dendritic membrane potential in the behaving mouse hippocampus


1Neurosci., Columbia Univ., New York, NY; 2Neurobio., Stanford Univ., Stanford, CA

Abstract: In the hippocampus, excitation, inhibition and a variety of ion channels interact at the level of dendrites of principal cells to give rise to a rich repertoire of electrical signaling that drives somatic action-potential generation and place-cell formation. Studying this input-output transformation is a formidable technical challenge. Sharp electrode and patch-clamp based
dendritic recordings amenable to in-vitro and anesthetized preparations have so far proven unsuitable to withstand movement artifacts encountered in awake running mice, even when head-fixed on a treadmill. Optical means of measurement are clearly needed. Imaging of calcium activity has sufficient signal-to-noise ratio to be useful in studying dendritic integration in the behaving animal, however, relying on depolarization it offers only a limited view. Membrane potential imaging, on the other hand, can resolve both excitation and inhibition, but sensors and their use have proven to be challenging. Here, using latest improvements in ASAP-family of genetically encoded voltage sensors we report on two-photon imaging of dendritic membrane potential in hippocampal CA1-area pyramidal cells of mice running on a treadmill.


**Poster**

**336. Learning and Memory: Physiology II**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 336.13/BB66

**Topic:** H.01. Animal Cognition and Behavior

**Support:** Empire Innovation Program, SUNY Research Foundation
National Institute of Food and Agriculture Hatch grant

**Title:** Modulation of cortical population activity in resolving proactive interference

**Authors:** *C. CAMMARATA, E. D. DE ROSA;
Cornell Univ., Ithaca, NY

**Abstract:** Proactive interference (PI), when previously learned information intrudes on encoding of similar items, can hinder learning and adaptive behavior. PI is exacerbated during normal aging, contributing to cognitive decline. Little is known about the neural population activity associated with overcoming PI. Here we recorded local field potentials (LFP) from the prelimbic (PrL) and posterior parietal (PPC) cortices of young (9 months, n=3) and aged (22 months, n=2) adult male Long Evans rats performing an odor discrimination task that contrasted learning a novel odor pair (Novel trials) with learning a pair that elicited PI (PI trials). Young rats displayed PI by initially having lower accuracy in PI trials than Novel trials (p<0.05) but resolved this interference so that the difference was no longer significant by the end of training (p=0.75). In Novel trials beta (10-30 Hz, p<0.01) activity in the PrL was significantly higher when rats responded correctly than when they responded incorrectly. Conversely PI trials with a correct response had lower beta activity in the PrL (p<0.01), and lower theta (10-30 Hz, p<0.01) and beta (p<0.01) activity in the PPC than those with an incorrect response. Aged rats were unable to
resolve PI, as indicated by significantly lower accuracy in PI trials compared to Novel odor trials even at the end of training (p= 0.046), and rats lacked the LFP modulation seen in young rats. This suggests that overcoming PI involves a disruption of the LFP activity associated with learning novel stimuli, and that the mechanisms for this disruption are lacking in aged rats. The neuromodulator acetylcholine (ACh) is necessary for PI resolution in young rats and declines with age, and ACh-dependent beta activity in the PrL and PPC supports flexible attention, so ACh is a strong candidate to regulate the LFP activity observed. We are currently replicating to above experiment in young (9-12 month, n=10) and aged (18-22 month, n=10) rats with I.P. injection of 0.25 m.g./k.g. scopolamine, a muscarinic antagonist that hinders PI resolution but not new learning at this dose, or methyscoplamine which cannot enter the brain. We predict that inhibiting ACh activity will prevent the suppression of low frequency activity seen in young rats, causing them to resemble aged rats neurally (a lack of LFP modulation) and behaviorally (inefficiency in resolving PI).

Disclosures:  C. Cammarata: None. E.D. De Rosa: None.

Poster

336. Learning and Memory: Physiology II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 336.14/BB67

Topic: H.01. Animal Cognition and Behavior

Title: Cholinergic modulation of hippocampal theta and hippocampus-dependent trace and contextual fear conditioning


Abstract: Hippocampal theta rhythm has been studied extensively, however, relatively little is known about the lower frequency or Type II theta. It was classically characterized by its presence during immobility and sensitivity to cholinergic antagonism. It is thought to play an important role in fear and anxiety responses, though exactly what function it serves in this regard and the underlying cellular and molecular mechanisms are still largely unknown. In order to study the generation and subsequent contribution of Type II theta in fear behaviors we conducted wireless local field potential recordings in stratum lacunosum-moleculare of dorsal CA1 in multiple lines of mice with cell type-specific knock-outs of cholinergic receptor sub-types (floxed M1 and alpha7 crossed with CamKII-cre, GAD2-cre, Chrna2-cre, SST-cre and PV-cre). The mice were subjected to a standard trace fear conditioning paradigm in order to assess the role of theta rhythms in the acquisition and retrieval of this hippocampus-dependent form of auditory fear conditioning. This high-throughput wireless approach allows for the simultaneous assessment of the functional behavioral impact of these genetic manipulations combined with
local field potential recordings. Freezing behavior and power in the lower frequency Type II band (4-7 Hz), the higher frequency (7-10 Hz) band, total theta power as well as the ratio of Type II to Type I were assessed prior to and during acquisition, during the context test and during the tone test. The behavioral results indicated that loss of the M1 muscarinic cholinergic receptor in both CamkII-Cre and GAD2-Cre mice impairs contextual fear conditioning whereas loss of the alpha7 nicotinic receptor subunit in SST-Cre and CamkII-Cre enhanced contextual fear conditioning. No differences were observed in trace auditory fear conditioning in these lines. Local field potential recordings showed a relative increase in type II theta with successful acquisition correlated with expression of freezing consistent with prior findings that type II theta is associated fear behaviors. This general approach provides a rich data set from which to determine the functional relationships between multiple cholinergic receptor subtypes in specific cell populations with the simultaneous readout of hippocampus-dependent learning and memory, and may also provide a peek into the potential interactions between type I and type II theta.


Poster

337. Human Long-Term Memory: Medial Temporal Lobe II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 337.01/BB68

Topic: H.02. Human Cognition and Behavior

Support: NIH R01-MH106512
NIH T32-NS047987

Title: Direct assessment of effective connectivity and plasticity in the human hippocampal-cortical network

Authors: *K. N. WARREN, S. SCHUELE, S. VANHAERENTS, J. L. VOSS; Northwestern Univ., Chicago, IL

Abstract: Episodic memory is supported by the hippocampus and a distributed network of interacting cortical regions. We have previously shown that noninvasive brain stimulation targeting this hippocampal-cortical network (HCN) increases resting-state fMRI correlations among network regions and influences episodic memory. However, direct electrophysiological measurements in humans of connectivity and plasticity in this network have not been well examined. Using human neurosurgical patients undergoing invasive monitoring for medically intractable epilepsy (n=5), we evaluated the effective connectivity and plasticity of regions in this network based on evoked potentials (EPs). EPs were measured in the hippocampus evoked by direct stimulation of an in-network site in lateral temporal cortex and an out-of-network
control site. Hippocampal EPs were measured before and after plasticity induction via 40 seconds of theta stimulation (5Hz) of the lateral temporal cortex, as noninvasive stimulation at this frequency induces changes in hippocampal-cortical network connectivity. We found increased amplitude of hippocampal EPs from temporal cortex, but not control regions, following plasticity induction. These findings provide direct electrophysiological evidence supporting the conclusion that stimulation regimens associated with plasticity induction cause changes in effective connectivity within the hippocampal-cortical network.


Poster

337. Human Long-Term Memory: Medial Temporal Lobe II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 337.02/BB69

Topic: H.02. Human Cognition and Behavior

Support: NIH/NIA Grant R01-AG049002

Title: Aging alters frequency specificity of noninvasive brain stimulation effects on hippocampal network involvement in recollection precision

Authors: *S. DAVE¹, M. S. HERMILLER¹, A. NILAKANTAN¹, S. VANHAERANTS¹, J. L. VOSS²; ²Med. Social Sci., ¹Northwestern Univ., Chicago, IL

Abstract: Memory precision can vary even when items are successful recalled, ranging from detailed and highly precise to generalized and less exact. Highly precise memories depend on the hippocampal-cortical network, and memory precision declines with normative aging. The hippocampal-cortical network exhibits neural synchrony at theta-band frequencies, and therefore noninvasive stimulation might have frequency-dependent effects on memory precision. In a recent study, we found that theta-burst stimulation (TBS; 5-Hz bursts) had a greater influence on item memory and hippocampal fMRI connectivity than did beta-frequency (20-Hz stimulation). In the current study, we sought to compare the effects of TBS versus beta-frequency stimulation on memory precision and to determine whether frequency-specific influences vary by age. In a within-subjects study (15 young adults (range: 21-28 years old), 15 older adults (range: 60-73 years old), participants received TBS, beta-frequency, or sham control stimulation prior to an episodic memory task that measured object-location recall precision. Stimulation was delivered to a left parietal cortical hub of the hippocampal-cortical network, based on individualized MRI-guided connectivity between the stimulation target and the left hippocampus. In young adults, both stimulation types increased spatial recollection precision relative to sham, but with fMRI
evidence suggesting that this occurred via distinct mechanisms. TBS increased precision-related fMRI connectivity between the hippocampus and visual cortex, whereas beta-frequency stimulation increased precision-related fMRI connectivity between the parietal cortex stimulation location and the visual cortex. Thus, TBS impacted hippocampal-cortical function whereas beta-frequency stimulation impacted cortical function, with either effect yielding enhanced recollection precision. In older adults, spatial memory precision improved following beta-frequency stimulation but not TBS, suggesting that aging impairs the ability for TBS to affect the hippocampus. These findings suggest that frequency as well as aging modulate the influence of stimulation on memory processing and that alternate stimulation regimens might be needed to influence hippocampal function in older adults, such as multiple-day stimulation. fMRI findings in the older adult sample will be discussed.


Poster

337. Human Long-Term Memory: Medial Temporal Lobe II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 337.03/BB70

Topic: H.02. Human Cognition and Behavior

Support: NeurTex Brain Research Institute

Title: Pre-encoding activity in the hippocampus is related to subsequent memory

Authors: Z. J. URGOLITES¹, *J. T. WIXTED², L. R. SQUIRE³, S. GOLDFINGER⁴, M. PAPESH⁵, P. N. STEINMETZ⁶;
¹Dept. of Psychiatry, UCSD, La Jolla, CA; ²UC San Diego, La Jolla, CA; ³Veterans Affairs San Diego Healthcare Syst., San Diego, CA; ⁴Arizona State Univ., Tempe, AZ; ⁵Louisiana State Univ., Baton Rouge, LA; ⁶NeurTex Brain Res. Inst., Dallas, TX

Abstract: Studies using fMRI have found that activity in the hippocampal region during encoding is higher for subsequently remembered stimuli compared to subsequently forgotten stimuli. It is generally supposed that memory-related activity during encoding is driven by the presentation of the to-be-remembered stimulus. However, another possibility is that activity immediately preceding stimulus onset substantially pre-determines the memory-related activity that occurs during stimulus presentation. If so, then pre-stimulus encoding activity might differ for subsequently remembered vs. forgotten items. To test this possibility, single neuron activity was recorded from intracranial microwires implanted in 30 epileptic patients while they performed a continuous recognition memory task. The task consisted of the auditory presentation of 300 words that were each repeated once at lags ranging from 0 to 32 intervening words (600
word presentations in all), and the patients had to decide whether each word was novel or repeated by pressing a key. Activity in left hippocampus was measured 1000 to 200 ms before word onset (i.e., before encoding) and 200 to 1000 ms after word onset (i.e., during encoding). Pre-stimulus activity for words later correctly judged as repeated (hits) was higher than pre-stimulus activity for words later incorrectly judged as novel (misses). In addition, across participants, the magnitude of this difference in pre-stimulus activity correlated with the overall ability to discriminate between repeated and novel words (i.e., d’). Activity measured 200 to 1000 ms after the onset of novel words yielded a similar pattern. The results suggest that activity in the hippocampus prior to stimulus presentation partially determines how well it will be encoded and subsequently remembered.


Poster

337. Human Long-Term Memory: Medial Temporal Lobe II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 337.04/BB71

Topic: H.02. Human Cognition and Behavior

Support: NeurTex Brain Research Institute

Title: Single-neuron phase coding of novelty in the human hippocampus

Authors: *J. MILLER¹, P. N. STEINMETZ², J. JACOBS¹;
¹Dept. of Biomed. Engin., Columbia Univ., New York, NY; ²NeurTex Brain Res. Inst., Dallas, TX

Abstract: From studies of patients with lesions, we have obtained strong evidence that the hippocampus is important for episodic memory. However, we do not have a detailed understanding of the specific patterns of neuronal activity in this structure that are used by humans and animals to differentiate and represent individual memories. To examine the electrophysiology of memory encoding in the human hippocampus, we recorded single-neuron activity from neurosurgical patients as they performed a verbal episodic memory task. We compared the rate and timing of individual neuronal activations between timepoints when subjects viewed old vs. new items to characterize the neural code for memory novelty in the hippocampus. As in previous work, we found some individual neurons increased their overall firing rates when subjects viewed novel stimuli. In addition, by examining the temporal relation between individual action potentials and network field potential oscillations, we found a new form of temporal coding for memory novelty. The spiking of individual neurons was phase locked to hippocampal oscillations in the 1-4-Hz “slow theta” band, and the precision of this
phase locking significantly increased when subjects viewed novel stimuli. This suggests that the hippocampus exhibits a temporal code for memory novelty based on phase coupling of individual neurons to network oscillations. By demonstrating that the hippocampal code for memory involves changes in spike timing in addition to changes in mean spike rate, it suggests that separate phases of network oscillations have distinct functional roles with regard to memory novelty.

Disclosures:  J. Miller: None. J. Jacobs: None. P.N. Steinmetz: None.

Poster

337. Human Long-Term Memory: Medial Temporal Lobe II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 337.05/BB72

Topic: H.02. Human Cognition and Behavior

Support: CIHR MOP125958

Title: Behavioural discrimination for gist of everyday scenes

Authors: *N. V. HOANG, F. N. AHMAD, M. MOSCOVITCH;
Rotman Res. Inst., Toronto, ON, Canada

Abstract: Behavioural discrimination between repeated and similar items is assumed to rely on a neural mechanism known as pattern separation. Pattern separation has been assessed in humans by the Mnemonic Similarity Task (MST), which measures recognition memory at test for previously presented items (targets), lures that are similar to targets, and novel items (foils) (Bakker et al., 2008). This study adapted the MST to measure if difficulty in lure discrimination at retrieval, as observed in the MST, is maintained when multiple exemplars of the same category are presented at both encoding and retrieval. The goal was to investigate if behavioural discrimination is mediated by prior knowledge of exemplars per scene category more so than by ‘pattern separation’ of overlapping items. Moreover, it is relevant to understand if the behavioural discrimination process is mediated by an individual’s recollection (remembering distinct representations) or familiarity (knowing gist-like representations) of the items. Thirty-two undergraduate students studied three exemplars per scene category, and were tested on 24 targets, 24 lures, and 24 foils. Contrary to findings reported by Stark and Stark (2017), there was no significant difference in recognition accuracy between lure and target discrimination. Participants showed higher false recognition to targets than lures. Similarly, target accuracy corresponded to greater recollection of details than familiarity; meanwhile, lure accuracy was more supported by familiarity of gist-like features than recollection. Our findings suggest that in the context of multiple exemplars, behavioural discrimination of exemplars per scene category may rely more on prior knowledge and less on pattern separation.
Disclosures: N.V. Hoang: None. F.N. Ahmad: None. M. Moscovitch: None.

Poster

337. Human Long-Term Memory: Medial Temporal Lobe II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 337.06/BB73

Topic: H.02. Human Cognition and Behavior

Support: ERC-StG 261177
ERC-CoG GEOCOG 724836
NWO-Vidi 452-12-009

Title: Organising knowledge for value generalisation

Authors: *M. M. GARVERT1, N. SCHUCK2, C. F. DOELLER1;
1MPI for Human Cognitive and Brain Sci., Leipzig, Germany; 2Max Planck Inst. For Human Develop., Berlin, Germany

Abstract: It has been suggested that the brain organises spatial and non-spatial information in a cognitive map. Such a representation of events and knowledge may facilitate goal-directed behaviour by enabling the generalisation of information across related states. Here, we combine a novel virtual reality task with computational modeling to investigate whether humans infer stimulus-reward associations that were never directly experienced based on learned knowledge about the relationships between stimuli in space. In this task, the spatial position of stimuli learned on day 1 predict rewards in a decision making task on day 2. We find that updates of stimulus-reward associations in the decision making task go beyond directly experienced associations and instead spread across stimuli located nearby in the cognitive map, enabling correct decisions even between stimuli whose values have never been directly experienced. Relational knowledge organised in cognitive maps can thus be used to extrapolate across related states and thereby facilitate novel inference. In most subjects, this behaviour can be captured well by a Bayesian model, where beliefs about the reward distribution are updated on a trial-by-trial basis. Participants whose choice behaviour can be particularly well captured by the model are also better at inferring values of stimuli they never chose. By including stimuli in the decision making task whose spatial locations are unknown, we further demonstrate that novel stimuli can be integrated into existing cognitive maps if their statistical structure is consistent with previously experienced regularities. Using fMRI, we investigate the neural dynamics underlying the spread of values across cognitive maps in hippocampal-medial prefrontal networks. Together, our novel approach opens up the possibility to connect seemingly disparate fields of spatial coding, learning and decision behaviour.

Disclosures: M.M. Garvert: None. N. Schuck: None. C.F. Doeller: None.
Title: Integrating knowledge from physical and conceptual spaces

Authors: *D. KUHRT*¹, J. L. S. BELLMUND²,³, R. KAPLAN¹, C. F. DOELLER²,¹;
¹Kavli Inst. for Systems Neuroscience, NTNU, Trondheim, Norway; ²MPI for Human Cognitive and Brain Sci., Leipzig, Germany; ³Donders Institute, Radboud Univ., Nijmegen, Netherlands

Abstract: How do we represent knowledge? One intriguing idea is that our knowledge is stored using a spatial representational format: the cognitive map. Studying spatial navigation has shed light on underlying neural mechanisms with recent work suggesting that cognitive map-like coding of physical space might also map conceptual knowledge. Examining how spatial and conceptual information is integrated, we created a two-dimensional conceptual space and a corresponding virtual model of a physical space, where space-defining features such as dimensionality, size, shape and informational content were carefully matched. Testing object-location memory, participants learned to navigate both spaces using identical egocentric controls and successfully created object-location associations. Further, participants were able to transfer knowledge about object positions from one space to the other. We probed object representations in a passive-viewing fMRI task both before and after learning, allowing us to assess the change in representation. We used multivariate pattern analysis to test if neural similarity after training scales with distances between objects and whether this scaling holds for the integrated map of both spaces. Preliminary fMRI analyses have uncovered pattern similarity changes in the hippocampal formation and prefrontal regions following knowledge acquisition in both spaces. Our findings demonstrate a transfer of knowledge between cognitive maps that differ in content, as well as domain. Taken together with findings from a parallel behavioural study using immersive virtual reality, our data highlight potential domain-invariant codes in navigation that transcend physical space and help us navigate our knowledge.

Poster

337. Human Long-Term Memory: Medial Temporal Lobe II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 337.08/BB75

Topic: H.02. Human Cognition and Behavior

Support: ZonMw InZicht 94312004

Title: Cognitive map formation through tactile and visual exploration

Authors: *L. OTTINK*¹, M. HOOGENDONK¹, T. M. VAN DER GEEST², R. J. A. VAN WEZEL¹, C. F. DOELLER³;

¹Donders Institute, Radboud Univ., Nijmegen, Netherlands; ²Hogeschool van Arnhem en Nijmegen, Arnhem, Netherlands; ³MPI for Human Cognitive and Brain Sci., Leipzig, Germany

Abstract: The human brain can form a cognitive map of an environment, which represents spatial information and can support navigation. Most research on this has focused on visual information. In this study, we investigated cognitive mapping of environments presented in a non-visual modality, thereby focusing on tactile information. This may have implications for visually impaired persons, to whom visual information is less available. Blinded and non-blinded sighted participants learned three different tactile maps of a city-like environment in three successive tasks. The maps differed in complexity, and each had five marked locations associated with an item. To assess cognitive map formation after map exploration, participants estimated Euclidean and path distances between item pairs, rebuilt the map, recalled the locations of the items, and navigated two routes. Both blinded and non-blinded participants overall performed well, indicating the formation of accurate cognitive maps. Interestingly, there were smaller differences between the groups than hypothesised. Blinded participants performed worse than non-blinded participants, but only on the more complex maps, suggesting no distinct advantage of the use of vision on the least complex map. Furthermore, there were no significant differences between maps or between the blinded and non-blinded participants for estimations of distances between item pairs. These results suggest that with and without visual information, metrics of a tactically presented environment are stored in a cognitive map. Interestingly, it furthermore suggests not just memory of explicit information of the map, but storage of, or the ability to infer, implicit spatial information. This is evidence supporting ideas of a modality-independent representation of space. This line of research will be extended to include visually impaired participants, and to additionally focus on the auditory modality. We will assess how spatial information presented in the tactile and auditory modality is integrated into a cognitive map in visually impaired persons, and how this compares to sighted participants. Furthermore, we will use functional magnetic resonance imaging to investigate the influence of presenting environments through these modalities on neural representations of space.

Poster

337. Human Long-Term Memory: Medial Temporal Lobe II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 337.09/BB76

Topic: H.02. Human Cognition and Behavior

Support: ERC-CoG GEOCOG 724836

Title: Spatial learning of abstract multimodal concepts

Authors: *L. B. SANDØY¹, J. B. JULIAN¹, L. SOMMER¹, C. F. DOELLER¹,²;
¹Kavli Inst. for Systems Neuroscience, NTNU, Trondheim, Norway; ²MPI for Human Cognitive and Brain Sci., Leipzig, Germany

Abstract: Spatial coding in the hippocampal formation has traditionally been studied in the context of navigation. However, recent studies suggest that the hippocampal formation may mediate a diverse range of cognitive functions beyond navigation, including conceptual learning. The hippocampal formation may be involved in concept learning since concepts often are defined based on a set of continuous feature dimensions, akin to the latitude and longitude of navigational space. Importantly, most real-world concepts are defined based on multimodal features. For example, different citrus fruits can be defined by their color and amount of sweetness, which are derived from visual and gustatory sensory modalities, respectively. Yet, whether the hippocampal formation is involved in learning multimodal concepts is unknown. Our main objective was to develop a multimodal conceptual learning task to investigate hippocampal representations of non-navigational domains. More specifically, are multimodal conceptual spaces represented similarly as navigational cognitive maps?

To address this question, we developed a computer-based multimodal concept learning task using pitch and color to create a two-dimensional continuous concept space. In this task, participants were trained to associate specific pitch/color combinations with distinct symbols. Participants then conducted memory tests for these stimulus-symbol associations using a behavioral task modeled after standard assays of spatial memory during navigation. Preliminary results show that participants were able to successfully maneuver around in this multimodal concept space. The results indicate that participants formed an integrated representation of the multimodal space, beyond simply learning the correct stimulus-symbol associations. Follow-up studies are currently exploring if representations of multimodal space obey the same principles as map-like representations of navigational space, as well as using fMRI to interrogate the role of the hippocampal formation in supporting such representations. Together, this research will reveal
how new concepts are learned, and whether similar processes guide conceptual learning and spatial navigation.


Poster

337. Human Long-Term Memory: Medial Temporal Lobe II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 337.10/BB77

Topic: H.02. Human Cognition and Behavior

Support: ERC-StG 261177
        ERC-CoG GEOCOG 724836
        NWO-Vidi 452-12-009
        NWO-Gravitation 024-001-006

Title: Conceptual relevance in cognitive space

Authors: *S. Theves\textsuperscript{1,2}, G. Fernandez\textsuperscript{3}, C. F. Doeller\textsuperscript{1,4};
\textsuperscript{1}MPI for Human Cognitive and Brain Sci., Leipzig, Germany; \textsuperscript{2}Donders Institute, Radboud Univ., Nijmegen, Netherlands; \textsuperscript{3}Donders Inst., Radboud Univ. Med. Ctr., Nijmegen, Netherlands; \textsuperscript{4}Kavli Inst. for Systems Neuroscience, NTNU, Trondheim, Norway

Abstract: The hippocampal formation encodes maps of the physical environment and a key question in neuroscience is whether its spatial coding principles also provide a universal metric for the organization of non-spatial, conceptual information. In support of this view, we recently demonstrated that the hippocampus encodes distances between points in an abstract space spanned by continuous stimulus-feature dimensions that were relevant to the acquisition of a novel concept. Here, we further probe whether this distance code reflects a mapping of just any task-relevant, or specifically conceptually-relevant dimensions. During fMRI scanning, we presented every-day objects, which had beforehand been associated with specific values on three abstract dimensions, of which only two were relevant to concept learning. We find that the hippocampal signal is significantly better explained by inter-object distances in a space defined along the conceptually-relevant dimensions as compared to distances in a space defined along all task-relevant dimensions. We further rule out that this result is explained by a difference in complexity between mapping two-dimensional versus three-dimensional information. These findings suggest that the hippocampus supports knowledge acquisition by encoding a combined space along conceptually-relevant dimensions.

Disclosures: S. Theves: None. G. Fernandez: None. C.F. Doeller: None.
Title: Integrating episodic and spatial context signals in the hippocampus

Abstract: Episodic and spatial memory are two major forms of memory. Episodic memory allows remembering events from the past whereas spatial memory allows forming a map-like representation of the environment. Interestingly, these two memory forms are supported by the same brain structure: the hippocampus. However, the exact relationship between episodic and spatial memory processes in the hippocampus still remains unclear. In this study, we test two different models assuming that the hippocampus supports both memory forms either via a parallel processing or a common coding mechanism, respectively. For this purpose, we conducted an fMRI experiment with a life-simulation task and a virtual reality game to manipulate episodic and spatial relations between objects. In the life-simulation task, subjects watched two different stories whereby regular objects were associated with one of the two stories (episodic contexts). In the virtual reality game, subjects delivered objects to stores in two different neighborhoods of a virtual city whereby regular objects were associated with one of the two neighborhoods (spatial contexts). Ultimately, this resulted in a $2 \times 2$ design with pairs of objects sharing both an episodic and a spatial context, pairs of objects sharing only one (either episodic or spatial) context and pairs of objects sharing no context. We presented all objects in picture viewing tasks to assess overlapping neural representations of objects caused by shared episodic and / or spatial contexts in cross-stimulus adaptation analyses. Preliminary results show differences between adaptation effects for pairs of objects sharing only an episodic or a spatial context as well as a trend of an interaction between episodic and spatial context processing in the hippocampus, characterized by a stronger adaptation effect for pairs of objects sharing both contexts. This indicates that our experimental approach is powerful enough to induce neural similarity between objects sharing episodic and / or spatial contexts in the hippocampus. Furthermore, our preliminary results provide evidence for both models of a parallel processing and a common coding mechanism for episodic and spatial memory in the hippocampus.

Disclosures: A. Nitsch: None. N. de Haas: None. L. Deuker: None. C.F. Doeller: None.
**Title:** A general framework for decoding behaviour from wide-band neural activity

**Authors:** *M. Frey*\(^1\), S. Tanni\(^2\), C. Perrodin\(^3\), A. O'Leary\(^4\), M. Nau\(^1\), J. Kelly\(^5\), A. Banino\(^6\), C. F. Doeller\(^7\), C. Barry\(^2\);

\(^1\)Kavli Inst. for Systems Neuroscience, NTNU, Trondheim, Norway; \(^2\)Cell and Developmental Biol. Dept., UCL, London, United Kingdom; \(^3\)Exptl. Psychology, \(^4\)Univ. Col. London, London, United Kingdom; \(^5\)Open Climate Fix, London, United Kingdom; \(^6\)Deepmind, London, United Kingdom; \(^7\)Max Planck Inst., Leipzig, Germany

**Abstract:** Decoding provides a way of placing a limit on the information present in a neural population. We used multichannel recordings from hippocampal and additional structures to assess the accuracy with which behaviour can be decoded from the underlying neural activity. Typical decoding algorithms use spike sorted action potentials detected from ongoing neural activity, which potentially introduce selection biases, and methods limited to action potentials inevitably discard the information contained in the local field potential. Here we decode behaviour directly from the wavelet-transformed electrophysiological signals. For this purpose, we developed a novel deep neural network architecture which takes as input the wavelet transformed signal and predicts continuous behaviour. The model consists of stacked two-dimensional convolutional layers with weights shared between channels and time, followed by a fully connected layer. This architecture captures both spatial and temporal information in the signal and is able to successfully decode complex behaviours, outperforming established location-decoding methods, such as a Bayesian decoder, trained on manually sorted spikes. We show that our model generalises across different brain regions and recording techniques, successfully decoding information about position, speed, head direction, and sound frequency from electrode and two-photon calcium imaging data.

**Disclosures:** M. Frey: None. S. Tanni: None. C. Perrodin: None. A. O'Leary: None. M. Nau: None. J. Kelly: None. A. Banino: None. C.F. Doeller: None. C. Barry: None.
Title: Predictive spatiotemporal integration in the human medial temporal lobe

Authors: *I. POLTI¹, M. NAU¹, R. KAPLAN¹, V. VAN WASSENHOVE², C. F. DOELLER³;¹
Kavli Inst. for Systems Neuroscience, NTNU, Trondheim, Norway; ²CEA, DRF/Joliot, NeuroSpin; INSERM, U992, Cognitive Neuroimaging Unit, Univ. Paris-Sud; Univ. Paris-Saclay, Gif Sur Yvette, France; ³Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany

Abstract: Converging evidence from single-unit recordings in non-human primates and human functional MRI (fMRI) suggests that the medial temporal lobe maintains a cognitive map of visual space. Entorhinal grid cells are one critical component of the cognitive map, and are thought to support vector computations. On the population level, grid cells putatively give rise to a six-fold rotationally symmetric (hexadirectional) fMRI signal. In parallel, the hippocampal-entorhinal circuit and the striatum have been implicated in temporal processing, feedback learning, and the representation of future states. Here, we combined fMRI with a tightly-controlled time-to-contact (TTC) estimation task. We examined activity in the hippocampal formation as a function of behavioral performance and feedback. Participants fixated on a dot that moved on linear trajectories at different speeds within a circular boundary. Whenever the dot stopped moving, participants extrapolated its trajectory and speed, ultimately pressing a button when the dot would have hit the boundary if it continued moving. This design allowed us to examine TTC estimates as a function of gaze direction for a variety of motion speeds. We found that behavioral precision and accuracy were modulated by temporal context and that activity in the hippocampal-entorhinal circuit and the striatum reflected feedback valence. We further examine how hippocampal-entorhinal and neocortical trial-by-trial activity and hexadirectional signals relate to behavioral performance in this task. Our approach provides new insights into how space and time are processed dynamically across the visual field and sheds new light on how the hippocampal formation guides human behavior.

Title: Does hippocampal volume and/or tissue microstructure explain performance differences on hippocampal-dependent tasks?

Authors: *I. A. CLARK¹, A. M. MONK¹, V. HOTCHIN¹, G. PIZZAMIGLIO¹, A. LIEFGREEN¹, N. WEISKOPF², M. F. CALLAGHAN¹, E. A. MAGUIRE¹;
¹Wellcome Ctr. for Human Neuroimaging, Univ. Col. London, London, United Kingdom; ²Dept. of Neurophysics, Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany

Abstract: The imagination of scenes, autobiographical memory, future thinking and spatial navigation are four cognitive functions known to be hippocampal-dependent. Marked differences exist across healthy individuals in their performance on tasks assessing these functions, but clear reasons for this variability are lacking. Task performance may be related to hippocampal structure. For example, damage to the hippocampi impairs these four cognitive functions, and healthy individuals with extreme navigation expertise (London taxi drivers) have increased posterior hippocampal grey matter volume. However, only a few studies have interrogated the relationship between hippocampal volume and these four cognitive functions in the general population, mostly focusing on autobiographical memory and navigation. Results have been mixed, and may have been influenced by relatively small sample sizes. Extant studies have also predominantly concentrated on hippocampal volume, but advances in neuroimaging techniques now permit examination of tissue microstructure in the form of myelination and iron biomarkers. Here, we investigated whether performance on scene imagination, autobiographical memory, future thinking and spatial navigation tasks was related to hippocampal volume and/or tissue microstructure. Behavioural and whole brain structural MRI data (0.8mm isotropic quantitative multi-parameter maps) were collected from 217 healthy male and female human participants aged between 20-41 years. Grey matter volume was assessed using voxel-based morphometry, and tissue microstructure using voxel-based quantification. Neither hippocampal grey matter volume nor tissue microstructure was related to overall scene imagination, autobiographical memory or future thinking performance, even at liberal statistical thresholds. There was also no significant correlation with a composite navigation measure. However, performance on a navigation subtask specifically assessing route knowledge was positively associated with anterior hippocampal grey matter volume. Overall, therefore, in this relatively large sample of young,
healthy participants, with a wide variance of performance on the hippocampal-dependent tasks of interest, neither hippocampal volume nor tissue microstructure could explain individual differences. We conclude that a healthy adult hippocampus likely has sufficient capacity for even the best performance on scene construction, autobiographical memory, future thinking and most spatial navigation tasks. It may be only in extreme situations, such as London taxi drivers, that the hippocampus exhibits measurable structural changes.


Poster

337. Human Long-Term Memory: Medial Temporal Lobe II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 337.15/BB82

Topic: H.02. Human Cognition and Behavior

Support: Wellcome Trust (101759/Z/13/Z)
Wellcome Trust (203147/Z/16/Z)

Title: Sleep and dreaming in people with focal bilateral hippocampal damage

Authors: *G. SPANO1, G. PIZZAMIGLIO1, C. MCCORMICK2, I. A. CLARK1, T. D. MILLER3, F. D. WEBER4, S. DE FELICE1, J. O. EDGIN5, C. R. ROSENTHAL6, E. A. MAGUIRE1;

Abstract: Sleep and dreaming have each been posited to play a role in memory processing and consolidation. Intact hippocampi are essential for the proper functioning of memory, and yet little is known about how hippocampal lesions affect sleep and dreaming. Here, we examined male human patients with focal bilateral hippocampal damage and matched healthy control participants in order to investigate the specific contributions of the hippocampus to sleep physiology and the frequency and content of dreams.
We characterised sleep phenotype by performing in-home polysomnography on three nights. Despite similar sleep efficiency, total sleep time, sleep onset, and sleep fragmentation, the patients spent significantly less time in slow wave sleep (SWS, also known as N3 sleep) compared to the control participants. By contrast, N1, N2, and REM sleep were comparable between the groups. The lack of SWS in the patients was further confirmed by a data-driven
approach, independent of sleep staging, which showed a similar significant decrease of delta band power in the EEG (1.8–4.0 Hz), the hallmark of SWS. We also scrutinized N2 sleep for features that might presage the decrease in SWS sleep, including slow oscillations (0.5–1 Hz), slow (9–12 Hz) and fast (12–15 Hz) spindles, and the coupling of slow oscillations and fast spindles. We found no differences between the patients and their controls on any of these measures.

We assessed dreaming during a separate night of sleep using a provoked awakening protocol. This involved participants being woken up at various times, including in non-REM and REM sleep. Despite being roused a similar number of times, patients had significantly fewer dreams compared to the controls. Moreover, when patients reported dreams, they were significantly less vivid, detailed, and episodic-like compared to those of the control participants. SWS sleep in particular has been linked with the offline consolidation of recently-formed memories. The near-absence of SWS in the patients, coupled with their less frequent and impoverished dreams, leaves them bereft of two key features that are known to be important for memory processing. Overall, these results serve to highlight that vital sleep functions depend on hippocampal integrity.


Poster

337. Human Long-Term Memory: Medial Temporal Lobe II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 337.16/BB83

Topic: H.02. Human Cognition and Behavior

Support: Wellcome Trust (210567/Z/18/Z)
Wellcome Trust (203147/Z/16/Z)

Title: Are scenes the smallest units of events? Evidence from hippocampal and vmPFC oscillatory dynamics

Authors: *A. M. MONK, D. N. BARRY, V. LITVAK, G. R. BARNES, E. A. MAGUIRE; Wellcome Ctr. For Human Neuroimaging, UCL, London, United Kingdom

Abstract: Our lived experience comprises a series of ongoing contiguous events. This allows us to construct a coherent autobiography, imbuing our lives with a sense of continuity, and a capacity to recall experiences even when the original events occurred many decades ago. How the brain achieves this monumental feat is a central question in neuroscience. In this experiment, we sought to understand how such events are built by the human brain, and in particular the roles
played by the hippocampus and ventromedial prefrontal cortex (vmPFC). We also tested the hypothesis that scene imagery is central to the representation of events, given the extant literature showing that the anterior hippocampus and vmPFC are involved in constructing such imagery. We leveraged the high temporal resolution of magnetoencephalography to examine oscillatory dynamics in twenty one healthy female and male participants while they watched simple movie clips built from individual frames that either depicted the evolution of events comprised of scenes, or events composed of non-scenes (abstract patterns). In both cases, the movie clips created a sense of an unfolding event over time, as small changes in the stimuli image-to-image showed the progression of an activity. We performed analyses of power in source space, inter-regional coherence and effective connectivity. Unfolding scene and non-scene events resulted in low frequency power changes in the hippocampus and vmPFC relative to non-event stimuli. However, there was higher coherence between activity in the anterior hippocampus and vmPFC during events built from scenes and, by contrast, higher coherence between the posterior hippocampus and vmPFC during events built from patterns. Dynamic causal modelling of scene-based events revealed the vmPFC drove oscillatory activity in the anterior hippocampus, whereas during non-scene events the posterior hippocampus drove activity in the vmPFC. These results extend previous findings by showing that not only do the anterior hippocampus and vmPFC support the construction of single scenes, but that unfolding events built from scenes are also supported by these regions, where the vmPFC exerts a driving influence. This aligns with perspectives that propose the vmPFC guides the construction of scene and event representations in the hippocampus.


Poster

337. Human Long-Term Memory: Medial Temporal Lobe II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 337.17/BB84

Topic: H.02. Human Cognition and Behavior

Support: Wellcome Trust Grant 210567/Z/18/Z
Wellcome Trust Grant 203147/Z/16/Z
Wellcome Trust Grant 203257/Z/16/Z
Wellcome Trust Grant 203257/B/16/Z

Title: Imaging the human hippocampus in real-world contexts using wearable MEG

Authors: *D. N. BARRY¹, T. M. TIERNEY¹, S. MELLOR¹, R. BOWTELL², M. J. BROOKES², G. R. BARNES¹, E. A. MAGUIRE¹;
Abstract: During conventional human neuroimaging, such as magnetoencephalography (MEG), participants must remain still with their heads immobilised in order to avoid compromising the localisation of brain activity. This imposes substantial limitations on the types of cognitive paradigms that can be deployed. However, the development of optically-pumped (OP) magnetometers represents a significant evolution of MEG technology. OP-MEG does not require cryogenic cooling, and so the sensors can be placed directly on the scalp. The resulting proximity to the brain increases the magnitude of the measured signal. However, the most significant advance associated with OP-MEG is the capacity for participant movement. OPM sensors can be mounted on a scanner-cast moulded specifically to a participant’s head, allowing the person to move freely when combined with an innovative approach to nulling the magnetic field surrounding a participant. The potential, therefore, exists to study more complex behaviours in which movement plays an integral part. For example, spatial navigation in a naturalistic setting involves changes in head direction and coordinated body movements.

To fully leverage the wearability of OP-MEG in such a real-world domain, one must be able to detect signals from deeply-located brain structures upon which behaviours like navigation depend, such as the hippocampus. Consequently, as a proof of principle, we first had healthy male and female participants perform a hippocampal-dependent task - the imagination of novel scene imagery - while being scanned using OP-MEG. We found that task-related modulation of theta power in the medial temporal lobe was observable, with a peak in the anterior hippocampus. We then proceeded to show that it is possible to examine head direction signals while participants move their heads naturalistically, including the concomitant vestibular inputs that are typically absent from standard MEG and fMRI studies. We conclude that OP-MEG heralds exciting new opportunities for investigating the neural correlates of a range of crucial cognitive functions in real-world contexts that have hitherto eluded conventional neuroimaging methods.


Poster

337. Human Long-Term Memory: Medial Temporal Lobe II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 337.18/BB85

Topic: H.02. Human Cognition and Behavior

Support: NSF
Title: Neural correlates of recognition memory in the human visual ventral stream

Authors: *N. M. DE LA ROSA-RIVERA*¹, K. LEGER², N. BLAUCH³, R. A. COWELL⁴;
¹Univ. of Massachusetts Amherst, Amherst, MA; ²Brandeis Univ., Waltham, NH; ³Ctr. for the
Neural Basis of Cognition, Carnegie Mellon Univ., Pittsburgh, PA; ⁴Psychological and Brain
Sci., Univ. of Massachusetts, Amherst, MA

Abstract: Long-term declarative memory has traditionally been tied to the medial temporal lobe
(MTL). An important question is whether such memory can be supported by regions outside
MTL under certain circumstances? Representational accounts of cognition hold that memory for
an item is supported by whichever brain region best represents that item. One theory, the
Representational-Hierarchical Account, claims that representations are organized in a hierarchy
extending from simple features in visual cortex, through conjunctions of features in temporal
cortex, to high-dimensional associative representations of events in hippocampus. This theory
makes two predictions. First, memory for simple stimuli should be computed outside of MTL, in
visual cortex. Second, cortical sites supporting memory discrimination should map onto the sites
containing the representations most useful for the memory task. If to-be-remembered items are
objects comprising conjunctions of visual features (color, shape), then cortical regions
supporting memory for the particular conjunctions should be those containing a conjunction
code, whereas regions supporting memory for the features should be those containing a feature
code.

We conducted an fMRI study to measure 1) the neural code elicited by visual stimuli and 2) the
neural correlates of remembering those stimuli. Stimuli were abstract objects defined by
conjunctions of color, shape and spatial frequency. In the study phase, participants viewed 24
stimuli 20 times each. In the test phase, subjects saw 3 item types: studied, novel (entirely novel
features) and recombined (novel conjunctions of familiar features, created by recombining
features of studied objects). At test, subjects responded “familiar”, “novel” or “recombined”. We
measured the neural code elicited in the study phase using a multi-variate technique that first
assesses feature information and conjunction information, then pits these against each other to
ask whether a region is biased toward a feature code or a conjunction code. We predict that
regions containing a feature code (in the study phase) should provide the best signal for
remembering novel features (in the test phase), which we assess by contrasting “recombined”
with “novel” items. Conversely, regions containing a conjunction code should provide the best
signal for remembering novel conjunctions, assessed by contrasting “familiar” with
“recombined”. Results in 3 subjects suggest a transition from feature-coding in V1 to
conjunction-coding in V3, and neural correlates of mnemonic discrimination in visual cortex.
Thus the paradigm is suitable for addressing the key questions in a larger sample size.

Disclosures:  N.M. de la Rosa-Rivera: None. K. Leger: None. N. Blauch: None. R.A. Cowell:
None.


**Poster**

**337. Human Long-Term Memory: Medial Temporal Lobe II**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 337.19/CC1

**Topic:** H.02. Human Cognition and Behavior

**Title:** Induced plasticity caused by regular and targeted mnemonic discrimination training: An fMRI approach

**Authors:** *J. GÜSTEN*\(^1,2\), D. BERRON\(^1,2,3\), E. DÜZEL\(^1,2,4\);


**Abstract:** With an increasingly ageing population, the possibility to improve or preserve cognitive function throughout life via training interventions has become a popular focus of research. When looking at transfer effects of cognitive training, the complexity of earlier training regimes makes it difficult to pin down those features underlying the training-related gains (Karbach, 2014). While using highly targeted paradigms may restrict the probability of transfer to other domains, understanding the precise pattern and mechanisms of transfer might be more important for theoretical advances than generality of transfer (Lövden et al., 2010). In the present training study, we were interested in studying patterns of transfer caused by highly specific mnemonic discrimination training, an ability that typically decreases with age (Stark, Yassa, Lacy, & Stark, 2013). Forty young participants (age range 18-30 years) performed a web-based object-scene mnemonic discrimination task (Berron et al., 2018), three times a week, for two weeks in a row. Each training session lasted around 60 minutes. During the task, participants saw blocks of object and scene images and had to indicate whether a stimulus was repeated identically (repeat) or presented in a similar version (lure) via button presses. In contrast to the original task, object and scene blocks were presented in a 6-back instead of 2-back task design. In a control task focused on processing speed and change detection, half of the participants had to identify moving targets on the screen by clicking them. Before and after training, whole-brain functional and structural MRI data was acquired using a 3T MAGNETOM Skyra fit scanner. This included a task-based fMRI session using the original object-scene mnemonic discrimination task. As expected, the training group showed significantly increased task performance compared to the control group. Given that the task is thought to rely on pattern separation (PS), we hypothesized to find group by timepoint interactions of PS-related activity along the two processing streams involved in object and scene memory, including the MTL subregions. First, we expected a change in the univariate measure of repetition suppression. Second, we performed an encoding-retrieval similarity analysis (ERS), hypothesizing that
multivariate activation patterns become more distinct in those regions involved in the task. The present study provides important insights on adaptation patterns emerging from highly targeted mnemonic discrimination training. These could yield a more mechanistic understanding of potential transfer effects of memory training interventions in the future.

**Disclosures:** J. Güsten: None. D. Berron: None. E. Düzel: None.

**Poster**

337. Human Long-Term Memory: Medial Temporal Lobe II

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 337.20/CC2

**Topic:** H.02. Human Cognition and Behavior

**Title:** Behavior-dependent directional tuning in the human navigation network

**Authors:** *M. NAU*¹, T. NAVARRO SCHRÖDER¹, M. FREY¹, C. F. DOELLER²; ¹Kavli Inst. for Systems Neuroscience, NTNU, Trondheim, Norway; ²MPI for Human Cognitive and Brain Sci., Leipzig, Germany

**Abstract:** Human scene-processing and navigation regions have been extensively studied, yet the relationship between their neural tuning and active spatial behavior remains poorly understood. We leveraged 7T-fMRI to monitor human brain activity at sub-millimeter resolution while participants navigated a virtual reality environment and performed an object location memory task. We used voxel-wise encoding modeling of the navigation behavior to predict each voxel’s time course to map position-invariant directional tuning across the cortex. By iterating through multiple basis sets of directional kernels, we also estimated the corresponding tuning width of each voxel. Our approach revealed a narrow-to-broad topography of directional tuning from posterior to anterior parahippocampal cortex as well as distinct tuning curves in visual, retrosplenial and entorhinal (EC) cortices. Next, we asked whether and how the estimated tuning curves reflect different mnemonic and behavioral states. While tuning strength, but not tuning width, of the posteromedial EC reflected how well participant performed in the spatial memory task, the opposite pattern could be observed in the retrosplenial cortex. A whole-brain analysis confirmed these results and demonstrated behavior-dependent directional tuning in a brain network including occipital, medioparietal and mediotemporal regions. Directional representations were stronger in visual and retrosplenial cortex during locomotion and stronger in parahippocampus and hippocampus when standing still. Our approach demonstrates the feasibility of using encoding models to study neural tuning during naturalistic behavior and sheds new light on the relationship between mnemonic performance, behavioral states and directional tuning in the human brain.

Poster

338. Neural Correlates of Language Processing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 338.01/CC3

Topic: H.02. Human Cognition and Behavior

Support: Jane and Aatos Erkko Foundation
Academy of Finland Grants 257811 and 276643
Emil Aaltonen Foundation

Title: Individual differences in interpretation of an auditory narrative shape BOLD-synchrony between subjects

Authors: *M. HAKONEN*1,2, A. IKÄHEIMONEN1, A. HÜLTEN1, J. KAUTTONEN1, M. KOSKINEN3, F.-H. LIN4, A. LOWE1, M. SAMS1, I. JÄÄSKELÄINEN1;
1Dept. of Neurosci. and Biomed. Engin., Aalto Univ. Sch. of Sci., Espoo, Finland; 2Advanced Magnetic Imaging Ctr., Aalto University School of Science, Finland; 3Fac. of Med., Univ. of Helsinki, Helsinki, Finland; 4Dept. of Med. Biophysics, Univ. of Toronto, Toronto, ON, Canada

Abstract: The interpretation of an identical narrative varies between listeners, because of differences in their life histories. We studied whether we can also find differences in listeners’ brain activity. We recruited 48 healthy volunteers, fluent in Finnish. Half of the subjects had both parents with Finnish cultural background. For the other half, either one or both parents had Russian cultural background. The subjects listened to a 71-min narrative during ultra-fast fMRI. The narrative told a story of two protagonists, one with a Finnish and the other with a Russian background. Afterward, the narrative was replayed in 101 segments, and the subjects were asked to produce associations related to each segment to describe what had been on their minds while they had heard the story in the scanner. Between-subject similarities of brain hemodynamic activity were estimated using inter-subject correlation analysis. The similarity in how the narrative was interpreted was estimated by comparing the semantic relatedness of the associated words between each pair of subjects in a semantic space (Word2Vec) generated from a large internet text corpus. An intersubject representational similarity analysis (RSA) between the BOLD-similarities and the semantic similarities of the associated words across the subjects revealed that the more semantically similar words two subject associated the more similar also were their BOLD responses in the temporal (superior temporal gyrus, middle temporal gyrus) and frontal cortices (medial frontal gyrus, left inferior frontal gyrus) as well as in regions associated with the default mode network. Our results suggest that multiple brain areas of the narrative processing hierarchy support the elicitation of individual narrative interpretations.
Brain areas where the similarity in the narrative interpretation between subjects correlated with BOLD-similarity between subjects ($q<0.05$, FDR corrected at the cluster level with 5000 permutations).


Poster

338. Neural Correlates of Language Processing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program#/Poster#: 338.02/CC4

Topic: H.02. Human Cognition and Behavior

Support: MICIU-FEDER project PGC2018-094765-B-I00
Generalitat de Catalunya SGR2017-977
ICREA Acadèmia distinguished Professorship awarded to CE
Title: Test title

Authors: *C. ESCERA¹, S. ARENILLAS-ALCÓN², T. RIBAS-PRATS², M. GÓMEZ-ROIG³, J. COSTA-FAIDELLA²;
¹Inst. of Neurosciences, ²Univ. of Barcelona, Barcelona, Spain; ³Inst. de Recerca Sant Joan de Déu, Esplugues de Llobregat (Barcelona), Spain

Abstract: The ability to encode the complex spectrotemporal features of speech sounds from birth is capital to acquire the phonetic repertoire during the first months of life. Previous studies with infants suggest the encoding of voice pitch variations and vowel formants structure to take place at different stages of infant development. However, no previous study has investigated the ability to encode simultaneously both elements of speech in newborns. We use the frequency-following response (FFR) to reveal the neural tracking accuracy of F0 and formant structure in a sample of healthy newborns (N=17; gestational age 37-42 wk; 6 males) compared to adults (N=14; mean age 26 yr; 4 males). FFRs were recorded to 4000 presentations (alternating polarities) of a single two-vowels (/oa/) 250 ms-long stimulus with a rising pitch ending (F0=113 Hz from 0 to 160 ms, then rising up to 152 Hz; /o/ from 10-80 ms, F1=452 Hz, F2=791 Hz; /a/ from 90-250 ms, F1=678 Hz, F2=1017 Hz). FFR to alternating polarities were averaged together to emphasize F0 encoding, and analyzed separately for steady (10-160 ms) and rising (160-250 ms) segments. They were also subtracted to isolate the encoding of fine structure for the /o/ (10-80 ms) and /a/ (90-160 ms) steady pitch segments. F0 encoding was similar in newborns and adults as revealed by similar SNR of the F0 spectral amplitude (t[29]=0.43, p=0.67) and pitch strength retrieved from the autocorrelogram (F[1,58]=2.245, p=0.139). Pitch tracking was less robust during the rising segment (F[1,58]=24.362, p<1e-4), but similar across groups. In contrast, the encoding of the format structure yielded significant differences across groups, as revealed by ANOVA with the factors group (F[1,58]=7.482, p=0.008) and vowel (F[1,58]=21.517, p<0.0001), as well as their interaction (f[1,58]=4.279, p=0.043). Post-hoc analyses revealed that the encoding of the first formant differed across vowels only in adults, and that the spectral amplitude of F1 for the /a/ (at 678 Hz) was larger in adults than in newborns, with no differences for the /o/ (at 452 Hz) across groups. Together, these results indicate that the encoding of voice pitch is fully functional already at birth but that some aspects of fine structure encoding develop later.


Poster

338. Neural Correlates of Language Processing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 338.03/CC5
Abstract: During speech comprehension, the brain decodes meaning based on the acoustic features of speech. Words are primary units of meaning and a crucial step of speech comprehension is to segment a continuous speech stream into discrete words. Finding the boundaries between words is not a trivial task, which relies on not only auditory analysis but also linguistic knowledge. Previous studies have found that, when listening to speech, low-frequency cortical activity can track multi-syllabic words even when the word boundaries are not conveyed by acoustic cues. These results suggest that the brain can apply linguistic knowledge to group syllables into multi-syllabic words. Nevertheless, these results are obtained when listening to either word lists or lists of unrelated sentences. Whether low-frequency cortical activity can track multi-syllabic words during more natural speech comprehension remains unclear. Here we constructed short stories in which all words are bisyllabic words and recorded neural responses to these stories using electroencephalography (EEG). The only task of the listeners is to answer comprehension questions after each story. We found that EEG responses of the listeners concurrently tracked both syllables and bisyllabic words. These results demonstrated that the brain groups syllables into words during natural story perception and this process is reflected by word-rate neural entrainment.

Disclosures: C. Luo: None. J. Zou: None. N. Ding: None.
Abstract: Previous studies have illuminated the neural basis of formation and overt production of sentences during conversation. There are common utterances, however, called “filler words” that interrupt the flow of a sentence. Filler words are typically words or sounds like ‘uh’ or ‘hm’ that signify the speaker’s hesitation and intention of finishing the sentence. We determined how the spatiotemporal dynamics of neural modulations differ between overt utterance of filler and normal phrases, by measuring event-related high-gamma activity at 70-110 Hz during extraoperative electrocorticography recordings. Three patients were presented with images of scenes and instructed to describe who was depicted in the image, what they were doing, at what time, and where. Naturally occurring filler words and normal phrases were tagged at the beginning and end of vocalization. Time-frequency spectral analysis, using a complex demodulation method, was conducted time-locked to the onsets and offsets of phrases. The data showed that filler phrases were specifically associated with high-gamma augmentation in dorsolateral prefrontal regions including the middle-frontal and inferior-frontal gyri on either hemisphere. The right lingual cortex showed high-gamma attenuation specifically during utterance of filler phrases. The precentral and superior-temporal gyri showed high-gamma augmentation commonly during utterance of filler and normal phrases. During spontaneous filler word utterances, the human brain may activate dorsolateral prefrontal regions which support the search for an optimal phrase to be verbalized next, while suppressing lower-order visual processing.


Poster

338. Neural Correlates of Language Processing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 338.05/CC7

Topic: H.02. Human Cognition and Behavior

Title: Brain networks for speech understanding and mathematics computation - Evidence from task-evoked and task-free BOLD activity

Authors: *S. GENG*¹, M. ZHU², Y. LIU¹;
¹Beijing Normal Univ., Beijing, China; ²State Key Lab. of Cognitive Neurosci. and, Beijing, China

Abstract: Previous studies have suggested that brain activities for language comprehension and mathematical computation are some overlapped but also different. The present study aims to
directly compare these two key characteristics achieved early in life and happens very often in our brains. Distinct from most of previous studies, the data from three modalities - task-induced BOLD response, resting-state brain functional connectivity (RSFC) and behavioral performance of 78 participants from the Human Connectome Project (HCP) database, were jointly analyzed in present study. As expected, the differences between language and mathematics network are consistently observed in both task-evoked BOLD response and RSFC, which means that the language network including bilateral superior temporal gyrus, sulcus temporal gyrus, middle temporal cortex, and left angular gyrus, and inferior frontal gyrus, whereas math network covering bilateral inferior parietal lobule, intraparietal sulcus, precuneus, supplementary motor area, and middle and superior frontal gyrus. Further, the ROI-based hierarchical networks from RSFC showed differentiated correlations with the task-induced networks, which means the lower level networks are more similar, and the higher ones are different on story and math. In addition, we also observed that the correlation between the RSFC and the task-induced BOLD activity varied with their behavioral performance. To sum up, the brain networks for speech understanding and arithmetic computation are distinct on both task-induced and task-free brain activity, and some inherent coherences are observable on task-based, task-free resting state BOLD activity and human’s behaviors.

Disclosures:  S. Geng: None. M. Zhu: None. Y. Liu: None.
phrases” (VPs), syntactic information must be disentangled from the acoustic support. Italian syntax provides a particularly felicitous set of data which could be used to approach this goal. Here, we investigated the electrophysiological correlates of these basic syntactic structures by using stereo-electroencephalography (SEEG), an invasive technique which allows a precise localization of cortical activation. Seventeen subjects undergoing SEEG implantation for the treatment of refractory epilepsy were enrolled in the study. We used a purposely designed protocol able to factor out acoustic information by construing pairs of phrases that have exactly the same acoustic content but that are interpreted as NPs, or “verb phrases” VPs depending on their syntactic context (homophonous phrases). Several contacts exhibited significant high gamma (150Hz - 300Hz) event related spectral perturbation (ERSP) increase during the hearing of the homophonous phrases with respect to both the baseline and the other strings of words. Moreover, responsive contacts that also exhibited a significantly different response according to whether the phrase were VPs or NPs were present in several cortical areas and no localization of the specific activity was identifiable. This suggests that syntax, at least when explored from an electrophysiological point of view, appears to be a much more integrated brain activity than expected, although language impairment may clearly be caused by focalised lesions in crucial hubs of the overall network as traditionally identified by clinical studies. Our findings pave the way for a deeper understanding of the electrophysiological mechanisms of syntax.

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Poster

338. Neural Correlates of Language Processing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 338.07/CC9

Topic: H.02. Human Cognition and Behavior

Support: R01DC016607-01A1S1

Title: The neural basis of program comprehension

Authors: *A. A. IVANOVA¹, S. SRIKANT¹, Y. SUEOKA¹, U.-M. O'REILLY¹, E. FEDORENKO¹,²; ¹MIT, Cambridge, MA; ²MGH, Charlestown, MA

Abstract: Computer programming is a novel cognitive tool that is gaining prominence in modern society. However, little is known about the neural systems that support this skill. Here, we test the involvement of two candidate brain networks in program comprehension: the language system and the multiple demand system. Participants (N=25) underwent an fMRI experiment during which they read snippets of Python code and had to predict their output upon
execution. The control condition involved reading short paragraphs of text describing similar problems and predicting the result. We find that the language system responds to both code problems and text-based problems; however, its response to code is primarily driven by frontal regions (lying within inferior frontal and middle frontal gyri) and not by temporal regions. The multiple demand system also responds to both code and text, with stronger responses observed for code. These results indicate that program comprehension and language comprehension share partially overlapping neural substrates and that program comprehension additionally recruits the multiple demand system. In sum, our work establishes programming as a novel domain for studying cognitive processing within the human brain and suggests that this cognitive skill might make use of both language-specific and domain-general neural systems.

Disclosures: A.A. Ivanova: None. S. Srikant: None. Y. Sueoka: None. U. O'Reilly: None.

Poster

338. Neural Correlates of Language Processing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 338.08/CC10

Topic: H.02. Human Cognition and Behavior

Title: Politeness in the oral complaints: Pauses and neural indicators during speech in English (L1/L2), Spanish (L1/L2) and heritage Spanish

Authors: *N. ENRIQUEZ¹, J. L. CONTRERAS-VIDAL², L. DIAZ³;
²Electrical and Computer Engin., ¹Univ. of Houston, Houston, TX; ³Univ. Pompeu Fabra, Barcelona, Spain

Abstract: We present a study on processing problem indicators in oral production in both Spanish and English in a simulated complaint task. Subjects are native English, native Spanish and heritage Spanish speakers. The aim was to describe the role of speech pauses as problem
processing indicators in oral production as a communication strategy and their differences between groups of speakers and the language used in the simulation.

Hypotheses: i) pauses are different quantitatively and qualitatively depending on the speaker’s competence; ii) results for heritage speakers will be closer to L1 group than to L2; iii) cognitive load will be higher using a second language and iv) pragmatic and cultural aspects do play a role in the amount and type of pauses in each language.

In order to test these hypotheses, we gathered two different corpora. Recla/SP (10050 tokens) which consists of a sample of 30 dialogues in Spanish (10 native, 10 heritage and 10 non-native) and Recla/EN (9270 tokens) which contains 30 dialogues in English (10 native, 10 heritage and 10 non-native).

We report preliminary findings on EEG data (32 channels, 500 Hz) during oral production. The bandpower in EEG data during 36 speech pauses was compared to baseline, where the subjects relaxed and looked at a blank paper in front of them for 1 min. The data was preprocessed by using the H-inf filter to remove eye-related artifacts, band-pass filtering the signal between 0.3 and 50 Hz, using the PREP pipeline for robust re-referencing, and ASR for removal of artefactual power bursts. At the onset of the speech pauses we found statistically significant (p<0.01) modulation in the left frontal areas for the delta band (1-4Hz), and activation of central-left regions in the alpha (8-12 Hz) and gamma (30-50 Hz) bands. The pattern was found both in English and Spanish speech production, with pre-frontal bandpower deactivation in the English task.

Linguistic data confirms the role of pauses as indicators of the difficulty of the task itself and the linguistic difficulty in carrying it out in L2, as stated in hypothesis (i), (ii) and (iii) above. The study also reveals significant differences between native, heritage and non-native speakers, related to hypotheses (i), (ii) and (iv). Specifically, in native speakers, pauses are indicators of difficulty in management decisions, while in non-native speakers pauses indicate difficulties with syntax and verb inflection. Also, as was stated in (ii) results for heritage are closer to L2.

Disclosures: N. Enriquez: None. J.L. Contreras-Vidal: None. L. Diaz: None.

Poster

338. Neural Correlates of Language Processing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 338.09/CC11

Topic: H.02. Human Cognition and Behavior

Support: Australian Research Training Program
Australian Research Council Future Fellowship
Maurice de Rohan International Scholarship
Title: The canonical frequency bands reflect sleep-based memory consolidation of a modified miniature language

Authors: *Z. R. CROSS*¹, L. ZOU-WILLIAMS², M. KOHLER⁴, M. SCHLESEWSKY², R. F. HELFRICH⁵, G. GASKELL⁷, R. T. KNIGHT⁶, I. BORNKESSEL-SCHLESEWSKY³; ¹Univ. Of South Australia, Adelaide, Australia; ²Sch. of Psychology, ³Univ. of South Australia, Adelaide, Australia; ⁴Sch. of Psychology, Univ. of Adelaide, Adelaide, Australia; ⁵Helen Wills Neurosci. Inst., ⁶Univ. of California Berkeley, Berkeley, CA; ⁷Dept. of Psychology, Univ. of York, York, United Kingdom

Abstract: Language is a prime example of a domain where information is extracted according to statistical dependencies, and which has been shown to be preferentially consolidated during sleep. However, there is limited evidence supporting a link between oscillatory-based models of hippocampal memory consolidation and the learning of sentence-level combinatorics. Here, we report results from an EEG study employing an 8-hour nocturnal sleep period and sentence judgement tasks of a modified miniature language. 36 monolingual English speakers (16 male, mean age=25.4) participated in one of two conditions (Sleep, Wake). Both conditions involved an implicit learning phase, baseline sentence judgement task, followed by either an 8hr sleep opportunity (Sleep, n=18) or an equivalent period of wake (Wake, n=18) and a delayed judgement task. We used non-linear directional cross-frequency coupling analyses to quantify the temporal relationship between NREM slow oscillations (SO) and spindles. We also quantified spectral activity in the θ, α and β bands during sentence judgement tasks. Individual differences in statistical learning ability (SLA) were controlled for. Linear mixed-effects modelling revealed that θ power increased from Baseline to Delayed testing, with this effect more pronounced in the Sleep versus the Wake group, and which predicted greater accuracy on the judgement task ($\chi^2(1)=14.61, p<.001$). By contrast, while an increase in α power at Delayed testing predicted better behavioural performance for the Sleep group, the reverse was observed for the Wake group ($\chi^2(1)=10.67, p<.001$). An increase in β power predicted higher accuracy ratings, with this effect being more pronounced for the Sleep group at Delayed testing ($\chi^2(1)=25.20, p<.001$). Additional models revealed that as the number of SO and spindle coupling events increased with an increase in task-evoked θ power, so did percent of correct responses ($\chi^2(1)=33.07, p<.001$). The relationship between task-evoked θ power and improved behavioural performance after sleep may reflect sleep-induced connectivity between the hippocampal complex and neocortex, which is supported by the finding that SO-spindle coupling and increases in task-evoked θ power predicted increased accuracy. Further, while the difference in α power between groups may reflect time-of-day effects, the difference in β power may reflect the propagation of predictions via a hierarchically organized predictive coding architecture that is instantiated by sleep-dependent synaptic downscaling. This predictive coding architecture, strengthened during sleep, likely facilitates the processing of a newly learned language.

Poster

338. Neural Correlates of Language Processing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 338.10/CC12

Topic: H.02. Human Cognition and Behavior

Support:  
PICT 2016-1256  
UBACYT 20020170200259BA  
PIP 11220150100787CO

Title: Different sources of predictions during natural reading: A co-registration study

Authors: *B. BIANCHI1, R. LOREDO2, J. R. CARDEN2, V. JAICHECHNO2, T. VON DER MALSBURG4, D. E. SHALOM3, J. E. KAMIEKOWSKI1;

1Computer Sci. Dept., Univ. De Buenos Aires, Buenos Aires, Argentina; 2Inst. de Lingüística,  
3Physics Dept., Univ. de Buenos Aires, Buenos Aires, Argentina; 4Dept. of Linguistics, Univ. of Potsdam, Potsdam, Germany

Abstract: During reading our brain predicts upcoming words. This predictions, if correct, could speed up the processing of the word when it is finally read. It have been amply shown that Predictability (the variable that estimates the probability of guessing the next word) have an impact on how we move our eyes across the text: more predictable words are fixated for shorter periods of time than less predictable words. Additionally, EEG experiments showed that the amplitude of the N400 potential is modulated by this variable: more predictable words correspond to less N400 amplitude. Classically, EEG experiments were performed avoiding eye-movements, because the muscular contraction interfere with the brain electrical signal. During the last years, helped by the advances in computational power, co-registration experiments, in which eye movements are allow, generated more natural contexts to study such a complex process.

In the present work we analyzed brain potentials during natural reading to address for the sources of predictions. Previously, in independent studies, it was shown that mnemonic predictions (i.e. predictions performed purely on long term memory, like when reading a proverb or a son lyric) and predictions done purely on the linguistic context have different impact, both on gaze duration and on the N400.

Categorical analyses of fixated-word-Predictability showed a N400 effect. A late effect on fixated word is also seen in correlation with the next word Predictability (parafoveal-on-foveal effect). The categorical analysis of the interaction between Predictability and Sentences Type also showed slight differences. Finally, analyses performed using nonparametric cluster based statistics and Linear Mixed Models replicate effects seen in the previous study on fixed gaze.

Poster

338. Neural Correlates of Language Processing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 338.11/CC13

Topic: H.02. Human Cognition and Behavior

Support: JSPS KAKENHI 18H00669

Title: Electroencephalogram for context retrieval and temporal processing in understanding the implicit intention of a speaker in discourses

Authors: *S. TOKIMOTO\textsuperscript{1}, N. TOKIMOTO\textsuperscript{2}; \textsuperscript{1}Mejiro Univ., Tokyo, Japan; \textsuperscript{2}Shobi Univ., Saitama, Japan

Abstract: In a daily conversation, a speaker often expresses his/her intention indirectly, and the comprehender understands the speaker's implicit intention by pragmatic inference. For example, we often use an interrogative for asking others to do something ("Can you reach the salt?"). This study examines the relationship between the retrieval of context and the event time in pragmatic inference to understand a speaker's implicit intention in discourses by an experiment recording the 64-channel electroencephalogram (EEG) elicited by Japanese discourses. Twenty native speakers of Japanese participated in the study. We auditorily presented the experimental discourses by three speakers, in which the first speaker introduced a topic, the second asked a yes-no question concerning the topic, and the third answered the question indirectly. The discourses were manipulated in the 2-by-2 manner for context explicitness and event time. That is, (1) Context Explicit or Implicit: We changed one word of the first utterance so that the contexts to derive the speaker's implicit intentions from were given explicitly or implicitly. The second and the third utterances were the same between the two conditions. (2) Past or Future: Whether the third speaker's utterance referred to his/her Past or Future behaviors.

The EEG data were analyzed time-locked to the words in the third utterances at which the implicit answers were drawn out. We observed a significant positive event-related potential (ERP) in the central-parietal region and a significant negative ERP in the occipital region in the time-window of 200-400 ms for Past in Context Implicit condition. We found no significant contrast between Past and Future in Context Explicit and between Context Explicit and Context Implicit in Future. We localized the generators of the ERPs by the dipole fitting of the EEG data and the clustering of the dipoles. Both of the generators of the occipital negativities observed for Past against Future in Context Implicit and for Context Implicit against Context Explicit in Past were localized in precuneus. These localizations in precuneus coincide well with the experimental findings for temporal processing by fMRI and our localization suggests a close
relationship between the retrieval of the context relevant to the implicit intention of a speaker and the taking the subjective time of the speaker.

**Disclosures:**  S. Tokimoto: None. N. Tokimoto: None.

**Poster**

**338. Neural Correlates of Language Processing**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 338.12/CC14

**Topic:** H.02. Human Cognition and Behavior

**Title:** VWFA resting connectivity in the skilled reading project

**Authors:** *B. T. CARTER¹, S. G. LUKE²;
²Psychology, ¹Brigham Young Univ., Provo, UT

**Abstract:** Introduction - The Skilled Reading Project is a study of the neural networks and structure supporting reading behavior in a group of undergraduates at Brigham Young University. Previously this dataset has been used to examine the functional networks associated with linguistic predictability in paragraphs of text. The visual word form area (VWFA) has been shown to associated with reading and functionally coupled with other regions specific to language. This exploratory analysis was conducted to determine which regions were coupled with the VWFA in a group of skilled readers. Methods - Participants underwent T1-weighted structural and T2*-weighted resting MRI. A group structural model was constructed first in the following manner: participant DICOMs were converted into NIFTI files, ACPC aligned, N4 bias field corrections were applied, affine registered and registered to MNI space via the MNI ICBM 2009 Nonlinear Symmetric atlas, then a group template was constructed via ANTs. The following preprocessing steps were taken: the first three TRs were removed, followed by despiking, slice timing alignment, registration to participant anatomy, 4 mm FWHM blur was applied, non-brain intensities were then masked, followed by scaling, and regressing out motion and bandpass filtering. 34 participant datasets survived motion censoring. Seed coordinates were selected based on an automated meta-analysis of 117 studies conducted via Neurosynth (Yarkoni, Poldrack, Nichols, Van Essen, & Wager, 2011). A 5 mm mask was constructed, individual time courses were extracted, correlation maps constructed and converted to Z-maps. A Student’s T-test was then applied to determine group level associations. Results - The peak associations were found in the left inferior frontal gyrus, right inferior parietal lobule, right inferior frontal gyrus, right insular cortex, and two peaks in the right orbital frontal gyrus. Conclusion - The functional connection the left inferior frontal gyrus demonstrates that the VWFA is primarily associated with language function in skilled readers. Connectivity with right sided regions may be evidence of increased ability to integrate language processing with higher level cognition. Further investigation is needed to determine the significance of this connectivity.

Poster

338. Neural Correlates of Language Processing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 338.13/CC15

Topic: H.02. Human Cognition and Behavior

Support:  DGAPA IN203818
          CONACYT Fronteras de la ciencia 225
          PAPIIT DGAPA 30

Title: Modulation of communicative intention through facial expression and its neural correlate

Authors: *J. RASGADO-TOLEDO, M. GIORDANO;  
Univ. Nacional Autónoma de México, Queretaro, Mexico

Abstract: Speakers use a variety of contextual media, such as facial emotional expressions for successful transmission of their message. Listeners must decipher the meaning of the verbal message by understanding the intention behind it (Recanati, 1986). A traditional approach to the study of communicative intention has been through speech acts (Escandell, 2006). The objective of the present study is to contribute to the understanding of the influence of facial emotional expression to the recognition of communicative intention in Spanish-speaking, well-educated young adults. The study was divided in several experimental phases. For all phases the CFEE face database was used (Du et al., 2014), and utterances were constructed with neutral emotional valence using NRC Emotion lexicons (Mohammad & Turney, 2010) and LEXMEX corpus to calculate the frequency of use. Phase one evaluated the relationship between begging, demanding, ordering and requesting with basic emotional facial expressions in a sample of 40 participants. Phase two, assessed the compatibility of anger, joy and sadness with sentences that had the linguistic characteristics of a demand, a request and an assertion in 30 participants. In phase three, 40 participants had to categorize a series of affirmative statements as demands, begging or assertions after viewing expressions of anger, joy and sadness. In phase four, the participants solved the behavioral task inside a scanner. We used the same set of stimuli, adding a blur face as a control, and random jittering. In this phase 22 right handed, neurotypical Mexican participants performed the task. A 3 Tesla GE MR750 scanner with a 32-channel head coil was used. 36 slices acquired using a T2* weighted EPI sequence, TR of 2000 and TE of 40 ms, FOV 25.6, with a matrix of 64 x 64 and a 4-mm slice thickness. BOLD signal was examined during the presentation of the sentences, including a regressor for each emotional expression. Analysis was made using Feat in FSL (Smith, et al., 2004). The results of phase one suggested a link between demanding with anger face, and a sad face with the act of begging. Phase two indicated an association between demands with anger, begging with sadness, and happiness with
assertions. Phase three showed that the sentences were categorized differently according to the facial emotional expressions that were presented. The neuroimaging data showed activation of regions involved in language, intentionality and face recognition during presentation of the statements. The results suggest that these areas could contribute to the contextual processing and different speech act categorization according to the emotional face presented.

Disclosures:  J. Rasgado-Toledo: None. M. Giordano: None.

Poster

338. Neural Correlates of Language Processing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 338.14/CC16

Topic: H.02. Human Cognition and Behavior

Support: NSF award BCS1533691
NSF IUCRC BRAIN Center Award Number: 1650536
Center for Advanced Computing and Data Science, University of Houston

Title: Modulation of EEG partial directed coherence in creative writing

Authors: *J. G. CRUZ-GARZA¹, A. SUJATHA RAVINDRAN¹, C. RIVERA GARZA², J. L. CONTRERAS-VIDAL¹;
¹Electrical and Computer Engin., ²Hispanic Studies, Univ. of Houston, Houston, TX

Abstract: With mobile EEG technology, it is now possible to study human neuroscience in context-aware settings where movement and social interaction can occur freely. We used portable dry EEG headsets (four channels: TP09, AF07, AF08, TP10, with reference around Fpz), video cameras, and journal entries, to record the brain activity of bilingual students (English and Spanish) as they participated in an undergraduate creative writing workshop in Spanish over four months. The workshop aimed to develop their creative writing skills by having the students physically experience different locations around the city of Houston and producing creative texts from their bodies’ experience. The students (N = 8) recorded their brain activity as they experienced areas in the city (Preparation phase), and as they created the first draft of their creative texts (Generation phase). We used Partial Directed Coherence (PDC) and compared it between the Preparation and Generation phases in their writing process. Higher PDC was found in the Preparation Phase at a significance level of p < 0.05, from right temporo-parietal to left anterior frontal areas of the scalp across all frequency bands analyzed: 1-50 Hz. In the Generation phase, we found the opposite directionality PDC in high frequency bands: 13-50 Hz. Information transfer from temporal-parietal to anterior-frontal areas of the scalp during the Preparation phase may be indicative of sensory processing and evaluation. High frequency from the anterior-frontal areas to temporo-parietal areas during the Generation phase may indicate the
consolidation of evaluation and decision making processes, translating them into a creative textual representation of those memories and sensory experiences.

**Disclosures:**  
J.G. Cruz-Garza: None.  
A. Sujatha Ravindran: None.  
C. Rivera Garza: None.  
J.L. Contreras-Vidal: None.

**Poster**

**338. Neural Correlates of Language Processing**

**Location:** Hall A  
**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 338.15/CC17

**Topic:** H.02. Human Cognition and Behavior

**Title:** Does it matter how it's written? The effect of misspelled words in sentence reading

**Authors:** *J. F. QUIÑONEZ-BELTRAN*¹, F. R. GÓMEZ VELÁZQUEZ², V. D. RUIZ-STOVEL²;  
¹Lab. de Neurodesarrollo Cognitivo, Univ. De Guadalajara, Guadalajara, Mexico; ²Lab. de Neurodesarrollo Cognitivo, Univ. de Guadalajara, Guadalajara, Mexico

**Abstract:** Reading comprehension depends on the integration of words in a sentence. The analysis of each word and the access to the meaning will be determined by the recognition of the word. If we know the word, this will trigger the word identity very rapidly. The process of matching the visual features of the word with the representations stored in our lexicon is automatic. This recognition primarily relies on two characteristics: the orthographic representation and the phonological representation associated to the word. In recent years in Mexico, the importance of spelling has been undermined, originating a popular belief that “it doesn’t matter how a word is written”. But what happens when we find words whose orthographic representations have been altered? In this study, we aim to determine if misspellings affect the semantic processing of sentences.

We designed a semantic decision task with 172 high cloze probability sentences. The sentences consisted of 6 words in which the last word was completed by a) a congruent word, b) a congruent word with a pseudohomophone error, c) a congruent word with a transposed letter error, d) an incongruent word, or e) an incongruent word with a pseudohomophone error. The participants were asked to read the sentences and decide if the sentence was semantically congruent or incongruent regardless of spelling errors. We recorded EEG activity while participants performed the task. As expected, we observed in the ERP results that the semantically incongruent sentences elicited an N400. On the other hand, sentences with spelling errors (pseudohomophone and transposed letter) elicited a P600. These results support the monitoring hypothesis, showing that there is a process of evaluation during language perception when the brain has to decide either to accept or reject a word, and that this could bring the system into a state of indecision that is reflected in electrophysiological activity.
Disclosures:  J.F. Quiñonez-Beltran: None. F.R. Gómez Velázquez: None. V.D. Ruiz-Stovel: None.

Poster

338. Neural Correlates of Language Processing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 338.16/CC18

Topic: H.02. Human Cognition and Behavior

Support: Penn Translational Neuroscience Initiative pilot grant

Title: Inhibitory transcranial magnetic stimulation to the left inferior frontal gyrus modulates verbal selection in controlled language tasks in a context dependent manner

Authors: *J. P. ZIMMERMAN*¹, A. KELKAR¹, D. Y. HARVEY¹, J. D. MEDAGLIA², R. H. HAMILTON¹;
¹Dept. of Neurol., Univ. of Pennsylvania, Philadelphia, PA; ²Psychology, Drexel Univ., Philadelphia, PA

Abstract: Background: In language production, it is often necessary to select from multiple appropriate competing words to convey intended meaning to listeners. Verbal selection has been strongly linked to processing in the left inferior frontal gyrus (LIFG), however it is unclear how processing in the LIFG differs in cases differing language task demands. Here we apply transcranial magnetic stimulation (TMS) to the LIFG of subjects to modulate performance on two controlled language tasks that differ in their degree of semantic context: verb generation (low semantic context) and sentence completion (high semantic context).

Methods: Thirty healthy right-handed subjects participated in the study. Subjects were randomized to receive either active TMS neuromodulation or sham TMS. TMS neuromodulation was applied using a continuous theta burst stimulation (cTBS) protocol known to produce LTD-like cortical inhibition. Subjects performed sentence completion and verb generation tasks before receiving TMS to the LIFG and then repeated the tasks after cTBS. Task order was counter-balanced across subjects. Selection cost was calculated as the difference between median reaction time on trials with high selection demands and trials with low selection demands. Effects of cTBS on verbal selection were assessed using a 2x2 ANOVA with factors of session (pre/post) and treatment (active/sham). Additionally, trial level reaction times were analyzed using a linear-mixed effects model to assess the effects of session, treatment and selection demands on reaction time in controlled language tasks.

Results: In the sentence completion task (high semantic context) there was a significant session*treatment interaction in the trial level reaction time analysis such that reaction times were faster compared to baseline following active cTBS and slower compared to baseline following sham cTBS. There was no significant session*treatment interaction on selection cost
in sentence completion. In the verb generation task (low semantic context), there is a significant session*treatment interaction in the selection cost analysis such that selection cost is increased following active cTBS neuromodulation compared to sham.

**Conclusion:** TMS neuromodulation to the LIFG modulates performance on controlled language tasks in a context dependent manner. In tasks with high semantic context there is a general effect on reaction times indicating that cTBS may speed up semantic processing. In low semantic context tasks there is no general effect on reaction times, but an increase in the cost of high selection demands indicating cTBS may be slowing down the verbal selection process.

**Disclosures:** J.P. Zimmerman: None. A. Kelkar: None. D.Y. Harvey: None. J.D. Medaglia: None. R.H. Hamilton: None.

**Poster**

338. Neural Correlates of Language Processing

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 338.17/CC19

**Topic:** H.02. Human Cognition and Behavior

**Support:** NSF Career Award #1652127

**Title:** Word analogy relations predicted from addition and subtraction of fMRI activation patterns

**Authors:** *M.-H. WU, A. J. ANDERSON, R. A. JACOBS, R. RAIZADA; Univ. of Rochester, Rochester, NY

**Abstract:** Consider a word analogy, such as “teacher is to chalk, as mechanic is to wrench”. Computational semantic models such as Word2Vec have found that simple addition and subtraction of word embeddings can solve such analogies; that is, the word embedding of teacher vec(“teacher”) is closest to the predicted embedding vec(“chalk”) + vec(“mechanic”) - vec(“wrench”). We used pattern-based fMRI analyses to ask whether answers to analogies can be inferred in a similar manner by adding and subtracting fMRI activation patterns elicted by the words. Participants viewed common English words, presented one at time, in the MRI scanner and were asked to form mental images of the displayed word. The stimulus set consisted of triplets made up of a person, building and tool related to one of 15 semantic categories (e.g., teacher / school / chalk in the teaching category). Analogical pairs were formed by first selecting two semantic categories and then picking two words of the same type from each semantic category. This underlying structure was unbeknownst to participants, and the study did not involve explicit analogical reasoning. For each participant, we identified voxels across the whole brain that had the highest inter-trial correlation. The fMRI pattern for each word was the averaged BOLD pattern across all its repetitions. Similar to Word2Vec, for each analogical pair,
we calculated the predicted pattern of the target word based on the patterns of the other words in the analogical pair. For example, the predicted pattern of “teacher” was pattern(“chalk”) + pattern(“mechanic”) - pattern(“wrench”). We ranked the correlations between the predicted pattern and the actual fMRI patterns of all words other than the three used in the calculation. This procedure was repeated for all four words in each possible analogical pair, and the scaled rank of the true word pattern was averaged and compared against chance across participants with a one-sided t-test. We observed a small but significant effect (p < 0.02), which suggests that fMRI patterns of a word can be predicted from that of other words in an analogical pair. Significant rank (p=0.03) was still observed when a more rigorous ranking procedure was applied, where we only compared the predicted pattern to the patterns of words sharing the same type as the true word (i.e. only ranking other person words against “teacher”); this eliminated the possibility that successful ranking was driven purely by voxel selectivity to word types instead of identity. Our work demonstrated that geometric regularities found in word embeddings can also be observed in word-induced fMRI activation patterns.


Poster

338. Neural Correlates of Language Processing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 338.18/CC20

Topic: H.02. Human Cognition and Behavior

Support: NIHI R01 NS099199

Title: Associations between resting-state and reading task lateralization of language-related cognitive networks

Authors: T. K. DAY¹, ³, T. M. MADHYASTHA²;
¹Dept. of Radiology, ²Radiology, Univ. of Washington, Seattle, WA; ³Inst. of Child Develop., Univ. of Minnesota, Minneapolis, MN

Abstract: Lateralization of language is perhaps one of the most well-known features of the human brain. This has been studied with many methods involving active language tasks, including functional magnetic resonance imaging (fMRI). However, resting-state (RS) fMRI provides a way to interrogate the resting configuration of the brain and areas related to language. Broca’s area (BA) and Wernicke’s area (WA), located in the left hemisphere (LH) and linked to production and understanding, are the primary language areas in the brain. Zhu et al. [1] identified regions of the cortex (“asymmetry seeds”) where RS functional connectivity (FC) to BA and WA vs. their right-hemisphere (RH) analogues was asymmetric, describing six asymmetry seeds where (1) FC to the left BA > right BA; (2) left WA > right WA; and (3) right
WA > left WA. However, they didn't link RS asymmetry to task asymmetry. In this study, we directly test whether RS asymmetry is related to the asymmetry during a language task. The Reading Brain Project [2] contains RS scans and five repetitions of a passive reading task \( (n = 50) \). We calculated FC from BA and WA in the passive reading task and at rest and, using regions of interest centered on the asymmetry seeds, calculated three measures (as above) of LH-RH FC asymmetry. We found that FC asymmetry during the passive reading task predicts FC asymmetry in RS in two out of the three measurements. FC in BA regions (described as primarily LH asymmetric) varied roughly equally across subjects between LH and RH in both RS and task. FC asymmetry in BA regions was correlated between the RS and task \( (r^2 = 0.235, p = .002) \). FC asymmetry in WA regions described as LH-asymmetric was not correlated between RS and task \( (r^2 = 0.154, p = 1.64) \), but was positively correlated with age \( (\beta = 0.006, p = 0.029) \). FC in WA regions described as primarily RH were primarily RH-asymmetric in both RS and task, and were correlated \( (r^2 = 0.356, p < .001) \). (All \( p \) values corrected for three multiple comparisons). There was no effect of age on BA or WA RH or gender in any analysis. Our findings suggest BA networks are the most robust in comparing RS and task FC asymmetry. The inconsistent WA measurements suggest more work is needed to understand the LH/RH components of the WA networks. Although other work has shown linkages between RS and task network configurations, this study links resting measurements of language networks to their task configuration, possibly allowing estimation of language network configuration in participants such as young children - who are unable to respond to an active language task. Preregistration: osf.io/njenv. Citation: [1] Zhu et al. (2014). *PLoS ONE* (9)1: e85880 [2] openneuro.org/datasets/ds001857

**Disclosures:** T.K. Day: None. T.M. Madhyastha: None.

**Poster**

**338. Neural Correlates of Language Processing**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 338.19/CC21

**Topic:** H.02. Human Cognition and Behavior

**Support:** SEP-CONACYT 220973

**Title:** N400 in lexical decision of words and pseudowords on a Yuto-Nahua language: The case of Wixarika

**Authors:** D. L. CANELA, A. E. CARRILLO, W. F. LARA GALINDO, *F. A. ROBLES;* Univ. of Guadalajara, Guadalajara, Mexico

**Abstract:** Several studies have shown that similar lexical processing effects in distinct languages are due to the grounding of language into neurobiology (Friederici, 2002; Willems, Özyürek &
Hagoort, 2008). However, except from some Asian languages, studies have replicated these findings only with European tongues. Wixarika is an agglutinative Yuto-Nahua language spoken in the northeast of Mexico. In this experiment, we compared the N400 for 10 Wixarika and 20 Spanish speakers (ages 18-27) in a lexical decision task using their mother tongue. Task consisted of hearing 50 nominal phrases for each condition: words (nuestra agua/tame haá/our water), and pseudowords type I (mi cuchillo-chicullo/my knife, xeme hikuri-hukiriti/your cactaceae) and type II (tu abeja-abeje/your bee, tame haá-hué/our water). N400 peak was found in central channels at 423 ms (-1.4 μV) for Spanish and at 331 ms (-1.7 μV) for Wixaritari speakers. When comparing N400 between conditions (words vs. pseudowords) we found no differences neither in Spanish amplitudes (F=1.01, p=0.32) nor latencies (F=2.30, p=0.14), neither in Wixarika amplitudes (F=0.376, p=0.55) nor latencies (F=2.60, p=0.15) speakers. When comparing groups, there were no differences in response latencies neither in words (t=0.574; p=0.571), pseudowords I (t=1.420; p=0.167) or pseudowords II (t=0.428; p=0.672). Also, there were not differences between groups in N400 amplitudes (t=1.410, p=0.170) or latencies (t=0.46, p=0.69). These findings line up with the proposal that states that N400 is produced by the temporal synchrony of a stimulus-driven activity (represented or non-represented in lexicon) with a broad neural network set by recent linguistic input (Federmeier & Laszlo, 2009).

Poster

338. Neural Correlates of Language Processing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 338.20/CC22

Topic: H.02. Human Cognition and Behavior

Support: CONACyT scholarship 440709
CONACyT (Fronteras No. 225-2015)
PAPIIT-DGAPA 17
CONACyT scholarship 755580

Title: Metaphors comprehension throughout development and its neural correlate

Authors: *E. NAVARRETE, E. VALLES-CAPETILLO, M. GIORDANO;
Lab. D-12, Inst. de Neurobiología, Querétaro, Mexico

Abstract: Introduction:
The objective of this research is to identify those brain areas associated with an aspect of
pragmatic language, specifically, metaphor comprehension through development from childhood
to adolescence. The design is descriptive and correlational. The technique used is task-related
functional Magnetic Resonance Imaging (fMRI).

Methods:
In a sample of 898 participants (9-16 years old, mean age = 12.08 ± 1.92, 468 female), a
qualitative instrument was used to evaluate metaphor comprehension. Significant differences
were identified for the comprehension level between genders (female > male) and developmental
stage (adolescents > children). Differences between stimulus conditions were also identified:
literal and absurd > metaphors. From this instrument 63 stimuli were selected (21 metaphors, 21
literal and 21 absurd) and were presented in audio format (male and female voices) to a sample
of 63 children (9 - 13 years old, mean age = 11.07 ± 1.83, 35 female). Comprehension scores and
reaction times were obtained. Using this information a fMRI task was designed, it included 3
runs (5.5 min each) with 21 events (7 literal, 7 metaphors, 7 absurd). Each event included one
auditory stimulus (2 s) + fixation cross (4-8 s) + question (does it make sense?: "yes" or "no") (6
s) + fixation cross (2-6 s). Presentation of stimuli was optimized with optseq2. Activation
averages were calculated for each condition and contrasts were made between conditions and
between each condition against each pair of the remaining conditions, using the FSL FEAT tool.

Results:
Initial results for the contrast between metaphor> literal and absurd conditions showed one
cluster of relevant activation with a maximum z-value of 3.8, which was located on the right
precuneus (Harvard Oxford Cortical Structures atlas). Other areas with z activation values
greater than 3 were the right lingual and left fusiform gyrus.
Conclusions:
It is currently known that the precuneus participates in four general functions: consciousness, spatial movement, self-consciousness, and recovery of episodic memory and visuospatial images. The lingual gyrus appears to be activated when people try to attribute meanings to symbols, while the fusiform gyrus participates in semantic association and specifically in metaphor interpretation. These initial findings suggest that brain areas involved in visual imagery and possibly in Theory of Mind, may be involved in metaphor comprehension in children.


Poster

338. Neural Correlates of Language Processing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 338.21/CC23

Topic: H.02. Human Cognition and Behavior

Support: ONR Grant N00014-16-1-2694

Title: Combining fMRI activation patterns and semantic vector representations to predict activation patterns for new concepts

Authors: *R. J. VARGAS, M. A. JUST;
Carnegie Mellon Univ., Pittsburgh, PA

Abstract: In the past two decades, fMRI has been used to study the semantic organization of concepts in the human brain. The activation patterns of concepts are used to discover underlying neurally-defined semantic dimensions and their spatial organization in the brain. Scanner cost and participant fatigue constrain each study to investigate small samples of a narrow set of concepts (typically 30-60 items) limiting the semantic coverage. Natural language processing of large text corpora (e.g. Wikipedia) has led to the development of semantic characterizations of concepts [e.g. GloVe (Global Vector)] based on their co-occurrence with other words. This project relates the neural representation of a concept to its GloVe representation, to create a mapping that can be used to generate predicted activation patterns for as-yet unassessed concepts. The goal is to investigate the semantic organization of human thought on a large scale using a comprehensive brain image library of concept representations that has been generated using this mapping. A kernelized ridge regression model is used to predict the neural representations (defined as the vector of activation levels of the 1800 most consistently activating voxels across the stimulus set) for all but one concept in a set, using each of the concepts’ 300-dimensional GloVe semantic vector representations. The resulting learned mapping is then applied to the GloVe representation of the left-out concept to generate a
predicted image. The predicted image is then compared to the actual fMRI activation pattern. The rank distance (among the distances of all the other concepts) of the predicted image to the actual image is used to determine a predicted image’s accuracy. This approach was applied to three existing fMRI studies of a set of concepts: 60 concrete nouns (e.g. apple, hammer; N = 11); 18 emotions (e.g. sadness, pride; N = 10); and 28 abstract concepts (e.g. truth, ethics; N = 9). The mean rank accuracies across concepts and participants are as follows: 0.76 for the 60 concrete nouns; 0.61 for the 18 emotion concepts; and 0.68 for the 28 abstract concepts, where chance is approximately 0.5. The learned mapping generated by the model was then applied to the library of 400,000 GloVe representations to generate predicted images for virtually every word in English. The similarity between the actual activation patterns for concepts and the library of predicted patterns revealed which semantically similar concepts the individual participant or group were thinking about. Factor analysis of the predicted fMRI space was used to discover neurally-generated semantic dimensions for concepts that have no corresponding fMRI data.

Disclosures: R.J. Vargas: None. M.A. Just: None.

Poster

338. Neural Correlates of Language Processing

Location: Hall A

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Program #: Poster #: 338.22/CC24

Topic: H.02. Human Cognition and Behavior

Support: NINDS-5R01-NS091139-05
NINDS-5U01-NS098969-03

Title: Dynamics of effective connectivity between the human cortex and subthalamic nucleus during speech production

Authors: *A. R. WEISS\textsuperscript{1}, A. KORZENIEWSKA\textsuperscript{1}, W. J. LIPSKI\textsuperscript{2}, A. BUSH\textsuperscript{2}, A. CHRABASZCZ\textsuperscript{2}, N. E. CRONE\textsuperscript{1}, M. RICHARDSON\textsuperscript{2};  
\textsuperscript{1}JHU Cognitive Neurophysiol. and BMI Lab, Dept. of Neurol., Johns Hopkins Med. Inst., Baltimore, MD; \textsuperscript{2}Brain Modulation Lab, Dept. of Neurolog. Surgery, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Speech production requires interactions across large-scale cortical networks, and human intracranial electrocorticographic (ECoG) studies have shown that increases in spectral power in the high gamma (70-220 Hz) frequencies are associated with causal interactions at the same frequencies (Korzeniewska, 2011). While recent work by Chrabaszcz et al (2019) has revealed that articulatory features are encoded by different locations within the subthalamic nucleus (STN), questions remain regarding the contribution of subcortical nodes to speech production. Further, the role of cortico-basal ganglia interactions in encoding linguistic and/or
speech motor processes remains poorly understood. Here, we probe event-related causality (ERC) at high gamma frequencies between the STN and cortex to investigate how speech motor encoders are transferred across the cortical-subthalamic network. ERC is a multichannel method based on the concept of Granger causality that estimates the time-course, directionality, magnitude, and spectral content of neural interactions. We simultaneously recorded ECoG and local field potentials (LFP) from subjects undergoing deep brain stimulation electrode implantation surgery for Parkinson’s Disease as they read aloud single consonant-vowel-consonant words and pseudowords. During surgery, patients were implanted with depth electrodes in the STN (the locations of which were altered over the course of surgery) and with ECoG strips over premotor cortex, primary motor cortex (M1), primary somatosensory cortex (S1), middle temporal gyrus (MTG), supramarginal gyrus (SMG), and superior temporal gyrus (STG). We aligned trials to either stimulus presentation or spoken response onset prior to analyzing high gamma event-related activity and ERC.

As expected, the word reading task elicited significant increases in high gamma activity in several cortical regions: in MTG, S1, STG, and SMG following the presentation of stimuli; in S1 immediately preceding speech production; and in S1 and STG immediately following speech production. ERC analysis revealed interactions between STN and these cortical regions, as well as between different STN locations. Interestingly, interactions between STN and S1, M1, STG, and SMG were reciprocal, and varied depending on STN recording location. These results indicate that a complex network between cortex and STN is activated during speech processing, reflected by high gamma modulation. Moreover, causal interactions in the high gamma frequency range occurring between these cortical regions and the STN vary depending on the recording location within the STN.


Poster

338. Neural Correlates of Language Processing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 338.23/CC25

Topic: H.02. Human Cognition and Behavior

Support: NSF DDIG 1823898

Title: Investigating the role of first language flexibility in bilingual language development: An ERP study

Authors: *A. LUQUE, K. MORGAN-SHORT;
Dept. of Hispanic and Italian Studies, Univ. of Illinois at Chicago, Chicago, IL
Abstract: In today's world, many adults find themselves in a situation in which it is beneficial or even necessary to learn a second language (L2). Yet, learning an L2 during adulthood is a complex and challenging endeavor that results in a great deal of variability in learning outcomes. Researchers who examine L2 learning are interested in identifying the characteristics shared among proficient bilinguals in order to shed light on the mechanisms that may contribute to successful L2 learning. One relevant characteristic of proficient bilingualism, as posited by a recent hypothesis (Bice & Kroll, 2015) may be that learners with more "flexible" first language (L1) systems may be better at acquiring an L2. In this study, we assessed first language (L1) flexibility among intermediate learners of Spanish (N= 21) using event-related potentials (ERPs). Participants completed an ERP picture-sentence matching task following Sanoudaki & Thierry (2015) and Luque et al., (2018), two studies that provided neurocognitive evidence of L1 flexibility among highly proficient bilinguals. Preliminary data analyses reveal evidence of L1 flexibility among some but not all L2 learners, suggesting that that the role that L1 flexibility plays for adult L2 development seems to differ along the continuum of bilingualism, with other factors, such as linguistic and cognitive, modulating this phenomenon. The implications of these preliminary results will be considered in the context of both theoretical and applied questions related to bilingual language development during adulthood. Future research directions will also be discussed, including research that examines the intersection of L1 flexibility with cognitive, socio-affective factors, and different contexts of L2 learning.

Disclosures: A. Luque: None. K. Morgan-Short: None.

Poster

338. Neural Correlates of Language Processing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: 338.24/CC26

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R21HD079884
BRAIN EAGER award 1451032
RISE - R25-GM060655

Title: Two times four = Dos por cuatro: Bilinguals engage similar neurocognitive processes in both languages when verifying multiplication facts

Authors: *V. R. CERDA, N. Y. Y. WICHA;
Biol., Univ. of Texas At San Antonio, San Antonio, TX

Abstract: Bilinguals typically learn multiplication tables through verbal rehearsal in one language. In turn, they are faster and more accurate at retrieving memorized multiplication facts in the language of learning (LA+) than the other language (LA-). These findings are explained by
math cognition models in two ways. The math facts may be uniquely represented and retrieved in LA+, requiring a less efficient neurocognitive process in LA-, such as translation or calculation. Alternatively, independent verbal memory networks for each language have differing strength of access when retrieving multiplication facts from memory. Evidence from bilingual language research suggests that a bilingual’s two languages are interconnected and engage similar neurocognitive processes for language tasks. This is especially true for bilinguals who learn both languages early in life. Here, we tested whether early Spanish-English bilingual children (n = 28) and adults (n = 29) use qualitatively or quantitatively different processes for math fact verification across their languages. We recorded event-related potentials (ERPs) as participants verified the correctness of spoken multiplication problems (“two” “four”), followed by a visually presented digit solution that was either correct or not (8 vs 9). The operands were presented in LA+ or LA- in separate blocks. Given that digit solutions were used across languages, any differences in the ERPs at the solution would be driven by the language used to access the math facts. For children, incorrect solutions elicited a larger negative-going ERP component, or N400, compared to correct solutions, reflecting access to semantic memory. This N400 effect was the same in both languages, implying that bilingual children process the problems for meaning when verifying solutions in both LA+ and LA-. Consistent with monolinguals, bilingual adults showed a larger positive-going ERP component for correct solutions, a P300, compared to incorrect solutions, reflecting detection of a highly memorized target. Critically, this effect was again the same across languages. Overall, even though bilinguals show a preference for performing math in one language, both adults and children engage similar processes in both LA+ and LA-, at least when verifying multiplication facts. These findings are consistent with the bilingual literature that shows comparable neurocognitive processes for language tasks in both languages. Moreover, our findings suggest that the bias toward monolingual math education may be unfounded from a neurocognitive perspective.

Disclosures: V.R. Cerda: None. N.Y.Y. Wicha: None.

Poster

338. Neural Correlates of Language Processing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 338.25/CC27

Topic: H.02. Human Cognition and Behavior

Support: NIH R01 HD092498

Title: Bilingual impact on left frontal lobe functionality for children's sentence processing

Authors: *I. KOVELMAN1, N. WAGLEY1, X. HU2, A. BARON4, L. BEDORE5, T. SATTERFIELD1, J. BOOTH6, J. BRENNAN3;
Abstract: How does bilingualism influence children’s emerging neural architecture for language? We examine this question through the Neuroemergentism framework (Hernandez, 2019) positing that bilingual experiences can alter brain development for higher cognitive function. It is generally established that developmental increase in language proficiency is associated with increased automaticity of language processing. At the neural level, greater proficiency is typically associated with increased left temporal and decreased left frontal activation. Yet, bilinguals often show heightened attention and sensitivity to linguistic input, which is associated with left IFG functionality. Within this theoretical framework, we predicted that bilingual children with greater dual language proficiency will show stronger left IFG activation during language tasks, relative to bilinguals with less balanced bilingual proficiency. We used fNIRS to test 82 Spanish-English bilinguals (7-11 yo) using a grammaticality judgment task. The ungrammatical sentences included early acquired -ing (He is dance), and later acquired -ed/s omissions (He dance). Bilinguals were exposed to Spanish at birth, English before age 5, and were educated in English-only schools in the US; they were English-dominant, had age-appropriate English proficiency, and lower/varied Spanish proficiency. The findings revealed that greater English proficiency was indeed associated with increased left STG/MTG activation and decreased left IFG activation. New findings revealed that consistent with our predictions, children with more balanced bilingual proficiency, or stronger Spanish proficiency in addition to age-appropriate English proficiency, showed greater left IFG activation. Importantly, more balanced bilinguals did not show greater difficulty with the task, as revealed by reaction time analyses. The findings suggest that balanced and systematic use of the two languages yields greater engagement of left IFG regions associated with heightened sensitivity to linguistic input. The findings thus inform theories of bilingualism as well as broader theoretical perspectives on child brain development by illuminating the impact of early-life language experiences on cortical organization for higher cognitive functions.


Poster

338. Neural Correlates of Language Processing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 338.26/CC28

Topic: H.02. Human Cognition and Behavior

Support: NIH R01-NS102500 to NK
Knowles Hearing Center, Northwestern University to NK
Title: Monolinguals catch up to bilinguals on executive control, but not neural processing of sound, over the course of adolescence

Authors: *J. KRIZMAN, V. MARIAN, N. KRAUS;
Northwestern Univ., Evanston, IL

Abstract: While the presence of a bilingual enhancement for executive control is considered by some controversial, bilingualism’s impact on sound processing is not. With respect to executive control, a major source of contention is that enhancements found in children can be absent in young adults. In contrast, auditory processing enhancements have been observed across ages. During development, either differences in maturation rate of a skill or overall attainment level for that skill can lead to a perceived enhancement. Therefore we hypothesized that the executive control enhancement is due to differences in maturation rate of that skill, while auditory processing enhancements are due to differences in overall attainment level. We predicted that if we assessed bilinguals and monolinguals early in adolescence (~14 yrs), when these abilities are still developing, and again late in adolescence (~18 yrs), when these abilities are more mature, we should see both executive control and auditory processing enhancements at the first time point, but only auditory processing enhancements at the second time point. Spanish-English bilinguals and English monolinguals’ executive control abilities were tested using the IVA+, an integrated test of auditory and visual attention and executive control, and subcortical auditory processing was tested using the frequency-following response, an obligatory response to sound originating in the auditory midbrain. In line with our predictions, bilinguals showed an executive control advantage at age 14, but the monolinguals caught up to them by age 18, while bilingual sound processing enhancements were present at both time points and were specific to speech sound features important for real-world language perception. These results suggest that diversity of language experience can accelerate development of executive control and strengthen subcortical auditory processing. While continued language experience may enable monolinguals to reach similar levels of executive control performance, the ongoing needs of bilingual communication maintains the heightened subcortical encoding of speech features. These results may help to explain the incongruent executive control findings in the literature and highlight auditory system plasticity in bilinguals.

Disclosures: J. Krizman: None. V. Marian: None. N. Kraus: None.

Poster

338. Neural Correlates of Language Processing

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 338.27/CC29

Topic: H.02. Human Cognition and Behavior
Support: NIH Grant R03HD079873

Title: Effects of language experience differ according to neuroanatomical metric

Authors: *H. CLAUSSENIUS-KALMAN*¹, K. A. VAUGHN¹, P. ARCHILA-SUERTE², A. E. HERNANDEZ¹;
¹Univ. of Houston, Houston, TX; ²Johnson Space Center, NASA, Houston, TX

Abstract: Although researchers generally agree that a certain set of brain areas underlie bilingual language processing, there is discrepancy regarding what effect timing of language acquisition has on these regions. We aimed to investigate the neuroanatomical correlates of age of acquisition (AoA), an issue that twelve previous studies have examined, but no two of which yield the same conclusion (likely influenced by methodological differences across studies). We analyzed gray matter density, volume, and thickness by using whole-brain analyses in 334 bilinguals and monolinguals. Neuroanatomical correlates of AoA differed depending on gray matter metric. In the present sample, analyses of GM density revealed AoA-related differences in regions associated with language planning, analyses of thickness revealed changes to areas involved in both language planning and articulation, and volume analyses revealed differences in one language area but were overall the least sensitive measure of AoA. Additionally, multiple regions not classically implicated in bilingualism were found, which emphasizes the important role of whole-brain analysis in neuroscience. This is the first study to investigate AoA and gray matter thickness, volume, and density all in the same sample. We argue that cognitive models of bilingualism should consider development and the role of neuroanatomical metric in driving results.


Poster

339. Language Acquisition and Coding

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 339.01/CC30

Topic: H.02. Human Cognition and Behavior

Support: National Natural Science Foundation of China 31771248
National Natural Science Foundation of China 31500873

Title: Rapid statistical learning in word segmentation

Authors: *J. ZHANG*¹, Y. SHAO¹, M. XU², N. DING¹;
¹Zhejiang Univ., Hangzhou, China; ²Ctr. for Brain Disorders and Cognitive Sci., Shenzhen, China
Abstract: Learning to segment a continuous speech stream into discrete words is a critical aspect of language acquisition. Word boundaries can be learned by statistical learning but several key questions remain elusive. For example, how many repetitions are required to learn a word? Do all the repetitions need to occur within a time window to enable learning? We here investigate how quickly statistical learning can occur and what are the neurophysiological underpinnings. In typical statistical learning paradigms, how often a word repeats covaries with the number of words to learn. To dissociate these two factors, we control the number of words to learn to one and manipulate the number of word repetitions and the interval between repetitions. Using this paradigm, we observed that the learners could discriminate words from others within 4 repetitions of the word. By adjusting the syllable rate, we find that the learning speed depends on how many syllables are played between two repetitions of a word, rather than the time duration between repetitions. We next investigated the neural basis of statistical learning using MEG. We find that the amplitude of the M100 response is reduced for the 2nd and 3rd syllable in the trisyllabic word and this effect quickly emerges after 3-4 repetitions of the word. Altogether, both behavioral and neural findings reveal that statistical learning of word boundaries only requires a few repetitions of a word and is neurophysiologically implemented by adaptation of auditory responses at ~100 ms latency.

Disclosures: J. Zhang: None. Y. Shao: None. M. Xu: None. N. Ding: None.
English bilinguals and English monolinguals (N = 24, ages 5 - 10) during fNIRS neuroimaging. The bilingual participants were exposed to Chinese at birth, and to English before age 5, attended English-only schools and afterschool programs in Chinese. The children completed morphological awareness tasks of compound (e.g., snow-man) and derivational (e.g., re-count, kind-ly) morphology during neuroimaging. The children also completed standard behavioral measures of language and literacy in English. Neuroimaging findings revealed that English morphological awareness task engaged left frontal and temporoparietal regions across participants. A direct comparison between bilinguals and monolinguals revealed that the bilinguals showed stronger left IFG and left Parietal as well as stronger overall right hemisphere activation. A more detailed investigation revealed that the compound condition elicited greater left Parietal activation whereas the derivational condition elicited greater left IFG activation in the bilinguals relative to monolinguals (q < 0.05, FDR corrected). Importantly, there were no significant differences between bilinguals’ and monolinguals’ neuroimaging task performance or English language and reading proficiency (p > 0.05). The findings suggest that early bilingual exposure to typologically different language such as Chinese can exert a significant impact on bilingual children’s neural organization for word structure processing in English in children with age-appropriate English language proficiency. The findings shed light on neurodevelopmental theories aiming to explain how early language experiences influence children’s cortical organization for language function.


Poster

339. Language Acquisition and Coding

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 339.03/CC32

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant K01MH103594
       NIH Grant 5R01NS090874-09
       NIH Grant R21MH109775

Title: Mapping the neural correlates of covert and overt verb generation using high-density diffuse optical tomography

1Mallinckrodt Inst. of Radiology, 2Mallinckrodt Inst. of Radiology; Dept. of Biol., 3Mallinckrodt Inst. of Radiology; Div. of Biol. & Biomed. Sci., 4Mallinckrodt Inst. of Radiology; Dept. of Physics, Washington Univ. Sch. of Med. in St. Louis, Saint Louis, MO
Abstract: Mapping the neural correlates of naturalistic speech has potential clinical applications, such as characterizing and monitoring language function in aphasic patients post-stroke or in patients with cochlear implants post-surgery. Traditional methods, such as functional magnetic resonance imaging (fMRI), pose limitations to studying naturalistic language processing due to a loud and constraining ambient environment, the requirement that participants lie supine and still, and contraindications in populations with implanted devices. Due to its silent and open imaging environment, high-density diffuse optical tomography (HD-DOT) provides a compelling surrogate for fMRI. As HD-DOT been extensively validated in adults, the purpose of the present study is to build upon previous studies to localize differential cortical activation to covert vs. overt verb generation.

14 adult subjects ages 20-42 (7 females, 5 right-handed; 7 males, 7 right-handed) participated in 1 or more HD-DOT scans consisting of 3 tasks: covert word reading (RW), covert verb generation (CV), and overt verb generation (OV). Stimuli for all runs were concrete nouns (n=765) with Zipf frequencies of at least 3.86 selected from the SUBTLEXus corpus. Nouns were presented visually using an event design paradigm with pseudorandomized inter-stimulus intervals (2-13 seconds), with each event block consisting of 5 nouns each displayed for 200 ms and presented 1 second apart. No noun was presented more than once per scan session and the order of nouns presented for each run was counterbalanced across participants. Additionally, audio responses from the OV runs were recorded.

We found brain responses of increasing magnitude for RW, CV, and OV, respectively, in expected areas of the brain. Results demonstrate additional cortical recruitment with increasing task complexity (RW to CV to OV), with RW primarily evoking responses in primary visual cortex, CV additionally recruiting Broca’s area and the visual word form area, and OV additionally activating right-lateralized primary motor cortex. Together, these results demonstrate that 1) HD-DOT localizes activations underlying language processing consistent with those acquired by fMRI and 2) covert language tasks cannot be used as a surrogate for more naturalistic tasks such as speech production. Compared to fMRI, HD-DOT’s naturalistic ambient environment and ability to image clinical populations precluded by fMRI motivates continued investigation of language processing. Future directions include analyzing audio responses to investigate performance differences and to isolate the effects of performance from the effects of handedness and sex.


Poster

339. Language Acquisition and Coding

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 339.04/CC33

Topic: H.02. Human Cognition and Behavior
Support: NIH R01 HD092498

Title: Reading acquisition through bilingual lens: fNIRS study of bilingual children

Authors: *K. Zhang*, 1 X. Sun1, J. Kim1, N. Nickerson1, Y. Li1, R. Marks1, F. Hu2, T.-L. Chou3, T. Tardif1, I. Kovelman1;

1Psychology, 2Ctr. for Human Growth and Develop., Univ. of Michigan, Ann Arbor, MI; 3Psychology, Natl. Taiwan Univ., Taipei, Taiwan

Abstract: How does the bilingual brain support reading acquisition? There are significant cross-linguistic differences between English and Chinese orthographies. In English, units of sounds map onto letters. In Chinese, units of meanings map onto characters. How do these differences influence children’s emerging neural architecture for learning to read? To answer this question, we compared emerging literacy skills in English and in Chinese of young English-Chinese bilinguals using fNIRS. The investigation focused on morphological awareness, children’s active sensitivity to smallest units of meaning, and a critical component of learning to read across languages. We asked young Chinese-English bilinguals (N = 15, ages 5 - 10) to complete tasks of compound (e.g., birth-day) and derivational (e.g., un-do, friend-ly) morphology in both of their languages during neuroimaging. The children also completed standard language and literacy measures in English and Chinese. The children were raised in Chinese-speaking families living in the US and were dominant in their English language proficiency. Neuroimaging findings revealed that in both languages, the morphological awareness task engaged left frontal and temporal regions (q < 0.05, FDR corrected). Yet, there were no significant differences in the brain activation between the bilinguals’ two languages, in either of the condition (q > 0.05). The findings pose two complementary interpretations. First, morphological processing across languages might be fundamentally similar. Second, young bilinguals might be developing similar neurocognitive mechanisms for processing word structure in each of their languages. Taken together, the findings shed light on the how language experiences with typologically-distinct languages influence neural organization for language processing in bilinguals.

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Poster

339. Language Acquisition and Coding

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 339.05/CC34

Topic: H.02. Human Cognition and Behavior

Title: Does transcranial direct current stimulation of the dorsolateral prefrontal cortex effect language learning in young adults?
Authors: *A. J. BOLLING, T. ENAM, V. KING, L. SCHROBILGEN, I. M. MCDONOUGH; The Univ. of Alabama, Tuscaloosa, AL

Abstract: Memory is the ability to encode, store, and retrieve information, which is critical for learning a foreign language. While children acquire new languages relatively easily, learning a new language as an adult can be quite difficult. One critical aspect of learning a new language is the ability to learn the pairing between words in the native and foreign language. fMRI research has suggested that the dorsolateral prefrontal cortex (dLIFC) is critical to learn new pairs of words. However, because fMRI is correlational, additional research is needed to further investigate the implicated brain region and how it is involved in language learning. Transcranial Direct Current Stimulation (tDCS) is a non-invasive procedure that uses electrodes to send small electrical currents through the scalp, modulating brain activity. The present confirmatory study aims to use tDCS to determine the degree that the dLIFC is causally involved in foreign-language learning and, in the process, learn how to improve language acquisition in adults. This study includes 3 sessions that each take place 24 hours apart. In the first two sessions, participants were stimulated for 20 minutes, and then learned 72 English-Swahili word pairs. The list of word-pairs was repeated once during each session to increase exposure. On the third day, participants were tested using cued-recall, where the Swahili word was shown, and participants typed the English equivalent. One week later, the test was retaken. The tDCS was administered at the F3 placement site using the International 10-20 system for EEG electrode placement at three stimulation levels (1.5mA, 1mA, and sham- a control) with n=13 per stimulation level (23 women). Participants were young adults in an introductory psychology course at the University of Alabama. We hypothesized that participants who received tDCS would have higher memory retention for the word pairs than those in the sham condition. We found that there were no significant effects of tDCS on the level of retention. This could be due to the small sample size, or it could mean tDCS does not have an effect on language learning. If the findings remain consistent with further research, it could suggest that the dLIFC is not causally involved in language learning as an adult.

Disclosures: A.J. Bolling: None. T. Enam: None. V. King: None. L. Schrobilgen: None. I.M. McDonough: None.

Poster

339. Language Acquisition and Coding

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program#/Poster#: 339.06/CC35

Topic: H.02. Human Cognition and Behavior

Title: The effects of different learning mechanisms on speech segmentation
Authors: *Y. CHEN, P. JIN, N. DING; Zhejiang Univ., Hangzhou, China

Abstract: Natural speech consists of continuous streams of sound with no reliable pauses between words. Therefore, one major challenge for learners in language acquisition is to discover word boundaries, a process known as speech segmentation. Explicit learning the phonology, semantic and orthography of the words is a critical learning mechanism underlying word segmentation. Meanwhile, statistical learning, an implicit process of becoming sensitive to statistical structure of the language, is also considered to facilitate speech segmentation even before knowing the words of the language. However, it is currently unknown whether these learning mechanisms have different effects on speech segmentation. Here, we used EEG frequency-tagged responses analyses to examine the neural entrainment of the statistically and explicitly learned nonsense words and real words. In our statistical learning experiment, we recorded EEG data while participants listened to a structured stream of trisyllabic nonsense words and then a trisyllabic real word stream. Statistical learning was supposed to occur during exposure of the nonsense word stream. In the explicit learning experiment, another group of participants were exposed to three streams of trisyllabic nonsense words each after learning either the phonology, the semantic or the symbol of the words. They also listened to the real word stream as in the statistical learning group. Learning performance was indexed by an EEG-based measure that quantified neural entrainment at the frequency of repeating words relative to that of individual syllables. The results showed the neural entrainment of real words was higher than nonsense words in statistical learning. However, the entrainment of real word was comparable to nonsense words whose phonology, semantic or symbol has been learned. Moreover, we found an interaction of group (statistical learning group and explicit learning group) and word type (real word and learned nonsense word), indicating poorer effect of statistically learned words than explicitly learned words. These results demonstrate that the effect of statistical learning is poorer than explicit learning, while the effect of explicit learning of nonsense word was comparable to real word. Our study illustrated the effects and mental representations of different learning mechanisms, providing insight into strategies of language learning.

Disclosures: Y. Chen: None. P. Jin: None. N. Ding: None.

Poster

339. Language Acquisition and Coding

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 339.07/CC36

Topic: H.02. Human Cognition and Behavior

Support: NIH R01 HD092498
Title: Bilingual Spanish-English literacy development: An fNIRS study of morphological awareness

Authors: *N. Nickerson, X. Sun, K. Zhang, R. A. Marks, J. Kim, I. Hernandez, V. Caruso, X. Xu, T. Tardif, T. Satterfield, I. Kovelman;
Univ. of Michigan, Ann Arbor, MI

Abstract: How does bilingual language experience influence how morphological awareness develops in the brain? Learning to read requires children to extract and decode meaning from sequences of morphological units, the smallest units of meaning with a word. This morphological structure varies across languages. In particular, while Spanish is more flexible in its morphological structure and complexity, English word order and structure is more fixed. During fNIRS neuroimaging, Spanish-English bilinguals and English monolinguals (N = 28, ages 5 - 10) completed English tasks of morphological awareness. Standard measures of language and literacy in both English and Spanish were also collected. English morphological processing elicited activation in left frontal and temporo-parietal regions across participants (q < 0.05, FDR corrected). Differences emerged between bilingual and monolingual participants, revealing stronger left parietal activation in bilinguals as well as stronger right frontal and temporal activation (p < 0.05). This difference in the brain basis of morphological processing is striking, as there were no significant differences between bilingual and monolingual children in either the neuroimaging task performance or their English language or reading proficiency. Our findings suggest that developing dual language proficiency in Spanish and English does indeed seem to influence young learners’ brain development for morphology. Such findings continue to inform our understanding of Spanish-English bilingual language and brain development through optical imaging.


Poster

339. Language Acquisition and Coding

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 339.08/CC37

Topic: H.02. Human Cognition and Behavior

Title: A systematic review and meta-analysis of brain differences in bilingual and multilingual adults

Authors: A. Danylkiv, *A. J. Krafnick;
Psychology, Dominican Univ., River Forest, IL
Abstract: Introduction While differences between bilingual and monolingual children have received much interest, there is growing evidence that speaking multiple languages may also be beneficial for aging adults in slowing brain decay (Gold et al., 2013). Compared to monolingual peers, individuals who speak more than one language perform better on executive function tasks (Grady et al., 2015), show greater activation in brain regions while performing the same task (Luk et al., 2010), and may have greater cognitive reserve and neural efficacy (Gold et al., 2013). Structural and functional neuroimaging studies of bilingual and multilingual subjects have shown considerable heterogeneity. Here we attempt to synthesize the neuroimaging literature through systematic review and meta-analysis. Methods PubMed (www.ncbi.nlm.nih.gov/pubmed/) and Google Scholar (google.com/scholar) were used to identify neuroimaging studies of bilingual adults. Search terms included (Bilingual AND neuroimaging), (Bilingual AND adult), (Bilingual AND brain). From these search results, manuscripts not using MRI or those primarily in children were filtered out. The patterns across studies were investigated for both functional and structural MRI (gray matter VBM), and studies with whole brain group comparisons and available Talairach or MNI coordinates were submitted to meta-analysis using GingerALE 2.3 (Eickhoff et al. 2009, 2012; Turkeltaub et al., 2012). Results Preliminary results of the structural MRI meta-analysis contained seven individual studies (three of which had no whole brain result foci to include). At an uncorrected voxel wise threshold of p<0.001 and a cluster threshold of 200mm³, four clusters were identified in bilateral anterior cingulate cortex, right cerebellum, and bilateral inferior frontal gyrus (see Figure). Discussion While some consistency is observed across VBM studies of bilingual and multilingual adults, this was only true at uncorrected thresholds. More work is needed fully understand neural plasticity related to speaking more than one language.

Disclosures: A. Danylkiv: None. A.J. Krafnick: None.
Title: Cortical activation during sentence processing among Chinese-English bilinguals and English monolinguals: An fNIRS study

Authors: *G. DING, K. A. J. MOHR, R. GILLAM, C. ORELLANA, A. HANCOCK; Utah State Univ., Logan, UT

Abstract: Research has shown that both monolinguals and bilinguals process language across specialized brain areas working collaboratively, but with greater variability among bilinguals. Age of acquisition (AoA) is typically considered to be one of the main factors influencing differences in brain activation patterns between first language (L1) and second language (L2). Essentially, the earlier a bilingual acquires the second language, the more like monolinguals the bilingual’s neural networking develops. This study examined behavioral and neurophysiological data of 10 monolingual children, 10 early-bilingual children and 10 late-bilingual adults to compare mechanisms underlying syntactic processing. They completed the Woodcock-Johnson III auditory working memory test (AWM) and then an English listening comprehension task during fNIRS scans. Bilingual children and adults received a similar task in Chinese. Auditory stimuli in both languages included three sentence types: Control (e.g., click the book), Subject-Verb-Object (SVO) and Passive (PAS). The semantic plausibility of the sentences was controlled, so that word order was the only relevant linguistic cue. Participants were asked to select the agent of each sentence. We expect that bilingual children would evidence similar activation patterns as monolinguals compared to adults, and that processing the more complex sentence type (i.e. PAS) would be more cognitively challenging. One-way and repeated-measure ANOVAs compared results, which showed that adults performed significantly better than children on AWM. Both children groups performed better on SVO than PAS in English and Chinese, but no significant differences on sentence type were found in bilingual adults. For fNIRS data, no main effects were found among three groups in English tasks. However, in comparing the two bilingual groups in both language tasks, a significant interaction (language*region of interest*sentence type*group) was found. For English tasks, adults showed reduced activation in left inferior frontal gyrus [LIFG] and left superior temporal gyrus [LSTG] for PAS compared to controls. Moreover, compared to Chinese (L1), more activation of middle prefrontal cortex [MPFC], LIFG, LSTG was found for SVO among adults in English (L2). In the
Chinese tasks, both bilingual adults and children showed increased activation in LIFG for PAS than SVO sentences. Findings of different activation patterns among adults in L1 and L2 suggests that AoA is an influential factor in sentence comprehension. Patterns for PAS sentences in both language tasks indicates that sentence complexity also influences sentence comprehension.

Disclosures:  G. Ding: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Research Office and the Lillywhite Endowment at Utah State University. K.A.J. Mohr: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Research Office and the Lillywhite Endowment at Utah State University. R. Gillam: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Research Office and the Lillywhite Endowment at Utah State University. C. Orellana: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Research Office and the Lillywhite Endowment at Utah State University. A. Hancock: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Research Office and the Lillywhite Endowment at Utah State University.

Poster

339. Language Acquisition and Coding

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 339.10/CC39

Topic: H.02. Human Cognition and Behavior

Support: Schmitt Program on Integrative Neuroscience
FA8650-14-C-7357
1652127

Title: An integrated neural decoder of linguistic and experiential meaning

Authors: *A. J. ANDERSON¹, J. R. BINDER², L. FERNANDINO⁴, C. J. HUMPHRIES⁵, L. CONANT⁶, R. D. RAIZADA², F. LIN⁷, E. LALOR¹;
²Dept of Brain & Cognitive Sci., ¹Univ. of Rochester, Rochester, NY; ³Dept. of Neurol.,
Abstract: The brain is thought to combine linguistic knowledge of words and non-linguistic knowledge of their referents to encode sentence meaning. Despite technological advances that now enable words’ meaning to be decoded from brain activity, there remains minimal direct neural evidence for this. Indeed, the dominant neural decoding method maps brain activation to a text-based semantic model that approximates meaning using patterns of word co-occurrences. We present direct evidence that both linguistic and non-linguistic experiences contribute to and can be decoded from neural representations of sentence meaning. We model attributes of peoples’ sensory, motor, social, emotional and cognitive experiences with words using behavioral ratings. We demonstrate that functional Magnetic Resonance Imaging (fMRI) activation elicited in sentence reading is more accurately decoded when this experiential attribute model is integrated with a text-based model than when either model is applied in isolation (participants were 5 males and 9 females). In decoding we exploit a representation-similarity-based framework which benefits from being parameter free, whilst performing competitively with parameter fitting approaches such as ridge-regression. We find that the text-based model contributes particularly to the decoding of sentences containing linguistically oriented “abstract” words and reveal tentative evidence that the experiential model improves decoding of more concrete sentences. Finally, we introduce a cross-participant decoding method to estimate an upper-bound on model-based decoding accuracy. We demonstrate that a substantial fraction of neural signal remains unexplained, and leverage this gap to pinpoint characteristics of weakly decoded sentences and hence identify model weaknesses to guide future model development.


Poster

339. Language Acquisition and Coding

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 339.11/CC40

Topic: H.02. Human Cognition and Behavior

Title: Sparse experimental design for encoding models

Authors: *L. XU, A. LEBEL, A. G. HUTH;
UT Austin, Austin, TX

Abstract: Encoding models predict neural or fMRI responses from natural stimuli but have high requirements on both data quality and quantity. This makes it difficult to scale such experiments to very large numbers of subjects or stimuli. Here we aim to solve this issue by using a sparse
experimental design, in which each subject is exposed to a different small fraction of the total stimulus set. This sparse dataset is used to fit a spatio-temporal shared response model (SRM) for the whole brain, which assumes that the same stimuli evoke synchronic and localized responses across subjects. The SRM learns brain response patterns from responses of same subject to different stimuli, and learns stimulus features from the responses of different subjects to the same stimuli. These response patterns and stimulus features are then used to reconstruct data for all the stimuli that were not played for each subject. The original and reconstructed data are then concatenated and used to fit separate encoding models for each subject with standard regression techniques. In a test experiment, 3 subjects listened to all 60 auditory stimuli (12 hours in total) and 6 other subjects listened to a small fraction (around 20/60) of the same auditory stimuli, while undergoing fMRI scanning. With SRM, the concatenated datasets improve encoding model performance by 47.3% (± 18.6) for 3 good subjects, and by 268.8% (± 120.8) for the other 3 poor subjects. In simulations, we tested our model on 40 subjects and 10 to 100 stimuli with the same total data collection budget (each subject sees 10 random stimuli), and found that the concatenated dataset with SRM performs much better than the baseline. Finally, we show how to optimize the design of these sparse experiments by treating the connections between stimuli and subjects as a bipartite graph, and then showing that certain graph properties (such as the eigenvalue gap and mean path length) are closely linked to model performance. In conclusion, our SRM model suggests the sparse experimental design for encoding models with lower cost of time and money on data collection, and also shows the potential to denoise noisy datasets. Further, the response patterns and stimulus features learned by SRM could also be interpreted to gain insight into stimulus features that are represented similarly across subjects.

study, we performed an fMRI experiment where two women and three men passively listened to five hours of natural language stimuli. We then used voxelwise modeling to predict BOLD responses on a held out test data set using five different feature spaces that capture different aspects of language processing. These feature spaces included a word embedding space that captures word-level semantics, a neural network-based semantic space that captures elements of contextual semantics, an articulatory space that captures lower-level language processing, a neural network-based timing space that captures a combination of semantics and prosody, and a word indicator space which served as a baseline for comparison to the other models. We then used variance partitioning to compare the predictive performance of each model in both cerebellum and cortex. The neural network semantic model with the longest context had the highest prediction performance in the cerebellum followed by the word-level semantic space. The articulatory space and the timing space had significantly lower performance than the semantic spaces and were not significantly different from each other. These results demonstrate that the cerebellum is not simply performing timing or motor predictions of language, but is involved in semantic processing. However, this pattern of predictive performance is mirrored in the cortex, suggesting that the cerebellum may merely be replicating cortical representations. To test for this possibility at a finer level of detail we compared representations of eleven different semantic categories across cortex and cerebellum. This analysis showed significant over-representation of the social semantic category in the cerebellum as compared to cortex, and under-representation of categories such as numbers and places. Collectively, this demonstrates that the cerebellum is highly involved in semantic processing of language and that it uniquely responds preferentially to social semantic categories.

Disclosures:  A. LeBel: None. S. Jain: None. A.G. Huth: None.

Poster

339. Language Acquisition and Coding

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 339.13/CC42

Topic: H.02. Human Cognition and Behavior

Support: Burroughs Wellcome Fund

Title: Visually grounded language encoding models for fMRI highlight the influence of sensory experience on semantic representations

Authors: *J. TANG¹, A. LEBEL², A. G. HUTH¹,²;
¹Computer Sci., ²Neurosci., The Univ. of Texas at Austin, Austin, TX

Abstract: To process natural speech, the brain relies on semantic representations which generalize acquired knowledge of the world. Currently accepted theories of grounded cognition
suggest that the brain’s semantic representations must be grounded in the sensory systems from which knowledge is initially acquired. However, little is known about how elementary representations from multiple modalities are integrated into semantic representations or how sensory information propagates to the semantic representations of abstract words.

To address these issues we fit voxelwise encoding models to predict BOLD fMRI responses to a natural language experiment, where subjects listen to 6 hours of natural speech from *The Moth Radio Hour*. In contrast to existing encoding models, which represent stimulus words using distributional word embeddings extracted from textual co-occurrence, we use word embeddings explicitly grounded in visual and tactile properties. To create grounded embedding spaces, we represent concrete words by the visual and tactile properties of their referents, extracted respectively through convolutional neural networks and property norms. A novel interpolation method is used to ground abstract words in the sensory properties of their distributional neighbors. As visual and tactile properties may capture overlapping information, we employ a variance partitioning scheme; encoding models are fit for each combination of the distributional, visually grounded, and tactile grounded features spaces, and set theory is used to find the unique response variance explained in each voxel by each subset of the features.

We show that in normally sighted (NS) subjects, visual information uniquely influences semantic representations in language areas around the visual cortex, while tactile information uniquely influences semantic representations near the somatosensory cortex. Repeating the experiment for early blind (EB) subjects, we find that the influence of tactile information on semantic representations extends beyond somatosensory-adjacent language regions into areas which are uniquely influenced by visual information in NS subjects. While the NS results suggest that sensory grounding is constrained by connectivity, the EB results show that in the absence of visual experience, semantic representations may be more heavily grounded in alternative sensory modalities.

**Disclosures:** J. Tang: None. A. LeBel: None. A.G. Huth: None.

**Poster**

339. Language Acquisition and Coding

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 339.14/CC43

**Topic:** H.02. Human Cognition and Behavior

**Support:** SEP-CONACYT 220973

**Title:** Processing syntactic and morphological violations in Spanish and Wixarika

**Authors:** A. GONZÁLEZ¹, *D. L. CANELA¹, F. A. ROBLES², W. F. LARA GALINDO³; ¹Univ. De Guadalajara, Guadalajara, Mexico; ²Univ. de Guadalajara, Colotlán, Jalisco, Mexico; ³Univ. de Guadalajara, Guadalajara, Mexico
Abstract: Differences in comprehension processing have been evaluated introducing task, stimuli or language acquisition variations leading to restricted discrepancies in brain physiological measures (Friederici & Alter, 2004). However, only recently grammatical distinctions started to be considered as a source of variance in ERP amplitudes or latencies (Tune et al., 2014). Present study was designed to contrast ELAN and LAN components between Wixarika, a Yuto-Nahua agglutinative language spoken in the northeast of Mexico, and Spanish speakers. In this study, we compared ERPs of 10 Wixarika and 20 Spanish speakers (ages 18-27) during an auditory lexical decision task with nominal phrases using: words (agua/haá/water), pseudowords type I (cuchillo-chicullo/knife, hikuri-hukiriti/cactaceae) and type II (abeja-abeje/bee, haá-hué/water). Nominal phrases were divided into three conditions: i) agreement (nehuká/ni panza/my belly), ii) violation of plural (ta kakaitsi*sus huarache(-es)*/his sho(-e)s*), iii) syntactic violation (xeme we*papate/nuestras (alta*) tortillas/our high tortillas). There were no differences between conditions neither for LAN amplitudes (Wixarika, F=0.864, p=0.377; Spanish, F=0.005, p=0.94) or latencies (Wixarika, F=0.001, p=0.992; Spanish, F=0.43, p=0.52) nor for ELAN amplitudes (Wixarika, F=1.72, p=0.23; Spanish, F=0.53, p=0.47) or latencies (Wixarika, F=2.09, p=0.19; Spanish, F=0.76, p=0.417). Groups comparisons showed differences only in ELAN latencies before syntactic violation (t=28, p=0.008) and in LAN amplitudes before morphological violation (t=28, p=0.040) only for pseudowords type II. Findings allow us to assert that both ERPs are sensitive to the distinct morphology and syntactic treatment they gave to phrases.
Title: Computations underlying human speech processing from phoneme to semantic meaning

Authors: *X. GONG, J. L. GALLANT, F. E. THEUNISSEN;
Univ. of California Berkeley, Berkeley, CA

Abstract: The transformation from sound to meaning in the brain is carried out by a hierarchy of processing modules that represent progressively more abstract features of sound. However, the computations underlying this transformation are still unknown. We hypothesized that, if cortical representations of semantic meaning are derived from a non-linear transformation of phonemic representations, then it should be possible to model cortical regions that represent semantic meaning in terms of nonlinear combinations of phonemes.

To test this hypothesis, we reanalyzed data collected in an earlier experiment which brain activity was recorded by functional MRI from seven human subjects while they listened to natural narrative speech (Huth et al., 2016; de Heer et al., 2017). We used voxelwise modeling to model every voxel in each individual subject in terms of several distinct feature spaces: single phonemes, combination of two and three phonemes, words and semantic meaning. We used a separate data set to evaluate voxelwise prediction accuracy, and we used variance partitioning to partial out the separate and joint contributions of each feature space. This allowed us to estimate the fraction of response variance accounted by each feature space separately in each voxel and each subject.

We find that regions in superior temporal sulcus (STS), inferior frontal gyrus (IFG), inferior posterior gyrus (IPG) and prefrontal cortex are predicted equally well by bi-phoneme combinations and semantic meaning. This result suggests that these voxels perform a logical AND operations on phonemic representations of speech sound in order to extract meaning. Furthermore, by examining the weights of voxelwise model, we found that these voxels are tuned to combinations of phonemes that occur most frequently in English. Finally, principal component analysis of the voxelwise model weights reveals that voxels located in STS and IFG are best predicted by meaningful, short phoneme combinations (e.g. “sh.iy” and “y.uw”), while...
voxels in IPG are best predicted by phonemic combinations that do not convey semantic meaning (e.g. “l.ih” and “t.ao”).

**Disclosures:**  
* X. Gong: None.  
* J.L. Gallant: None.  
* F.E. Theunissen: None.

**Poster**

**339. Language Acquisition and Coding**

**Location:** Hall A  
**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM  
**Program #/Poster #:** 339.16/CC45  
**Topic:** H.02. Human Cognition and Behavior  
**Title:** Functional connectivity of the right pars opercularis in American sign language  
**Authors:**  
* T. IKUTA¹, E. MALAIA², R. WILBUR³;  
¹Univ. of Mississippi, Oxford, MS; ²Univ. of Alabama, Tuscaloosa, AL; ³Purdue Univ., West Lafayette, IN  
**Abstract:** Signed languages differ dramatically from spoken languages in that the linguistic signal is encoded spatially. Right hemisphere involvement appears to be critical for sign language processing (Emmorey et al., 2013); however, the specific role and mechanism of this involvement is not well understood. Prior work has identified important roles of right IFG and right STG in both resting state activity (Malaia et al., 2014) and language processing in Deaf signers (Malaia et al., 2013). To better understand the roles of these regions in sign language processing and comprehension, we conducted voxelwise connectivity analysis on fMRI data of 12 Deaf signers and 11 hearing non-signers (Malaia et al., 2013), during presentation of signs and gestures. During four functional series of 5 minutes and 40 seconds, video clips of signs and gestures were presented. Data preprocessing and statistical analyses were conducted using FMRIB Software Library (FSL), as well as Analysis of Functional NeuroImages (AFNI). To conduct voxelwise functional connectivity, each of the regions of interest (left and right pars triangularis (BA 45), pars opercularis (BA 44), and pars orbitalis (BA 47)) was segmented by Freesurfer (Sischl, 2012) on the MNI 1mm space. Using threshold-free cluster enhancement, contrast images were estimated by the threshold of p<0.001. The right pars opercularis in Deaf participants demonstrated significantly higher connectivity to right Superior Temporal Gyrus (STG; MNI [64-324 2]) (FWE corrected p=0.044) (Figure 1). The five other ROIs (right pars opercularis, and BA 45 and BA 47, left and right) did not show significant differences in connectivity between two groups. The present results suggest that information flow between phonotactic (syllable-segmenting) and semantic system in sign language is right-lateralized. Our finding provides evidence for integration of right-lateralized spatial processing brain networks into linguistic processing algorithms for sign language.
Disclosures:  T. Ikuta: None. E. Malaia: None. R. Wilbur: None.

Poster

339. Language Acquisition and Coding

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 339.17/CC46

Topic: H.02. Human Cognition and Behavior

Title: Low-frequency neural activity reflects rule-based chunking during speech listening

Authors: *P. JIN, T. ZHOU, N. DING;
Zhejiang Univ., Hangzhou, China
**Abstract:** During language comprehension, it has been debated whether the brain groups words into higher level chunks such as phrases and sentences. It has been shown that cortical activity tracks phrases and sentences during speech listening, which has been taken as strong evidence for mental construction of multi-word chunks. Nevertheless, it is recently argued that neural tracking of phrases and sentences can be potentially driven by the syntactic or semantic properties of individual words. Here, we test whether cortical activity merely tracks properties of words or also mentally constructed multi-word chunks. When processing a sequence of nouns, two tasks separately require the listeners to group either semantically similar words into chunks or semantically dissimilar word into chunks. It is demonstrated that neural activity actively tracks task-required chunks rather than passively reflecting properties of words. The results indicate that cortical activity mainly tracks multi-word chunks constructed by rules, instead of properties of individual words.

**Disclosures:** P. Jin: None. T. Zhou: None. N. Ding: None.

**Poster**

339. Language Acquisition and Coding

**Location:** Hall A

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**Topic:** H.02. Human Cognition and Behavior

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JSPS KAKENHI Grant 18H02709
JSPS KAKENHI Grant 16K19510

**Title:** Predictability of electrocorticogram coherence using distributed semantic representation

**Authors:** *N. SATO*¹, R. MATSUMOTO²,³, A. SHIMOTAKE⁴, M. MATSUHASHI⁵, M. OTANI³, T. KIKUCHI⁶, T. KUNIEDA⁷,⁸, H. MIZUHARA⁸, R. TAKAHASHI³, A. IKEDA⁴; ¹Future Univ. Hakodate, Hakodate, Japan; ²Div. of Neurol., Kobe Univ. Grad. Sch. of Med., Kobe, Japan; ³Dept. of Neurology, Kyoto Univ. Grad. Sch. of Med., Kyoto, Japan; ⁴Dept. of Epilepsy, Movement Disorders and Physiol., Kyoto Univ. Grad. Sch. of Med., Kyoto, Japan; ⁵Human Brain Res. Center, Kyoto Univ. Grad. Sch. of Med., Kyoto, Japan; ⁶Dept. of Neurosurg., Kyoto Univ. Grad. Sch. of Med., Kyoto, Japan; ⁷Dept. of Neurosurg., Ehime Univ. Grad. Sch. of Med., Ehime, Japan; ⁸Kyoto Univ. Grad. Sch. of Informatics, Kyoto, Japan

**Abstract:** We recently proposed an analytical method for evaluating subpopulation network structure based on a theoretical coupling of subpopulation network structure and electrocorticogram (ECoG) coherence. The theoretical coupling was first demonstrated by our computational simulation of local field potentials (LFPs) using a biologically plausible network
and further applied to an ECoG signal analysis using distributed semantic representation. In this method, the subpopulation network structure was characterized by individual weights of distributed features at each electrode and these were calculated by multiple regression analysis with a constraint on the theoretical coupling. In the present study, we evaluated the stability of the analytical method based on the theoretical coupling using ECoG signals measured from ten participants during a picture naming task. A set of semantic vectors of picture names was defined by principal components of 100-dimensional word vectors that were calculated using the Word2Vec algorithm and a large text database. The plausibility of the semantic vectors was validated by their behavioral correlation where speech delay was significantly shorter for pictures of which semantic vectors were similar to those of a previously presented picture. The predictability of ECoG coherence using a given picture name was tested for each time period, frequency band, and electrode pair by a 20-fold cross-validation procedure. The individual weights of distributed features were estimated from a training dataset and the predicted coherence to test the set pictures was calculated by using the estimated weights, which was subsequently compared with the true coherence. Cross-subject analysis showed significant predictability of long-range coherence between temporal and frontolateral regions in a wide frequency range of 2-32 Hz and a time period of 0-0.8s after the onset of picture presentation (p<0.05, false discovery rate (FDR) corrected). Importantly, the predictability of coherence was higher for higher-dimensional semantic features, where more than one feature (cumulative contribution was 0.17 from the original 100-dimensional word vector) are required for the analysis. This suggests that high-dimensional semantic features contributed to the prediction of the coherence and that the analysis based on the theoretical structure-coherence coupling using distributed semantic representation suitable for ECoG signal analysis. In a future study, it is important to assess various types of networks associated with distributed representation that are compatible with recent machine learning techniques for visual and auditory signals.


Poster

339. Language Acquisition and Coding

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 339.19/CC48

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant DC014279
NSF GRFP DGE 1644869
NSF IGERT DGE 1144854
Title: Characterizing the spatiotemporal pattern of neural activity and word representation during visual word recognition


Abstract: Visual word recognition (VWR) is the process of mapping the written form of a word to its underlying linguistic item. Characterization of healthy VWR neural mechanisms holds promise for improving treatment of reading deficits and deepening our understanding of literacy. While noninvasive neuroimaging studies have identified putative brain regions and event-related potentials involved in VWR, the spatiotemporal flow of information through the brain remains unclear. In this study, we analyzed high gamma neural activity of more than 13000 electrodes from more than 140 intracranial neurophysiology patients as they read visually-presented words. We find that over 3000 electrodes show a task-sensitive response. Latency analyses reveal that on average, occipital lobe electrodes respond fastest, followed by temporal lobe, with the slowest responses from frontal and parietal lobes. Middle occipital gyrus and fusiform gyrus are among the fastest regions on average. By clustering the responses of all task-sensitive electrodes, we identified a variety of response types including excitatory and inhibitory responses, onset and offset responses, and responses sustained for the duration of the word presentation. We observe that occipital lobe has the highest proportion of excitatory responses while frontal lobe has an almost even excitatory/suppressive balance. Furthermore, we investigated the neural representation of the stimuli’s visual, phonemic, lexical, and semantic features. From each feature set, we predicted each electrode’s response and investigated the properties of the best-predicted electrodes. Visually-predicted and phoneme-predicted electrode groups had mostly low latencies and excitatory peaks. Visually-predicted electrodes were spread between occipital and frontal lobes. Lexically- and semantically-predicted electrode groups included more suppressive responses, with linguistically-predicted electrodes distributed across frontal, temporal, and occipital lobes, and a plurality of semantically-predicted electrodes in frontal lobe. We further investigated the encoding of these electrode groups of the feature sets over time, showing that visual features peak quickly after word onset, followed by phonemic, linguistic, then semantic features. Together, these results provide a high-resolution look at the spatiotemporal pattern and representation of neural activity during visual word recognition in the human brain.

**Poster**

**339. Language Acquisition and Coding**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 339.20/CC49

**Topic:** H.02. Human Cognition and Behavior

**Support:** NIH grant 1U01NS098968

**Title:** Evolution of cognitive state during a conversation

**Authors:** *A. E. HADJINICOLAOU*¹, G. BELOK¹, A. C. PAULK¹, C. V. IRWIN³, J. W. LEE⁴, Z. WILLIAMS², S. S. CASH¹;


**Abstract:** The simple act of conversation involves a rapid sequence of complex behaviors that must be modulated in response to a rapidly changing context. An understanding of how different networks are recruited when the brain shifts between the necessary cognitive states would provide crucial insight into the systems that govern communication, how they are disrupted by neuropsychiatric disease, and how such disruptions might be restored by therapeutic intervention. To examine such processes under natural conditions, we analyzed intracranial brain activity from epileptic patients (implanted with cortical and subcortical electrode arrays for clinical purposes), recorded during loosely structured conversations (45-120 min) with the investigator. Spoken dialog was captured by an audio recorder (H4n, Zoom) and transcribed (in-house software; CoreNLP, Stanford) to yield word lexical categories and their timing within the intracranial recordings. Here we present data from N=4 participants, whose brain activity was collected using a 128-256 channel neural signal processor recording system (Blackrock Microsystems). Recordings were indexed by time windows centered on the verbal onset of either (1) speaking events (participant speech), or (2) listening events (non-participant speech). Intracranial data were analyzed in MATLAB (MathWorks) with the NPMK (Blackrock Microsystems) and FieldTrip (Donders Institute) toolboxes. We used the Similarity Networks (SIMNETS) framework to identify candidates for functionally related recording channel groups, based on the difference in coherence (70-115 Hz) patterns between pairs of time windows (0.5 s duration, before and after speaking and listening events). We observed a widespread increase in alpha-beta band (8-20 Hz) power (relative to baseline), together with a simultaneous decrease in
gamma band (30-120 Hz) power, prior to verbal onset of participant speech. Conversely, alpha-beta power fell when the participant was listening to speech, while gamma power increased after the onset of investigator speech. For certain channel clusters identified by SIMNETS, we were able to characterize neural trajectories associated with shifts in state from receptive speech processing to the articulation of internally generated responses. We speculate that other intermediate states may be invoked during the act of conversation. Our results indicate the involvement of distributed cortical networks in the volitional shift from a receptive, listening state to a productive state.


Poster

339. Language Acquisition and Coding

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 339.21/CC50

Topic: H.02. Human Cognition and Behavior

Support: NINDS R01-NS088606
NINDS R01-NS091139

Title: Statistical testing for event related causality (ERC) among human brain networks using multivariate autoregressive modeling at short time scales

Authors: *A. KORZENIEWSKA¹, P. J. FRANASZCZUK², N. E. CRONE³;
¹Dept. of Neurol., Johns Hopkins Univ., Baltimore, MD; ²Human Res. and Engin. Directorate, US CCDC Army Res. Lab., Aberdeen Proving Ground, MD; ³Neurol., Johns Hopkins Hosp., Baltimore, MD

Abstract: Causal interactions among brain networks in normal or pathological brain states typically occur on very brief time scales. To analyze event-related causality (ERC) in such transient systems, we have used a short-window algorithm for estimating the coefficients of a multivariate autoregressive model (MVAR, Ding et al., 2000) of non-stationary ECoG signals. This method requires multiple repetitions of a given brain process (e.g. testing trials or seizures) to obtain one estimate of an MVAR model, followed by one estimate of the short-time direct directed transfer function (SdDTF, Korzeniewska et al., 2008), representing the directions, intensities, and spectral contents of the direct causal interactions among brain networks, within the time-frequency plane. To test the significance of changes in these interactions in the post-stimulus epochs relative to interactions during baseline epochs (across trials of a stimulus-response-structured cognitive task), we previously fitted the SdDTF time-frequency plane with 2D penalized thin-plate splines constrained by a mesh of knots (Ruppert et al., 2003). This
statistical approach was computationally inefficient, and the required spline interpolation sometimes failed to converge, making it impractical for use in time-sensitive clinical and research applications. Moreover, the mesh of knots introduced artifactual “waves” in the results. Here we report a new approach to statistical testing that overcomes these limitations. In this approach, we construct a joint 95% confidence interval using bivariate smoothing with a rectangular kernel (BSRK). Thus, the variance of the smoothing reflects the variability of SdDTF values, rather than reinforced perfect fits at knots, with poorer goodness of fitting between them. Besides, BSRK is free of the problem of convergence of spline interpolation, and can be applied to other methods of time-frequency analyses. Furthermore, BSRK allows for analyzing much longer time series (e.g. epileptic seizures), because it does not need to hold multiple thin-plate splines in computer memory. The method is also many times faster than the previous method. Comparisons between the results of BSRK and spline-based statistics show that they produce very similar patterns of task-related effective connectivity. However, event-related causality estimates based on bivariate smoothing with rectangular kernels do not suffer from the same artifacts as the old method. Moreover, due to its time and memory efficiency, bivariate smoothing with rectangular kernel is uniquely suitable to both research and clinical applications, e.g. planning epilepsy surgery.

**Disclosures:** A. Korzeniewska: None. P.J. Franaszczuk: None. N.E. Crone: None.

**Poster**

**339. Language Acquisition and Coding**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 339.22/CC51

**Topic:** H.02. Human Cognition and Behavior

**Title:** EEG correlates of the verbal transformation effect

**Authors:** *M. K. BRUCKER*<sup>1</sup>, N. CASTRO<sup>2</sup>, J. S. BRUMBERG<sup>3</sup>, D. E. THOMPSON<sup>1</sup>;<br> <sup>1</sup>Electrical and Computer Engin., Kansas State Univ., Manhattan, KS; <sup>2</sup>Univ. of Washington, Seattle, WA; <sup>3</sup>Speech-Language-Hearing, Univ. of Kansas, Lawrence, KS

**Abstract:** The verbal transformation effect (VTE) is experienced when an audio recording of a word or phrase is played repeatedly for a listener. A VTE is when the listener perceives the original word as a word or non-word that may be either similar sounding or phonetically different. This study reports the initial investigation of the detection of VTEs with electroencephalography (EEG) by first considering actual changes in the auditory stimulus to help uncover the neural processing of this complex auditory verbal illusion. EEG data were acquired from 21 subjects listening to six different audio stimuli, who were instructed to press a button any time they heard a change in the presented stimulus. The task was split evenly between trials that included an actual, objective change in the auditory stimuli, and
those that never objectively changed. Here, we consider transformations where the audio objectively changed between one of the three pairs of stimuli at a rate likely similar to that of illusory verbal transformations. The three pairs (numbered 4/7, 5/8, and 6/9) were presented in switching blocks and were analyzed separately. Stepwise linear discriminant analysis (SWLDA) with 10-fold cross validation was used to classify target and non-target transformation events based on the known transformation times. The 95% confidence bounds for balanced accuracy were then compared to chance to determine statistical significance. The data files of subject 4 and subject 16 were excluded due to recording errors.

Of the 19 tested blocks, the majority were detected significantly above chance level. For pairs 4/7, 5/8, and 6/9, the number of significant results was 14, 13, and 14, respectively. Thus, 41 out of 57 stimulus blocks (71.9%) across 19 subjects presented statistically significant classification accuracies of actual switches between stimuli.

The results of our study support a detectable event-related potential (ERP) associated with switching auditory stimuli in which the switch is presented at a rate similar to expected illusory perceptions due to the VTE. However, the classification accuracies are not ideal and may not be sensitive enough for detection of illusory changes during the VTE. Future work will investigate which ERP components and electrode montages contribute to the correct classification of the VTEs and whether other classification techniques can be used to improve the accuracies. The methods used will then be extended to the detection of the endogenous, illusory VTE changes.


Poster

339. Language Acquisition and Coding

Location: Hall A

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Program #: Poster #: 339.23/CC52

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01-DC004290
NIH Grant UL1-RR024979

Title: The effect of lexical status on acoustic encoding in human auditory and perisylvian language cortices: Preliminary results from intracranial recordings

Authors: *M. E. SARRETT¹, K. SCHREIBER¹, H. KAWASAKI², M. A. HOWARD, III², B. MCMURRAY¹;
¹Psychological and Brain Sci., Univ. of Iowa, Iowa City, IA; ²Neurosurg., Univ. of Iowa Hosp. and Clinics, Iowa City, IA
Abstract: Speech perception is challenging. Fine-grained acoustic cues must be distinguished quickly to categorize the signal into phonemes. Psycholinguistics suggests listeners maintain fine-grained acoustic detail for longer than the fleeting moment it appears in the auditory signal (Connine, Blasko, & Hall, 1991; McMurray, Tanenhaus, & Aslin, 2008)—subsequent information can bias how listeners categorize ambiguous speech sounds. Further, listeners can use later top-down information about whether a stimulus makes a word to alter categorization (Ganong, 1980). For example, an ambiguous sound between /d/ and /t/ is perceived as a /d/ in the context of “dash” but a /t/ in the context of “task”. This requires listeners to maintain fine-grained detail throughout the word or later, and reinterpret this information using lexical status. Studies using fast optical imaging, MEG, and EEG suggest listeners maintain such detail for at least 200 msec (Toscano et al, 2018; Gwilliams et al, 2018; Sarrett et al, in prep). However, it is unclear exactly what brain regions are involved, and whether feedback from higher level areas alters extended auditory representations. We used electrocorticography (ECoG) to address these questions using a variant of Ganong (1980). Participants heard 15 minimal pairs manipulated along a continuum from /b/ to /p/ that differed by which endpoint formed a word. For example, “beach” and “peach” are equi-biased; “boke” and “pake” are p-biased (“boke” is not a word); and “bake” and “pake” are b-biased. Participants were 4 neurosurgical patients with medically intractable epilepsy undergoing chronic monitoring. Participants heard each token and categorized its initial sound. We used multivariate pattern analysis (support vector regression) to recover the cue continuum from local field potentials and high gamma activity (Nourski et al, 2015) in auditory and perisylvian language cortices. Preliminary analyses indicate that fine-grained acoustic cues can be recovered even from high level language processing hubs at least as 300 msec, and that there is an early time window when acoustic cues are processed independent of top-down factors. Analyses in later time windows suggest that lexical status feeds back even to the earliest levels of cortical processing.


Poster

339. Language Acquisition and Coding

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 339.24/CC53

Topic: H.02. Human Cognition and Behavior

Title: Using multivariate pattern analysis on ECoG to characterize neural language pathways

Authors: *A. CURTIS*1,2, O. WOOLNOUGH2, K. FORSETH2, P. ROLLO2, N. TANDON2,3; 1Rice Univ., Houston, TX; 2Univ. of Texas Hlth. Sci. Ctr., Houston, TX; 3Mem. Hermann Hospital, Texas Med. Ctr., Houston, TX
Abstract: The dual route model theory of reading originates from data on patients who, due to brain lesions, rely exclusively on one of two routes - a phonological route for grapheme-to-phoneme conversion or a lexico-semantic route for direct lexical access. However, in healthy subjects both routes are engaged simultaneously - implying that a network level representation rather than focal neural activation is more representative of overall processing. To evaluate the validity of the dual route model, we analyzed high spatiotemporal resolution of intracranial recordings with a logistic regression model to evaluate the phonological and lexical streams of reading and to qualify their dynamics. 35 intractable epilepsy patients implanted with depth or subdural grid electrodes were asked to read aloud written stimuli comprising of (i) regular words, (ii) exception words (orthographically irregular), (iii) pseudo-homophones (orthographically novel but phonologically familiar) and (iv) pseudowords (orthographically and phonologically novel). A neural decoding model using logistic regression tracked the spatial distribution of task-relevant information over time. Features including filtered time courses and pairwise connectivity were used as inputs into the model, to maximize stimulus classification accuracy. We found high classification accuracy between words and pseudowords in the first 250 ms following stimulus onset. This decoding accuracy was primarily driven by recordings in the anterior superior temporal gyrus and mid-fusiform cortex. Additionally, around the time of articulation, we saw higher word vs. pseudoword accuracy (both pre- and post-articulation). Decoding performance at these time windows was mainly influenced by the inferior frontal gyrus and partly indexed by a large differential in gamma power between words and pseudowords in this region. This contrasting neural response for words vs. pseudowords lends credence to the dual route theory, which proposes that these two stimulus classes utilize separate mechanisms for word identification and verbalization. Temporal neural decoding enables the tracking of task-relevant information without imposing prior anatomical or functional assumptions, yielding insight into which cortical regions and nonlinear dynamical features are useful for categorical distinctions.

Disclosures: A. Curtis: A. Employment/Salary (full or part-time) ;; Rice University, University of Texas Health Science Center at Houston, Memorial Hermann Hospital, Texas Medical Center. O. Woolnough: A. Employment/Salary (full or part-time) ;; University of Texas Health Science Center at Houston. K. Forseth: A. Employment/Salary (full or part-time) ;; University of Texas Health Science Center. P. Rollo: A. Employment/Salary (full or part-time) ;; University of Texas Health Science Center at Houston. N. Tandon: A. Employment/Salary (full or part-time) ;; Memorial Hermann Hospital, Texas Medical Center.

Poster

339. Language Acquisition and Coding

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 339.25/CC54

Topic: H.02. Human Cognition and Behavior
Title: Improving language encoding for fMRI with transformers

Authors: *S. JAIN*, A. LEBEL, A. G. HUTH;  
 1Computer Sci., 2The Univ. of Texas at Austin, Austin, TX

Abstract: Compositionality is the effect of combining information across a sequence of words to extract meaning. It is crucial for language understanding as it goes beyond individual words and examines their meaning in context. Recent work has shown that language models (LM) capture this effect by combining semantic information across words in a sequence to learn contextual representations that can effectively predict the next word. Representations extracted from a Long Short-term Memory (LSTM) LM may then be used to understand how the brain handles compositionality. This is done by building encoding models that use these contextual representations as features for language stimuli to predict the brain responses they elicit. The transformer has recently emerged as a highly successful alternative to LSTMs for language modeling. The crux of this model lies in its multi-head attention mechanism that selectively attends to and combines different information across words without recurrence. This allows transformers to model global dependencies across much longer sequences, possibly learning richer contextual representations. In this work, we extended previous approaches to further understand compositionality in the brain by using a 12-layer transformer LM for language encoding. Different representational spaces were extracted by conditioning on the layer, stage of processing within each layer, and amount of context used. To test this model, we used data from an fMRI experiment wherein 3 subjects (1 female) listened to 13 hours of natural language stimuli. Each feature space was used to build a separate encoding model through ridge regression. Model performance was assessed by testing how well each encoding model could predict responses in a held-out dataset. Our findings indicate that the transformer significantly outperforms earlier models for all subjects by a very large margin. Unlike LSTMs, performance steadily improves with the amount of context, corroborating the claim that transformers can capture compositionality over much longer sequences. Across the 12 layers, middle layers best capture high-level semantic areas in the brain while earlier and later layers best predict lower-level areas. We find that attention heads in each layer behave very differently and seem to encode diverse information, predicting distinct parts of the cortex. These results suggest that the transformer can effectively capture diverse semantics and varying levels of compositionality, allowing it to successfully predict responses across much of the cortex and providing deeper insight into how the brain uses compositionality for language understanding.

Disclosures: S. Jain: None. A. LeBel: None. A.G. Huth: None.
Decoding of semantic meaning at single cell resolution during human language perception

**Authors:** *J. CAI*¹, B. L. GRANNAN¹, M. JAMALI¹, E. FEDORENKO¹², Z. WILLIAMS¹³;¹Massachusetts Gen. Hosp. - Harvard Med. Sch., Boston, MA; ²McGovern Inst. for Brain Res., MIT, Cambridge, MA; ³Harvard-MIT Div. of Hlth. Sci. and Technol., Boston, MA

**Abstract:** How many neurons do we need to decode the semantic meaning of a word in human language? Current work demonstrates that individual neurons in the left dominant prefrontal cortex of humans respond to the semantic meanings of words. Here, we perform predictive decoding analysis on experimental data to study how prefrontal cortical neurons represent the semantic meaning of individual words when provided in the context of sentences. Using micro-electrodes, we recorded 220 neurons from 11 participants when they listened to audio recordings containing both 8-word sentences and 8-word lists. All words were clustered into 9 main semantic domains using a dimensionality reduction approach. Population decoding was performed with a multinominal logistic regression of the population firing rate in order to predict the semantic domain of each word. The control analysis involved shuffling the labels of the word categories and repeating the decoding analysis. Our results show that decoding accuracy reached 30% when predicting the semantic domains in sentences, compared to 11% accuracy in the control. Interestingly, decoding performance dropped to chance when listening to the same words but in the context of random word-lists. Finally, Bayesian information criterion (BIC) analysis showed that a random selection of 28 neurons from the population resulted in the optimized decoding models. In sum, our work demonstrates that prefrontal neurons encode semantic content within the human language network and that these neuronal responses can be used to decode the semantic meaning of words over the course of individual sentences.

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*J. Cai:* None.  
*B.L. Grannan:* None.  
*M. Jamali:* None.  
*E. Fedorenko:* None.  
*Z. Williams:* None.
Title: High-density intracranial recordings reveal evidence of speech tracking in dorsal premotor cortex

Authors: *J. BEREZUTSKAYA, C. BARATIN, Z. V. FREUDENBURG, N. F. RAMSEY; Brain Ctr. Rudolf Magnus, Univ. of Utrecht, Utrecht, Netherlands

Abstract: A lot remains unknown about how the human brain processes continuous perceived speech. Previous reports have shown that apart from the core network of regions (transverse, superior temporal and inferior frontal gyri), a number of other brain areas support speech perception. In particular, the role of premotor cortex (PMC) remains debated. Recent work has focused on the ventral portion of PMC and its role in encoding various properties of speech. At the same time, the dorsal portion of PMC is becoming more and more of interest as its involvement appears inconsistent across studies and recording modalities. Thus its specific role in speech processing remains to be further clarified. In this study we investigated the involvement of PMC in continuous speech perception using high-density (HD) intracranial electrode recordings. Two participants implanted with HD grids watched a full-length feature film. We then analyzed their brain responses (high frequency band) in PMC in relation to the speech fragments of the film. First, in each subject we observed that a set of electrodes in dorsal PMC exhibited significantly higher responses to speech compared to non-speech fragments, such as music, noises, animal cries (t-stat.>4, p<.001, cor.). Then we correlated the responses of these electrodes to the speech envelope and found significant amount of speech tracking (Spearman’s rho>=.3). Dorsal PMC electrodes also showed significantly higher correlation to electrodes in the auditory cortex during speech compared to non-speech fragments (t-stat.>3, p<.01, cor.). Finally, dorsal PMC electrodes seemed to filter out the noise from the speech envelope (as they exhibited a better correlation to isolated speech track than same fragments of speech in mixed track: t-stat.>3, p<.01, cor.) and focused on tracking of the phrasal structure of speech (following of the ON/OFF speech pattern: max. F₁=1492, p<.001, max. F₂=733, p<.001). Interestingly, these effects were strong in participants with HD grids, but lessened substantially in participants with standard density electrode grids, suggesting that HD electrode recordings can provide a finer detail of information about the underlying brain function. Overall, our results suggest that dorsal PMC is largely involved in tracking of perceived speech by filtering out the noise from the
speech stream and following the phrasal grouping patterns in speech. In addition, we report that HD intracranial recordings may uncover a finer level of detail in the acquired brain signal which can be crucial for elucidating the role of cortical regions supporting complex cognitive function, such as continuous speech perception.

**Disclosures:**  J. Berezutskaya: None. C. Baratin: None. Z.V. Freudenburg: None. N.F. Ramsey: None.

**Poster**

**339. Language Acquisition and Coding**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 339.28/CC57

**Topic:** H.02. Human Cognition and Behavior

**Support:**  NINDS Grant NS091390  
NIMH Grant MH112846  
Neurosurgery Educational Research Fund

**Title:** Studying perceived language and semantic meaning in the prefrontal cortex at a single-cell resolution

**Authors:** *B. L. GRANNAN*¹, M. JAMALI², J. CAI², E. FEDORENKO⁴, Z. WILLIAMS⁵;  

**Abstract:** Humans can extract the meaning of language over remarkably fine temporal scales and myriad contexts. Functional imaging studies suggest that this human ability is subserved by a widely distributed frontotemporal and subcortical network of brain areas. The degree to which semantic meaning is represented by single neurons, however, remains unknown. Here we test the hypothesis that neurons in the dominant, associative prefrontal areas, which are surgically accessible during intraoperative neuronal recordings, are involved in the rapid computation of semantic- and sentence-level content during language perception. We performed acute recordings of left dorsal prefrontal neurons in 10 right-handed participants as they listened to naturalistic sentences that varied in content and theme. Using a dimensionality reduction approach, we tested for single-neuron responses that were selective for certain semantic domains by comparing firing rates in response to words from one domain with responses to all other words. In total we recorded from 220 prefrontal neurons, of which 47 (21.3%) were selective for at least one semantic domain (p<0.025 after false-discovery rate correction for multiple domains tested). The majority of these neurons (N=31, 66.0%) were selective for only one domain, and no neurons were selective for more than three. These neuronal response patterns, however, were not
static but rather reflected variations in the intended meaning of words based on the sentence context in which they were heard. When participants listed to non-ordered random word lists, only 5.5% of neurons demonstrated selective activity. Moreover, most neurons reflected the intended meaning of word homophones that were phonetically identical but belonged to different semantic domains. In conclusion, dominant prefrontal neurons demonstrate firing rate activity that is selective for and representative of the semantic content within speech. This is the first study to assess the role of prefrontal neurons during language comprehension and supports the conclusion that dominant frontal neurons are involved in the rapid, context-dependent extraction of intended meaning during language perception.


Poster

340. Software Tools: Analysis I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: 340.01/CC58

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

Title: Unsupervised spike clustering via ensemble of autoencoders

Authors: *J. EOM1, S. KIM1, H. JANG1, H. SHIN1, Y. HUH2, D. HWANG1;
1Yonsei Univ., Seoul, Korea, Republic of; 2Catholic Kwandong Univ., Incheon, Korea, Republic of

Abstract: Spike sorting refers to the technique of detecting the signals (spikes) generated by neuronal activities and assigning the spikes to the neurons they belong to. Although many spike sorting algorithms have been developed to help analyze the recorded signals, manual spike sorting has been preferred by researchers since it provided the most precise performance. Therefore, in order to process a large amount of cellular recording data, spike sorting took an enormous amount of time. The number of neurons (i.e., clusters) has to be set because this is the most critical step required to accurately sort the spikes. Previous attempts tried to estimate the number of neurons by extracting principal features from dimensionality reduction such as diffusion map and combining these features with clustering algorithms. In this study, we propose a deep learning method to find the number of neurons based on unsupervised feature extraction using an ensemble of autoencoders (AE), which is being widely reported to produce superior performance in terms of dimensionality reduction and feature extraction compared to conventional methods. After denoising the acquired signals, additional pre-processing such as sampling the signal at various intervals or calculating the gradient between sampled points have been tested to extract the optimal form of input data. As a result, using the gradients between every 3-point interval has shown the best performance in deciding the optimal number of
neurons when used as AE input. It also reduced the memory used for training and testing the
network compared to using entire signal. The proposed network architecture is an ensemble
model of 3 different AE. Each AE consists of 1 to 3 hidden layers. The deeper the AE, the
compressed feature contains higher-level information. The proposed ensemble network utilizes
the combination of the compressed features from 3 different models to have a more expanded
hidden representation of the input signal. After that, these vectors are clustered using K-means
clustering algorithm. The results of clustering are evaluated by Silhouette method to decide the
optimal number of neurons. K with the highest Silhouette score becomes the optimal number of
neurons. Because the Silhouette score exhibits a slight fluctuation, we executed 20 times of
clustering and averaged the Silhouette scores. The performance of the proposed method to find
the optimal number of neurons with the gradients at every 3-point interval shows over 90%
accuracy. It is comparable or slightly higher than the conventional methods, with fewer features
required (Proposed: 9, Diffusion map: 10, Wavelet transformation: 15).

Hwang: None.

Poster

340. Software Tools: Analysis I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 340.02/CC59

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

Support: NIH Grant R01MH112746

Title: PsychRNN: An open-source Python package for training artificial recurrent neural
networks on cognitive tasks

Authors: *J. T. STONE¹, D. B. EHRLICH², D. BRANDFONBRENER⁴, A. ATANASOV⁵, J.
D. MURRAY³;
¹Computer Sci., ²Neurosci., ³Psychiatry, Yale Univ., New Haven, CT; ⁴Computer Sci., New
York Univ., New York, NY; ⁵Theoretical Physics, Harvard Univ., Cambridge, MA

Abstract: Modeling using recurrent neural networks (RNNs) is quickly becoming a popular
strategy to probe the mechanisms behind cognitive tasks. RNNs are able to accomplish many
popular cognitive tasks but are vastly more manipulable and observable than classic model
organisms such as monkeys and humans. Modeling is a fast, inexpensive way to investigate the
abilities and limitations of proposed mechanisms behind cognitive tasks, and can lead to deeper
understanding of current proposed mechanisms and new mechanistic hypotheses. These insights
can in turn guide experimental focus to better distinguish between current proposals.
Here we introduce an accessible and extensible Python package for training RNNs on a variety
of cognitive tasks. Our package provides an accessible framework, requiring only knowledge of Python and NumPy, for easily defining new cognitive tasks and customizing tasks included with PsychRNN. The deep learning details are abstracted away so that researchers do not need machine learning or TensorFlow (TF) knowledge to get started modeling RNNs. PsychRNN includes multiple initializations, loss functions and regularizations as well as a framework to easily add more. For projects that require additional customization and researchers who have TF knowledge, the TF-based backend is easily extensible.

Our package focuses on facilitating specification of neurobiological constraints on synaptic architecture and on learning. We include optional implementations of Dale’s principle, neurobiologically plausible sparse connectivity, regional connectivity differences and individual unit time-constants. PsychRNN additionally can return intermediate network solutions so researchers can investigate how network solutions develop over the course of training.

The design of PsychRNN further enables novel investigations. Task modularity makes it easy to investigate how parametric variations in task demands determine network solutions. This modularity means that with only one task definition, it is easy to vary many different task parameters iteratively. This ease of use enables more investigation and experimentation for less researcher time.

PsychRNN also allows researchers to implement task shaping, where training is adjusted in closed loop based on performance. Experimentalists regularly train animals to perform tasks by shaping tasks—PsychRNN allows investigation of how shaping trajectory choice could affect the observed results. This could lead to discovering faster ways of training animals or possible confounds that may result from task shaping in animal training.

PsychRNN is available at https://github.com/dbehrlich/PsychRNN.

**Disclosures:**  
**J.T. Stone:** None.  
**D.B. Ehrlich:** None.  
**D. Brandfonbrener:** None.  
**A. Atanasov:** None.  
**J.D. Murray:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BlackThorn Therapeutics. F. Consulting Fees (e.g., advisory boards); BlackThorn Therapeutics.

**Poster**

**340. Software Tools: Analysis I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 340.03/CC60

**Topic:** I.06. Computation/ Modeling/ and Simulation

**Support:** NIH DP2 MH119735  
NIH R00 MH104605

**Title:** The topology of time: Characterizing transitions in simulated neural dynamics using topological data analysis
Abstract: A key challenge in understanding orders and disorders of brain dynamics is finding mechanistic representations of observed dynamical patterns for systematic comparisons. Such representations should not only detect phase transitions in the underlying dynamical system but also detect changes in the dynamical landscape. Here, we aim to develop a topological representation of dynamics that recovers a priori known transitions in simulated neural dynamics. More specifically, neural dynamics and corresponding fMRI blood-oxygen-level dependent (BOLD) signals were simulated using a dynamic mean field model (Deco et al, 2013) constrained by biologically realistic parameters. Phase transitions were induced by varying the level of global coupling between brain areas over time. Subsequently, we constructed topological representations of simulated BOLD dynamics, building on an existing method of topological data analysis (Mapper; Saggar et al. 2018). We found that recurrence plots defined on the topological representation recovered phase transitions and shifts in the underlying dynamical landscape induced by varying global coupling. Furthermore, this topological method was compared to its relatively traditional counterparts - recurrence plots generated directly from BOLD signals and that generated from dynamic functional connectivity. Recurrence of BOLD signals identified phase transitions but was insensitive to the change in the global coupling leading to such transitions. Recurrence of dynamic functional connectivity reflected whether, but not how, the global coupling was changing. In contrast, recurrence in the topological representation provided rich information on both the transitions and the underlying parameter changes. This work takes important steps toward a theoretically validated topological representation of neural dynamics, which not only detects sudden changes in the dynamic organization of the brain but also anticipates such changes in the gradual shifting of the dynamic landscape. It bears potential as a dynamic-based tool for diagnostics and early prevention of neural disorders.

Disclosures: M. Zhang: None. M. Saggar: None.
Authors: *M. G. MOORE*¹, C. YUN³, J. KARIMI ABADCHI⁴, M. YAN², M. H. MOHAJERANI⁵, M. REIMERS¹;
¹IQ Inst. and Neurosci. Program, ²Dept. of Computat. Mathematics Sci. and Engin. and Dept. of Mathematics, Michigan State Univ., East Lansing, MI; ³Dept. of economics and Dept. of information sciences, Cornell Univ., Ithaca, NY; ⁴CCBN/University of Lethbridge, Lethbridge, AB, Canada; ⁵Dept. of Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: Wide-field optical mapping (WFOM) methods can resolve neurodynamics on fast time-scales over the entire surface of a rodent cortex. The Allen Mouse Brain Connectivity Atlas (AMBCA) gives detailed axonal projections across cortex. We investigate whether connections inferred from dynamic network models applied to WFOM data resemble the axonal projections reported in the AMBCA. We model the cortex as a dynamic network whose nodes correspond to excitation averaged over a pixel. Local excitation-inhibition dynamics are modeled as an auto-regressive AR(p) process, and long-range excitatory axonal connections are modeled as feed-forward connections between pixels with lags estimated from distance and axon conduction speed. Using simulated data, we show that connectivity can be inferred accurately via Least-squares parameter estimation, and with significantly increased efficiency when Tikhonov regularization and other optimization methods are applied. Furthermore, we find that inferring connections based on correlation can fail when inputs are coherent across many pixels, e.g. inputs from thalamus, leading to false-positives. We show that simultaneous estimation of connectivity and coherent inputs can resolve this issue. We also show that such models can exhibit long-range synchronization even when the nodes are driven by noise, and that the output in the synchronized regime is dominated by a low-rank signal with a consistent spatial structure, and a temporal component given by a convolution of the coherent input, when such inputs are present. We compare simulations based on the AMBCA connections to observed dynamics, and attempt to estimate connections in existing WFOM data.


Poster

340. Software Tools: Analysis I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 340.05/CC62

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

Title: Context-aware *in-silico* synthesis of protoplasmic astrocyte morphology

Authors: *E. ZISIS, L. KANARI, S. DAGAR, D. KELLER, H. MARKRAM*; EPFL, Blue Brain Project, Geneva, Switzerland
Abstract: The astrocytic morphology gives rise to functional properties that are important for the brain circuitry. Thus, neurons are no longer considered as separate functional units, but rather as a puzzle piece in the greater functional ensemble of the neuronal-glial-vascular circuitry. Astrocytic cells in the brain form anatomically exclusive territories that minimally overlap with their neighbors. In these microdomains each cell establishes the connectivity with the vasculature and the neuronal synapses. The astrocytic morphology is dominated by contextual elements of its environment: the proximity to its neighbors, the vasculature and the distribution of the neuronal synapses within its domain. Thus, in order to build a generative model of protoplasmic astrocytes we need both the topological information of their structure and the geometrical constraints that govern their environment and bias their morphological characteristics.

We present a novel method that combines the topology synthesis of branching structures using as input topological barcodes (Kanari et al., 2018) with contextual cues, such as the bounding geometry and the attraction to the synaptic point cloud. Starting with a vasculature dataset, we first distribute astrocytic somata positions satisfying both their spatial densities and their nearest neighbor distributions. We then generate a Laguerre tessellation from the somata positions and radii, which reflects the bounding microdomain of the astrocytes. The microdomain hull is consequently used to establish the connectivity with the vasculature and the neuronal synapses in the neuronal circuit. The interplay of topology and spatially embedded information fuels the synthesis algorithm for protoplasmic astrocytes. We synthesized realistic astrocytic morphologies embedded in space and validated both single cell’s morphology and collective circuit wide properties against biologically reconstructed astrocytic morphologies and literature data. The synthesis pipeline lays the groundwork for functional models of energy provision to the neuronal microcircuit in which endfeet reconstruction and surface area can be used for compartmental reaction-diffusion simulations. Furthermore, the indirect communication of neuronal synapses through the astrocytic syncytium could provide us with insights on the plasticity dynamics in the NGV ensemble. Finally, calcium induced intra-cellular and inter-cellular wave simulations require specification of the geometry of domains, morphologies and the degree of astrocytic overlap. Our workflow ensures the reconstruction of the anatomical architecture that renders possible all the above.


Poster

340. Software Tools: Analysis I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 340.06/CC63

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

Title: Computational synthesis of dendritic morphologies within the rodent neocortex
Abstract: A neuron’s morphology determines its functional properties and its connectivity, both of which influence the dynamical properties of neuronal networks. It is therefore essential for the accurate digital reconstruction of detailed brain networks to replicate the anatomical properties of neuronal morphologies. In particular, the digital reconstruction of a large-scale, physiologically realistic network, such as the Blue Brain Project’s cortical microcircuit published in (Markram et al. 2015), requires large numbers of detailed neuronal morphologies, which are difficult to acquire experimentally, due to limitations of the reconstruction techniques. To populate such large-scale networks with unique neuronal morphologies, the computational generation (i.e., synthesis) of detailed morphologies that respect the branching properties of the biological cells is therefore required.

The principles that determine how dendritic and axonal arbors take shape are still largely unknown. For this reason, there is a plethora of synthesis methods for the artificial generation of neuronal morphologies (Ascoli et al. 2001, Koene et al. 2009, Cuntz et al. 2010), each of which contributed to the comprehension of neuronal growth, though no single algorithm has managed to reproduce a wide variety of neuronal shapes without appropriate fine tuning of the input parameters. Here, we apply the topological descriptor of branching morphologies that was introduced in Kanari et al. 2018 to the computational synthesis of dendritic morphologies from all layers and morphological types of the rodent cortex. Each generated morphology was validated against biological dendrites, based on a set of morphological features not directly used as input.

The synthesis algorithm driven by the topological architecture of dendrites generated realistic morphologies for a large number of distinct cortical neuronal cell types. Our results demonstrate that the topology-based synthesis algorithm implicitly captures correlations of features within the dendritic shapes, without the need for manual identification of these dependencies. In addition, electrical simulation of the synthesized cells in Neuron generates traces that reproduce the electrical properties of biological reconstructions. The topology-driven synthesis thus reproduces the morpho-electrical properties of a broad range of different branching shapes. Since the topology-based synthesis is generalizable to a large number of different dendritic shapes, it is suitable for the generation of unique neuronal morphologies to populate the digital reconstruction of large-scale, physiologically realistic networks.

**Poster**

**340. Software Tools: Analysis I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 340.07/CC64

**Topic:** I.06. Computation/ Modeling/ and Simulation

**Support:** This work was supported by funding from the ETH Domain for the Blue Brain Project. The Blue Brain Project’s HPE SGI 8600 system, BlueBrain V, was funded by the ETH Board and hosted at the Swiss National Supercomputing Center (CSCS).

**Title:** Impact of higher-order network structure on emergent cortical activity

**Authors:** *M. NOLTE*¹, E. GAL², E. B. MULLER¹, H. MARKRAM¹, M. W. REIMANN¹;

¹Blue Brain Project, EPFL, Geneva, Switzerland; ²Edmond and Lily Safra Ctr. for Brain Sci., The Hebrew Univ., Jerusalem, Israel

**Abstract:** Synaptic connectivity between neocortical neurons is highly structured, including first-order structure, such as strengths of connections between different neuron types and distance-dependent connectivity, and higher-order structure, such as an abundance of cliques of all-to-all connected neurons and small-world topology. The relative impact of first- and higher-order structure on emergent cortical network activity is unknown. Here, we compared network topology and emergent activity in two neocortical microcircuit models with different null-models of synaptic connectivity, both with similar first-order structure, but with higher-order structure - arising from morphological diversity within neuronal types - removed in one model. We found that morphological diversity within neuronal types creates heterogeneous degree distributions with hub neurons, raises in-degrees at the bottom of layer six, contributes to the abundance of cliques, and increases small-world topology. The increase in complexity of the higher-order network structure was accompanied by more nuanced changes in neuronal firing patterns, including increased activity and response reliability at the bottom of layer six. Without this structure, the dependence of pairwise correlations on the positions of neurons in directed cliques was strongly reduced. Our study shows that circuit models with very similar first-order structure of synaptic connectivity can have a drastically different higher-order network topology, and that the higher-order topology imposed by morphological diversity within neuronal types has a clear impact on emergent activity.

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Title: Digital reconstruction and simulation of thalamic microcircuitry

Authors: *E. IAVARONE1, J. YI2, Y. SHI1,2, H. MARKRAM1,2, S. L. HILL3,1;
1EPFL, Blue Brain Project, Geneva, Switzerland; 2EPFL, LNMC, Lausanne, Switzerland;
3CAMH, Krembil Ctr. for Neuroinformatics, Toronto, ON, Canada

Abstract: The thalamocortical system consists of different thalamic nuclei and their reciprocal interactions with the neocortex. It is involved in numerous functions, for instance transmission of sensory information and transition between brains states, such as sleep and wakefulness. Computer simulations facilitate the integration and standardization of different sources of experimental data and help us understand the structural and functional complexity of neural circuits. This approach has been recently published in the context of the Blue Brain Project as a detailed reconstruction and simulation of a cortical microcircuit [1].

In this work, we followed and extended the pipeline developed in [1] to develop a digital reconstruction of a thalamic microcircuit, including the somatosensory thalamus and the corresponding region of the thalamic reticular nucleus, which provides the main source of inhibition to the thalamus. We performed targeted experiments to capture electrophysiological and morphological cellular data from the adult mouse. We used this data, combined with data from the literature and open access datasets [2], to automatically constrain the firing properties of single neuron models [3]. We defined the microcircuit geometry as in [1] and populated it with neuron densities derived from the Blue Brain Cell Atlas [4] and compared them with stereology studies. The 3D morphological reconstructions constituted the basis to derive the detailed microconnectome between neurons in somatosensory thalamus and in the reticular nucleus. We then validated the micro-connectome with sparse experimental and literature data, which characterized the number of afferent and efferent synaptic boutons and the synaptic physiology. We included afferent sensory projections from the medial lemniscus and corticothalamic feedback, in order to model extra-thalamic inputs to the microcircuit.

We identified in vitro experiments that could be used to simulate and validate network phenomena, such as thalamic oscillations known as sleep spindles. The experimental data, model artifacts and associated metadata are continuously integrated in Blue Brain Nexus [5], in order to make them easily re-usable and track their provenance. The thalamic microcircuit model...
provides foundations and principles that can be extended to model the whole thalamus, and ultimately the thalamocortical system.

2. https://www.janelia.org/project-team/mouselight/neuronbrowser
5. https://bluebrainnexus.io/


Poster

340. Software Tools: Analysis I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 340.09/CC66

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

Support:  EPFL Blue Brain Project (funded by the Swiss ETH board)
NIH grant number R01NS11613 (Yale University)
the European Union Seventh Framework Program (FP7/20072013) under grant agreement n° 604102 (HBP)
European Union’s Horizon 2020 Framework Programme for Research and Innovation under Grant Agreement n° 720270 (Human Brain Project SGA1)
European Union’s Horizon 2020 Framework Programme for Research and Innovation under Grant Agreement n° 785907 (Human Brain Project SGA2)

Title: Improving performance of cellular simulation through more effective DSL translation and optimized compute engine for CPUs and GPUs

Authors: *J. G. KING¹, P. S. KUMBHAR¹, M. L. HINES², O. AWILE¹, L. KEEGAN¹, T. CAREL¹, I. MAGKANARIS¹, F. SCHUERMANN¹;
¹Campus Biotech, EPFL - Blue Brain Project, Geneva, Switzerland; ²Neurobio., Yale Univ., New Haven, CT

Abstract: The Blue Brain Project (BBP) seeks to build biologically detailed models of neuronal tissue and perform simulated experiments to help advance the understanding of the brain. Recent work has seen the realization of a tissue reconstruction consisting of 9 million neocortical neurons with 80 billion synapses. Such volume of data is a challenge to simulate and to aid in this endeavor, we participate to advance software capabilities for scalability and performance. From its inception, BBP has used NEURON since it has a rich feature set developed over the last thirty years. In recent years, CoreNEURON has been under development as a new compute
engine for NEURON. CoreNEURON uses a better optimized memory layout which allows for simulations up to 7 times larger to run on the same compute infrastructure. The internal data structures are more easily adaptable to take advantage of modern computing architecture capabilities. During this work to advance CoreNEURON, we also investigated better methods for translation of the domain-specific languages (DSLs) as used to describe biological models simulated in NEURON such as ion channels and synapses. This new NMODL translation allows for qualitative analysis of the user source code and restructures the final organization for better optimization of the final generated code, yielding more efficient execution in the built application. NMODL is capable to implement multiple SIMD and SPMD targets applicable to modern hardware. Benchmarks were executed on Intel Skylake, Intel KNL, and AMD Naples platforms, with individual compute kernels showing a speedup of up to 20x. In production simulations, overall performance gains of ~10x were measured. Further NMODL yields a 2x speed up when compared to a previously published SIMD optimized version that heavily relied on auto-vectorization by the compiler.


Poster

340. Software Tools: Analysis I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 340.10/CC67

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

Support: This work was supported by funding from the ETH Domain for the Blue Brain Project. The Blue Brain Project’s HPE SGI 8600 system, BlueBrain V, was funded by the ETH Board and hosted at the Swiss National Supercomputing Center (CSCS).

Title: Data-driven integration of hippocampal CA1 synapse physiology in silico

Authors: *A. ECKER¹, A. ROMANI¹, S. SÁRAY², S. KÁLI², M. MIGLIORE³, A. MERCER⁴, H. MARKRAM¹, E. B. MULLER¹, S. RAMASWAMY¹;
¹EPFL, Blue Brain Project, Geneva, Switzerland; ²Inst. of Exptl. Medicine, Hungarian Acad. of Sci., Budapest, Hungary; ³Natl. Res. Council, Palermo, Italy; ⁴UCL Sch. of Pharm., London, United Kingdom

Abstract: The physiology of synaptic connections in rodent hippocampal CA1 has been the subject of intense experimental study in recent decades. Yet, the resulting knowledge remains disparate and difficult to reconcile. Here, we present a data-driven approach to integrate the current state-of-the-art knowledge on the synaptic anatomy and physiology of rodent
hippocampal CA1, including axo-dendritic innervation patterns, number of synapses per connection, peak conductances and short-term plasticity into a single coherent resource. First, we undertook an extensive literature review of paired-recordings of hippocampal neurons, and compiled experimental data on their synaptic anatomy and physiology. The data collected in this manner is sparse and inhomogeneous, due to the diversity of experimental techniques used by different labs, which necessitates the need for an integrative framework to unify these data. To this end, we extended a previously developed workflow for the neocortex to constrain a unifying \textit{in silico} reconstruction of the synaptic physiology of CA1 connections. Our work identifies gaps in the existing knowledge and provides a complementary resource towards a more complete quantification of synaptic anatomy and physiology in the rodent hippocampal CA1 region.


**Poster**

**340. Software Tools: Analysis I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:/Poster #:** 340.11/DP15/CC68

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
**Topic:** I.07. Data Analysis and Statistics

**Support:** NSF Grant no. DGE 1106400

**Title:** An automated tool for detecting dynamical chaos in the brain

**Authors:** *D. TOKER\textsuperscript{1}, F. T. SOMMER\textsuperscript{2}, M. DESPOSITO\textsuperscript{3};
\textsuperscript{1}Univ. of California, Berkeley, Berkeley, CA; \textsuperscript{2}Univ. California, Helen Wills Neurosci Inst., Helen Wills Neurosci. Inst., Berkeley, CA; \textsuperscript{3}Helen Wills Neurosci. Inst., Univ. of California Berkeley, Berkeley, CA

**Abstract:** Dynamical chaos - i.e., exponential sensitivity to small perturbations - is thought to play a key functional role in the brain. A method for detecting chaos from neural recordings should therefore be a key feature of the neuroscientist's toolkit. But, classic chaos-detection tools are highly sensitive to measurement noise, which has made it difficult to distinguish between stochastic, periodic, and chaotic neurobiological processes. To aid in the experimental study of chaotic neural dynamics, we integrate several new mathematical tools into a fully automated processing pipeline, which can accurately assess the presence and degree of chaos in a diverse range of measurements, biological or otherwise, even when those measurements are noisy, and even for difficult edge cases. Our tool is freely available online.

Poster

340. Software Tools: Analysis I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 340.12/CC69

Topic: I.07. Data Analysis and Statistics

Support:  Startup Funds from University of Missouri

Title: A low-cost, open-source control and timing system for training animals on behavioral tasks

Authors: H. B. SMITH, O. K. BOTONIS, *I. OZDEN;
Biomedical, Biol. and Chem. Engin., Univ. of Missouri, Columbia, MO

Abstract: We report a low-cost, open-source control system for training lab animals on behavioral tasks. Our system is based on custom electronic circuits integrated with an Arduino Due board. The system can be programmed with a Python-based graphical user interface to design a custom training paradigm with the capability of acquiring behavioral data from many (>10) sensors and delivering TTL control/timing pulses from many output ports (>10) simultaneously. The system also incorporates several timing circuits, two programmable function generators and two audio channels, which can be used during training or behavioral tasks. We demonstrate the utility of our system by presenting the design of a system for training mice to perform an odor-discrimination-based Go/No-Go task, which requires controlling a 4-odor olfactometer with 6 solenoid valves, obtaining behavioral timing data from 2 sensors, triggering a laser for optogenetic stimulation, providing water reward and delivering auditory cues as feedback. The design files and the software of our system are user-friendly, modular and open-source.

Disclosures:  I. Ozden: None.  H.B. Smith: None.  O.K. Botonis: None.
**Title:** Robust and generalizable tracking of body parts of head-fixed mice


**Abstract:** Movement is a critical aspect of behavior and can explain a substantial fraction of neural variability. It is therefore important to monitor movements in great detail, especially for behavioral data collected at diverse sites. The International Brain Laboratory (IBL) built 21 standardized behavioral training boxes in six institutions around the world in which mice are filmed using a single Point Grey Chameleon3 camera. Using standardized camera placement and parameters, highly similar videos of head-fixed mice performing a visual detection task are acquired. At full capacity, behavioral training in the IBL produces around 100 hours of video per day. To robustly track body parts in this large dataset of videos, we evaluated DeepLabCut; a toolbox for markerless tracking based on deep learning. We trained networks on a general dataset composed of videos of different mice in different training rigs. The networks generalize well and can be used to track body parts in any IBL behavioral video with average error between 1.2 mm (for paws) and 0.1 mm (for the pupil). We show that DeepLabCut can replace traditional methods used for eye tracking, lick and sniff detection and paw tracking with a single side view video. First, a coarse network is used to detect Regions of Interest (ROIs). Subsequently, an automated crop is made around these ROIs. Finally, specialized DeepLabCut networks are applied to the cropped videos. We trained specialized networks to track pupil movements, sniffing and licking, and paw movements. This pipeline is fully automatized, starting from video acquisition to saving time series of body parts of interest. Combining detailed tracking of body movements with physiological recordings will be a powerful tool for investigating the neural underpinnings of behavior.

Title: Pathfinder: Open source software for analyzing spatial navigation search strategies

Authors: *M. B. Cooke, T. P. O'Leary, P. K. Harris, J. S. Snyder; Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The Morris Water Maze is a widely used task developed to test spatial navigation in rodents. Rodents are trained to learn the location of a platform that offers escape from the pool. Classically, measures such as latency to the escape platform and total distance traversed have been used in order to score spatial learning on this task. However, these measures offer little insight into the underlying navigational strategies that the animals employ. Recently, a number of studies have begun to classify water maze search strategies in order to clarify the precise spatial and mnemonic functions of different brain regions, and to identify which aspects of spatial memory are disrupted in disease models. While search strategies can be intuitively defined relative to escape locations and maze geometry, they have not been widely adopted, presumably due to the lack of available software. In order to address this issue, we developed Pathfinder, an open source application for analyzing spatial navigation behaviour. Here we show that Pathfinder effectively identifies a variety of search strategies, that vary in their spatial specificity, as rodents learn the location of the escape platform. Our software supports inputs from commonly-used, commercially-available software packages (Ethovision, Anymaze, and Watermaze, with more compatibility to be added soon), is optimized for classifying search strategies (Direct Swim, Focal Search, Directed Search, Spatial Indirect, Chaining, Scanning, Random Search, Thigmotaxis), and can also be expanded easily to work with other species and spatial navigation tasks. Pathfinder has the ability to automatically determine the platform location as well as the size of the pool and related pool parameters. It can generate heatmaps of trials, analyze navigation with respect to multiple goal locations and, due to the open source nature of the project, can be easily updated to accommodate future developments in the study of spatial navigation.

Disclosures: M.B. Cooke: None. T.P. O'Leary: None. P.K. Harris: None. J.S. Snyder: None.
Behavioural analysis toolbox for mice engaged in string-pulling task

String pulling is a bimanual task in which rats and mice use hand-over-hand movements to pull a string to obtain food rewards. The task requires little training as the animals have a natural tendency to pull strings. Making videos of mice engaged in the string-pulling task and their offline analysis for identification and tagging of body parts in individual frames allows quantification of the topographical and kinematic parameters for the movements of head, body, and forehands which can then be used to assess fine motor functions. Currently, manual analysis of video data acts as a bottle neck limiting data collection. Here we present a Matlab® based toolbox with an easy to use graphical user interface to aid researchers in analyzing string-pulling video data. The user defines colors of body, ears, nose, hands, and string which are used to find respective masks using color-based image segmentation. Heuristics based algorithms are then used to identify and tag the body, ears, nose, and forehands. If automatic tagging fails, the user can manually tag any body part. Using the centroids and shapes of tagged body parts, the software estimates and plots their kinematic parameters such as body length and angle, head yaw, roll and pitch, and paths as well as speeds of both hands. The toolbox is open source and users can modify according to their specific needs.
IronClust > Drift-resistant, real-time spike sorting based on anatomical similarity for high channel-count silicon probes

Abstract: Extracellular electrophysiology can record the activity of a large neural population with single spike resolution. Spike sorting is then needed to resolve individual cellular activities by grouping together similar spike waveforms distributed at a subset of adjacent electrodes. Silicon probes are widely used to measure the spiking activities from behaving animals, but the probes can drift in the brain due to animal movements or tissue relaxation following a probe penetration. Probe drift often causes errors in conventional spike sorting methods that assume stationarity in spike waveforms and amplitudes. However, some of the latest silicon probes offer whole-shank coverage by electrodes of sufficient density that algorithms could potentially compensate for such drift along the probe axis. We introduce a drift-resistant spike sorting algorithm, IronClust, for high channel-count, high-density silicon probes, which accurately handles both gradual and rapid drift. We exploit the fact that a linearly drifting probe revisits anatomical locations at later times. We apply density-based clustering to temporal subsets of the spiking events when the probe occupied similar anatomical locations. This anatomical similarity between disjoint time segments is determined from activity histograms, which capture spatial structures in the spike amplitude distribution on each electrode, prior to clustering. For each spiking event, the clustering algorithm (DPCLUS) computes the distances to a subset of its neighbors selected by their peak channel locations, and by anatomical similarity. Based on the k-nearest neighbors, one then finds the density peaks based on the local density values, and the nearest distances to the higher-density neighbors, and recursively propagates the cluster memberships toward a decreasing density gradient. The accuracy of our algorithm was evaluated using validation datasets generated using a biophysically detailed neural network simulator (BioNet), which generated stationary, slow monotonic drift, sinusoidal, and fast random drift. IronClust achieved ~8% error on the stationary dataset, and ~10% error on the gradual or random drift datasets, which significantly outperformed existing algorithms. We also found that additional columns of electrodes improve the sorting accuracy in all cases. By exploiting GPU code, IronClust achieves over 11x real-time sorting speed for 60 channels, which is over 2x faster than other leading algorithms. In conclusion, we present an accurate and scalable spike
Sorting tool that is resistant to probe drift, by taking advantage of anatomically-aware clustering and parallel computing.

**Disclosures:**  
**J.J. Jun:** None. **J. Magland:** None. **C. Mitelut:** None. **A.H. Barnett:** None.

**Poster**

**340. Software Tools: Analysis I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 340.17/CC74

**Topic:** I.06. Computation/Modeling/and Simulation

**Support:** LBNL-internal LDRD “Neural Systems and Engineering Lab” (KEB)

**Title:** Enhancing parametric models of neuroscientific data involving diverse covariance structures with the Union of Intersections (UoI) framework

**Authors:** *A. KUMAR, K. E. BOUCHARD;*  
Biol. Systems and Engin., Lawrence Berkeley Lab. and UC Berkeley, Berkeley, CA

**Abstract:** A pervasive but under-appreciated aspect of neuroscientific data is the presence of strong correlations between predictive features. For example, when estimating functional connectivity, there are often strong correlations between different populations of neurons. In the context of parametric estimation (e.g., linear regression), correlated design hampers both feature selection and estimation. The widely used Lasso tends to select only one out of a group of highly correlated features. Other estimators such as the Elastic Net exhibit a grouping effect, wherein clusters of correlated features will tend to be assigned similar coefficient values. These fundamental issues are often not appreciated by experimentalists, and jeopardize conclusions drawn from estimated models. In this empirical study, we demonstrate that Union of Intersections (UoI), a statistical-machine learning framework we have recently developed to enhance feature selection and estimation, exhibits state of the art performance on generic, sparse, correlated regression problems. The superior selection and estimation accuracy of UoILasso and UoIElasticNet is demonstrated on synthetic datasets. We systematically vary the covariance matrix of predictors from a block diagonal structure to one with exponentially decaying off-diagonal elements. This approach contrasts with most methodological developments in this space, which address very specific covariance structures. Furthermore, we impose varying degrees of signal to noise, heterogeneity in the model coefficients, and model sparsity. Our numerical studies reveal a ‘fundamental’ tradeoff between false positive and false negative control. This tradeoff, modulated by the signal-to-noise ratio of the problem, bounds the selection accuracy attainable by known estimators. As the UoI framework aggressively controls for false positives through a form of stability selection, it is able to outperform competing algorithms in sparse settings. Moreover, UoILasso and UoIElasticNet do not exhibit grouping effects, and are able to recover
heterogenous coefficient estimates, in agreement with the generative model. We validate our findings by estimating functional connectivity from rfMRI, human ECoG (speech cortex), rodent mECoG (auditory cortex), and NHP multi-single unit recordings (M1 during reaching), showing that UoI-based methods recover sparser models with no loss of predictive accuracy. Taken together, these results demonstrate the potential of the UoI framework to enhance the scientific interpretability of sparse parametric models involving correlated variables that frequently arise in data-driven neuroscience.

Disclosures: A. Kumar: None. K.E. Bouchard: None.

Poster

340. Software Tools: Analysis I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 340.18/CC75

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

Support: LBNL-internal LDRD “Neural Systems and Engineering lab” (KEB)

Title: Unsupervised extraction of dynamical features from noisy data with dynamical components analysis

Authors: *D. G. CLARK*¹, J. A. LIVEZEY¹, K. E. BOUCHARD¹,²; ¹Lawrence Berkeley Natl. Lab., Berkeley, CA; ²UC Berkeley, Berkeley, CA

Abstract: Complex brain functions are produced by the dynamic coordination of many neurons. High-dimensional data from multi-neuronal recordings stand to elucidate these population-level dynamics. Linear dimensionality reduction methods provide a computationally-efficient way to extract relevant dimensions from such data, however widely used techniques which extract dimensions based on maximizing variance, e.g. Principal Components Analysis (PCA), are prone to extracting noise rather than dynamics. At the same time, quantifying the dynamical complexity of time series data is of interest in many domains, including neuroscience. Predictive information (PI), defined as the mutual information between the past and future, provides a robust quantification of time series complexity: PI is zero for purely random time series, saturates for purely regular time series, and can grow without bound for time series with rich dynamical structure. We present Dynamical Components Analysis (DCA), a linear dimensionality reduction method which discovers a subspace of high-dimensional time series data in which PI is maximized. Here, we describe DCA, demonstrate its superior ability to recover dynamics in synthetic data and evaluate its performance on neuroscience datasets. DCA's PI-based objective function can discriminate between noise (zero or small PI) and meaningful dynamics (large PI). Using synthetic examples, we contrast DCA with Slow Feature Analysis (SFA), which does not capture long-timescale dynamics, and Forecastable Components
Analysis, which maximizes temporal regularity rather than dynamical complexity. We apply DCA to neural datasets from motor cortex and hippocampus and demonstrate that the dimensions extracted by DCA allow for more accurate decoding of behavioral variables than those extracted by PCA and SFA, implying that DCA more robustly extracts underlying neural dynamics. Finally, we show that DCA can be used as a preprocessing step for popular latent variables methods such as GPFA and LFADS, dramatically reducing the runtime of these algorithms without changing their inferred structure. Overall, our results show that DCA is a powerful and computationally-efficient linear dimensionality reduction method for extracting dynamics from high-dimensional neural data.

**Disclosures:** D.G. Clark: None. J.A. Livezey: None. K.E. Bouchard: None.

**Poster**

**340. Software Tools: Analysis I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 340.19/CC76

**Topic:** I.07. Data Analysis and Statistics

**Title:** SpikeForest - A large-scale reproducible spike sorting validation platform with an interactive web interface

**Authors:** *J. MAGLAND*¹, J. JUN¹, E. LOVERO¹, A. J. MORLEY², A. BARNETT¹; 
¹Flatiron Inst., New York, NY; ²MRC BNDU, Univ. of Oxford, Oxford, United Kingdom

**Abstract:** Extracellular electrical recording is a popular and direct method to measure the simultaneous spiking activity of a large neural population. The key computational extraction of distinct neuronal units and firing times is known as spike sorting. However, there is a growing number of automated spike sorting codes, and much uncertainty and folklore about their accuracy in various experimental conditions. Several papers report comparisons on a case-by-case basis, but there is a lack of standardized measures and validation data. Furthermore, there is a potential for bias, such as sub-optimal tuning of competing algorithms, and a focus on one brain region or probe type. Without a fair and transparent comparison, genuine progress in the field remains difficult.

We address this challenge by developing SpikeForest, a reproducible, continuously updating platform which benchmarks the performance of spike sorting codes across a large curated database of electrophysiological recordings with ground truth. With contributions from over a dozen participating labs, our database includes hundreds of recordings, in various brain regions, with thousands of ground truth units (and growing). As well as extracellular recordings with paired intracellular ground truth, we include state-of-the-art simulated recordings, and hybrid synthetic datasets. We wrapped many popular sorting algorithms (including HerdingSpikes2, IronClust, JRCLUST, KiloSort, Kilosort2, MountainSort, SpyKING CIRCUS, and YASS) under
a common Python interface that performs automatic caching of results, and guarantees reproducibility via singularity containers and transparency of parameter choices. This also enables researchers themselves to install and run all tested sorters with a single interface. The large scale of our analysis demands an automatically updating nightly batch on a high performance compute cluster, where hundreds of sorting jobs are run in parallel (> 5000 CPU/GPU hours). Results are uploaded to a MongoDB database which is then accessed by our public-facing web site. This site allows intuitive comparison of metrics (precision, recall, overall accuracy, and runtime) across all sorters and recordings, and interactive visual “drilling down” into each sorting output at the single unit or event channel-trace level. The web technology is built on Node.js/React, and the D3 library for rendering.

In short, via reproducible ground truth studies, SpikeForest will continuously validate community progress in automated spike sorting, and guide neuroscientists to an optimal choice of sorter and parameters for a wide range of probes and brain regions.


Poster

340. Software Tools: Analysis I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 340.20/CC77

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant 5DP1NS096787

Title: SpineYOLO: A web-based, deep learning tool for identification of dendritic spines

Authors: *M. S. SMIRNOV¹, B. SCHOLL², E. GONZALEZ¹, J. M. CHRISTIE³, R. YASUDA²;
¹Max Planck Florida Inst. for Neurosci., Jupiter, FL; ³Synapse Physiol. Group, ²Max Planck Florida Inst., Jupiter, FL

Abstract: Abnormal density and morphology of dendritic spines are associated with many mental diseases. Since hundreds of molecular players are involved in the regulation of spine morphology, scalable and high-throughput tools to analyze spine morphology are necessary to understand the link between molecules and spine morphology. Toward this goal, we present SpineYOLO, an open source and online tool capable of automatically identifying dendritic spines in fluorescent tissue. SpineYOLO relies on a deep learning, end-to-end approach to minimize any need for parameter tuning. The algorithm is capable of identifying bounding boxes around dendritic spines with an average precision (AP50) of 72%. We demonstrate that SpineYOLO is easily generalizable to different types of spines, including spines in mouse
hippocampal neurons, ferrets cortical neurons and Purkinje neurons, when combined with a straightforward one-click-per-spine data labeling tool. SpineYOLO can be used on a single computer, shared over a network, or accessed online via a web browser. This tool will be useful for high-throughput spine morphology analysis, and its performance will improve over time due to the collection of crowd-sourced training data through its web interface.

**Disclosures:** M.S. Smirnov: None. B. Scholl: None. E. Gonzalez: None. J.M. Christie: None. R. Yasuda: None.

**Poster**

**340. Software Tools: Analysis I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 340.21/CC78

**Topic:** I.07. Data Analysis and Statistics

**Support:** OD025349

**Title:** SPARC initiative: Data alignment for proteomic, physiological, molecular and anatomical datasets from 36 research groups

**Authors:** G. PINE1, T. GILLESPIE2, *A. BANDROWSKI1, S. TAPPAN3, M. E. MARTONE2; 2Neurosci., 1UCSD, La Jolla, CA; 3R&D, MBF Biosci. - MicroBrightField Inc., Williston, VT

**Abstract:** The SPARC (Stimulating Peripheral Activity to Relieve Conditions) initiative is a coordinated effort among 36 different research groups with the unified purpose to produce microscopic, genomic and physiological data that can be used to explore interactions of the peripheral nervous system and organs. In addition to the investigators, the consortium comprises of 3 cores: DAT, MAP and SIM. Consortium members are publishing their datasets through the DAT (data) core; datasets are enhanced by tools and processes available through MAP (mapping) core and a simulation platform will be provided by the SIM (simulation) core. Critical to success of such an ambitious project is a set of standards and best practices to ensure that data accruing from multiple, independent labs can be harmonized and integrated. The MAP core has created a SPARC-specific common ontology set, which uses community ontologies such as UBERON and FMA heavily, but extends them where this is needed by the SPARC investigators. The solution is to use interlex and SciGraph services, which work seamlessly over search applications, and to integrate access to these services within external and internal curation tools which all draw from a common ontology set even while that ontology set is being updated. Extension of MBF Bioscience software to support interlex and SciGraph services allows SPARC investigators to seamlessly utilize curated ontology sets at the time of initial annotation and enables simple resurfacing to SciGraph for missing term requests. Creating mechanisms to easily generate rich metadata enhances the quality and utility of all segmented data generated for the
SPARC program. During the initial stage of the project, consortium members submitted 44 unique datasets. In tandem with these datasets, researchers published a total of 57 corresponding protocols to protocols.io. These datasets will be made available on the SPARC data portal 12 months after initial submission, but about 10 datasets will be published ahead of time in July 2019. Each protocol(s) will be linked with the dataset, once the data becomes public and vice versa. Overall experiences with the SPARC community has highlighted the benefit of adapting common and flexible data structures, to serve the needs of the consortium.


Poster

341. Electrical Methods to Modulate Neural Activity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 341.01/DD1

Topic: I.08. Methods to Modulate Neural Activity

Title: A current balance phenomenon causes temporal interference stimulation

Authors: *P. GROVER\(^1\), J. CAO\(^1\), C. GOSWAMI\(^1\), B. D. DOIRON\(^2\);
\(^1\)Carnegie Mellon Univ., Pittsburgh, PA; \(^2\)Mathematics, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Objective and Rationale: We use single neuron models to understand mechanisms behind Temporal Interference (TI) stimulation [Grossman et al., Cell 17] (aka “Interferential Stimulation” [Smith 71]). TI stimulation has created interest because of its ability to stimulate deep inside the brain without shallow stimulation. Despite decades of work, mechanisms of TI stimulation remain unclear. Understanding mechanisms can help improve the precision of TI stimulation [Cao, Grover, IEEE TBME’19].

Methods and Results: This work provides the first understanding of TI stimulation mechanisms using minimal single neuron models. We observe that both 2D neuron models (e.g. FitzHugh-Nagumo and Izhikevich’s “simple” model) and 4D (Hodgkin-Huxley-type) neuron models exhibit TI stimulation for wide ranges of parameter choices. We observe that neurons (both 2D and 4D) that exhibit TI have an “accommodation-like” phenomenon: in 4D, a high-freq. inward current generated by opening and closing of fast sodium channels (in direct response to the pure sine current) is balanced by outgoing currents from relatively steady potassium (K+) channels (assisted by leakage) over a single sine cycle. Consequently, in response to high frequency pure sine currents, the membrane potential displays a stable subthreshold limit cycle. However, when the envelope is modulated at a sufficiently high slope, the K+ channel is slow to respond, while the sodium currents get a sharp boost, resulting in firing.

When a current imbalance exists for a pure sine input, the neuron does not exhibit TI stimulation
as it fires in response to both pure and modulated high-frequency sinusoids. Some 4D neuron models (e.g. excitatory pyramidal cells and HH-squid neurons) exhibit TI, whereas others (e.g. PV neurons) do not.

Conclusions: Our understanding of its mechanisms predicts that not all neurons will exhibit TI stimulation. Examined neurons complement this observation: PV neuron models do not seem to exhibit TI stimulation, whereas models of Hodgkin-Huxley squid neurons and excitatory pyramidal cells do exhibit TI stimulation.

Disclosures: P. Grover: None. J. Cao: None. C. Goswami: None. B.D. Doiron: None.

Poster

341. Electrical Methods to Modulate Neural Activity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 341.02/DD2

Topic: I.08. Methods to Modulate Neural Activity

Support: Wellcome Trust MIT fellowship 097443/Z/11/Z (N.G.)
UK Dementia Research Institute Foundation Fellowship (N.G.)
NIH Director’s Pioneer Award 1DP1NS087724 (E.S.B.)
NIH Director’s Transformative Research Award 1R01MH103910-01 (E.S.B)
New York Stem Cell Foundation-Robertson Investigator Award (E.S.B)
MIT Center for Brains, Minds, and Machines NSF CCF-1231216 (E.S.B.)
Title: Suppression of essential tremor via phase-locked driven disruption of temporal coherence

Authors: S. SCHREGLMANN¹, D. WANG², R. PEACH³, X. ZHANG⁶, J. LI⁴, E. PANELLA⁵, E. S. BOYDEN⁷, M. BARAHONA³, S. SANTANIELLO⁶, K. P. BHATIA¹, J. C. ROTHWELL¹, *N. GROSSMAN⁴;


Abstract: Aberrant neural oscillations hallmark the pathophysiology of numerous neurological and psychiatric disorders. Here, we first report a method to accurately track the phase of neural oscillations in real-time by a Hilbert transform that avoids the characteristic Gibbs distortion at the end of the signal, aka endpoint-corrected Hilbert transform (ecHT). The ecHT method maintains the same computational complexity class of the original Hilbert transform allowing implementation in simple digital hardware. We then used the ecHT method to show that the aberrant neural oscillation that hallmarks treatment-resistant essential tremor (ET), the most common adult movement disorder, can be noninvasively suppressed via transcranial electrical stimulation of the cerebellar activity at a fixed phase difference to the ipsilateral tremor movement. The suppression of the ET activity was sustained after the end of the stimulation and can be phenomenologically predicted, post-hoc, from the features of the tremor movement before the start of the stimulation. Finally, we used a highly-comparative time-series feature extraction (> 8000 features) with statistical learning and neurophysiological computational modelling to show that the suppression of ET activity can be mechanistically attributed to a disruption of the temporal coherence in the tremor movement that can be originated in a higher bursting entropy at the cortico-olivo-cerebello-thalamic circuitry. The suppression of aberrant neural oscillation via phase-locked driven disruption of temporal coherence may represent a powerful neuromodulatory strategy to treat neurological and psychiatric disorders.

Disclosures: S. Schreglmann: None. D. Wang: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); D.W. has applied for a patent on the technology, assigned to MIT.. R. Peach: None. X. Zhang: None. J. Li: None. E. Panella: None. E.S. Boyden: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); E.S.B., has applied for a patent on the technology, assigned to MIT.. M. Barahona: None. S. Santaniello: None. K.P. Bhatia: None. J.C. Rothwell: None. N. Grossman: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); N.G. has applied for a patent on the technology, assigned to MIT..
**Abstract:** Introduction Transcranial focused ultrasound (tFUS) is a noninvasive neuromodulation tool that can penetrate the skull and achieve high spatial specificity (mm). The goal of this work is to address the fundamental question of whether intrinsic cell-type specific factors such as ion channel distributions and dendritic morphology etc. can lead to difference in response to tFUS stimulation without introducing external assistive agents. We studied this question in wide type rats and optogenetics-tagged mice. Methods Single-element transducer (500 kHz) in pulsed mode was used on both a wild-type rat and opto-tagged mice models. We set the ultrasound pulse repetition frequency (PRF) at 5 levels, i.e. 30, 300, 1500, 3000 and 4500 Hz, and used 3D printed ultrasound collimators to guide the focused ultrasound wave unilaterally onto primary somatosensory cortex of the two rodent models. The sonication duration was maintained at 67 ms while keeping the cycle per pulse number constant throughout all the PRFs. 32-channel intracranial electrode was employed to record extracellular action potentials, and based on the action potential waveform, we separated the neurons with the temporal features into 2 major groups, regular spiking units (RSU, presumably as excitatory neurons) and fast spiking units (FSU, presumably as inhibitory neurons) in the wide-type rats. In order to validate the effect of tFUS on excitatory and inhibitory neurons, opto-tagged mice models were developed through breeding of transgenic animals to achieve cell-type specific expression of channel-rhodopsin. Neuron types in the mice model were identified through responsiveness to optical stimulation and were then tested using the 30 and 300 Hz PRF tFUS. In both models neuronal spiking rates were used to measure the response to tFUS. Results For the first time in in vivo rodent models, we found that at low PRF of 30 Hz, the excitatory neurons are significantly less responsive to the ultrasound stimulation than those with the PRF at 3000 Hz (p < 0.005) and the PRF at 4500 Hz (p < 0.001), whereas the spiking rates of inhibitory neurons do not change significantly across all PRF levels. In the opto-tagging mice model, the CamKII and PV neurons also demonstrate significantly contrasted spiking activities in response to the two levels of PRFs (p < 0.05). Conclusion Neurons exhibit intrinsic unequal sensitivity to tFUS. The ability to
noninvasively stimulate selected cell-types has a positive impact in understanding of the brain and ability to treat neurological disorders. Here we show early evidence that tuning tFUS PRF can preferentially target specific neuron types.

**Disclosures:** X. Niu: None. K. Yu: None. E.I. Krook-Magnuson: None. B. He: None.

**Poster**

341. Electrical Methods to Modulate Neural Activity I

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 341.04/DD4

**Topic:** I.08. Methods to Modulate Neural Activity

**Support:** Harvard Mind Brain Behavior Interfaculty Initiative (MBB)

**Title:** Late enhancement of sustained attention with cortical priming prior to rTMS

**Authors:** *G. EDWARDS, F. CONTÒ, L. BATTELLI; Ctr. for Neurosci. and Cognitive Systems@UniTn, Rovereto, Italy

**Abstract:** Low-frequency rTMS (LF-rTMS) to the intra-parietal sulcus (IPS) in the healthy hemisphere of patients with parietal lesion improves visual attention 30 minutes post-rTMS. It is hypothesized LF-rTMS inhibits the healthy hemisphere, causing upregulation of functional communication in the remaining attention network and increased attention performance. To aid clinical intervention, enduring effects are crucial. We hypothesize LF-rTMS effect on IPS may extend by controlling brain state through priming. Here, we prime the cortex using high-frequency transcranial random noise stimulation (HF-tRNS) prior to LF-rTMS, based on the hypothesis that HF-tRNS increases neuronal excitability. Experiment one tested four fMRI-guided stimulation protocols: 1) HF-tRNS & LF-rTMS, 2) LF-rTMS alone, 3) HF-tRNS alone and 4) Control. Sustained attention was recorded up to 94 minutes post-stimulation via bilateral multiple object tracking (MOT). MOT requires participants to track two moving discs among two moving distractor-discs in each visual field. Results showed an effect of stimulation on MOT performance (p=0.024). At 80-94 minutes after HF-tRNS & LF-rTMS, participants performed 7% better than Control (p=0.015). Priming with HF-tRNS over the IPS prolonged the LF-rTMS effect. In experiment two, we replicated experiment one and enhanced stimulation impact by requiring participants to perform MOT during HF-tRNS. MOT functionally activated bilateral IPS alongside external activation by HF-tRNS to boost priming. Specifically, MOT performance increased by 15.22% at 80-94 minutes following tRNS & rTMS relative to sham (p=0.023). Furthermore, MOT performance improved 13.56% following HF-tRNS without LF-rTMS at the 80-94-interval (p=0.036). Priming the cortex with behavior alongside HF-tRNS boosted the late attention increase found in experiment one following HF-tRNS & LF-rTMS. Further, the effect of HF-tRNS also intensified when applied with behavior. These results
suggest priming may be a useful tool in lengthening stimulation effects in clinical rehabilitation protocols.

**Below:** Results experiment 1. Stimulation effects baselined to control. Control performance presented below. ● p<0.05

**Right:** Results experiment 2. Participants thresholded to 75% correct prior to stimulation, illustrated by dotted line. ● p<0.05

**Disclosures:** G. Edwards: None. F. Contò: None. L. Battelli: None.

**Poster**

341. Electrical Methods to Modulate Neural Activity I

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 341.05/DD5

**Topic:** I.08. Methods to Modulate Neural Activity

**Support:** NIH Grant F31 HL145831

NIH Grant R21 NS109571

**Title:** Temporal interference stimulation to activate respiratory motor pools

**Authors:** *M. D. SUNSHINE*¹, E. NEUFELD³, A. M. CASSARA⁴, N. GROSSMAN⁵, K. J. OTTO², E. S. BOYDEN⁶, D. D. FULLER¹;

¹Physical Therapy, ²Univ. of Florida, Gainesville, FL; ³Computat. Life Sci., IT'IS Fndn., Zuerich, Switzerland; ⁴IT'IS Fndn., Zurich, Switzerland; ⁵Dementia Res. Inst., Imperial Col. London, London, United Kingdom; ⁶MIT, Cambridge, MA
Abstract: Temporal interference (TI) stimulation can activate neurons in deep brain structures using minimally invasive electrodes. Here we have adapted TI stimulation to target spinal respiratory neurons. Our overall hypothesis is that TI stimulation can target neuro-modulation to the ventral cervical spinal cord to regulate diaphragm activation during acute or chronic hypoventilation associated with opioid overdose or neurotrauma. We have systematically characterized, using experimental studies in adult rats and computational modeling approaches, the delivery of TI stimulation using epidural electrodes, in order to understand and optimize targeted diaphragm activation. We have been able to control the stimulation by focusing the interference maximum to the phrenic motor pool. Simulations were helpful in predicting and optimizing electrode locations and current ratios. A ratio of dorsal:lateral currents of about 2.0:0.8mA produced maximal diaphragm activation. Diaphragm activation with minimal blood pressure changes was achieved by varying the carrier frequency. To determine if TI acts via pre- vs. post-synaptic mechanisms, additional experiments were conducted used pharmacologic agents to block presynaptic input to phrenic motor neurons. Blocking glutamatergic receptors using intraspinal CNQX/AP5 delivery reduced the TI evoked response to 75±58%, 65±31%, and 53±21% of baseline using 100µA, 200µA, and 300µA lateral currents. Blocking glycine (strychnine) and GABA (bicuculine) receptors increased the evoked response to 168±81%, 143±64%, and 101±66% of baseline. Thus, both excitatory and inhibitory pre-synaptic inputs to phrenic motoneurons appear to be activated by T-I stimulation. We also validated the efficacy of TI stimulation following acute spinal cord injury (C2 hemisection) in anaesthetized rats. TI epidural stimulation could both activate and regulate diaphragm motor units after cervical spinal cord injury. We conclude that appropriately targeted TI can depolarize phrenic motor neurons with >50% of the evoked response resulting from direct motor neuron depolarization. The evoked diaphragm EMG response was dependent on the amplitude of the two TI currents; this observation helps constrain possible mechanisms of interaction underlying TI neuromodulation.


Poster

341. Electrical Methods to Modulate Neural Activity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 341.06/DD6

Topic: 1.08. Methods to Modulate Neural Activity
Title: Deep brain stimulation via temporal interference stimulation using implanted DBS electrodes. A feasibility study

Authors: *A. M. CASSARA1, E. NEUFELD1, H. BERGMAN2, E. S. BOYDEN3, N. GROSSMAN4, N. KUSTER1,5;  
1IT’IS Fndn., Zurich, Switzerland; 2Hebrew Univ., Jerusalem, Israel; 3MIT, Cambridge, MA; 4Imperial Col. London, London, United Kingdom; 5ETHZ, Zurich, Switzerland

Abstract: Temporal Interference (TI) Stimulation has been considered a breakthrough in non-invasive-brain-stimulation (NIBS) due to the possibility to focus and steer stimulation in deep brain structures in a controlled way, and without stimulating brain regions close to electrodes or the skin. In this study, we performed an in silico feasibility evaluation to determine the potential of use TI in patients with implanted DBS electrodes to access a large variability of deep brain regions. Electromagnetic (EM) simulations within reference anatomical head models were used to calculate the E-field generated by DBS electrodes for different combinations of activated electrode pairs, different orientations of the DBS implant and site of implantation. We investigated the possibility to steer stimulation in regions proximal to single DBS electrodes, as well as the possibility to target more distant brain organs using multiple implanted DBS electrode (e.g. bilateral implants), such as the one between right and left sub-thalamic nuclei. We investigate and compare traditional E-field exposure by individual electrode pairs against the distribution of TI modulation envelop for input currents used in standard DBS applications, and we use neuroelectric models of realistic neurons and axons to investigate the impact of TI on the neuroelectric activity of realistic positioned neurons and fibers. Safety issues of unwanted stimulation of brain regions outside of designated targets are also evaluated. This study is the basis for forthcoming experimental measurements to advance treatment planning and technology development of TI hardware.


Poster

341. Electrical Methods to Modulate Neural Activity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 341.07/DD7

Topic: I.08. Methods to Modulate Neural Activity

Support: ERC Synergy Grant

Title: Multi-locus transcranial magnetic stimulation for closed-loop brain stimulation
Authors: *R. J. ILMONIEMI*¹, G.-L. ROMANI², U. ZIEMANN³;
¹Neurosci. and Biomed. Engin., Aalto Univ., Espoo, Finland; ²Inst. for Advanced Biomed. Technologies, Gabriele D’Annunzio Univ. of Chieti-Pescara, Chieti, Italy; ³Eberhard Karls Univ., Tübingen, Germany

Abstract: Current protocols to treat the brain with transcranial magnetic stimulation (TMS) almost exclusively target a single cortical spot, such as the left dorsolateral prefrontal cortex in the case of depression or the motor cortex in the case of neuropathic pain. Furthermore, in many cases, the treatment target is inaccurately defined, its location determined only by head-surface landmarks or by moving the TMS coil a certain distance from a location where stimulation produces motor responses. The resulting stimulation may then activate different functional sites in different individuals, causing variability in treatment efficacy.

We are developing technology (called multi-locus TMS or mTMS) for stimulating multiple cortical sites simultaneously or at arbitrary time intervals. Instead of predefining the stimulation sequence, we will use algorithms in a closed-loop mode so that the stimulation targets, stimulus timing and pulse intensity are determined by real-time EEG, muscle-activity, or behavioral measures. In this dynamic approach, we aim at modifying network connectivity or other properties of neuronal activity into desired directions, ultimately for therapeutic purposes to normalize dysfunctional network connectivity in neurological and psychiatric disorders.

With our 2-coil mTMS prototype, we have demonstrated automatic brain scanning, feedback-guided algorithmic motor-hotspot determination, and the possibility to investigate short-distance surround inhibition and facilitation mechanisms. Motor hotspot determination can now be done with far fewer pulses than with the conventional manual approach, much faster, more reliably, and without user bias. In our ConnectToBrain project (2019-2025) funded by the European Research Council, we will build a large mTMS coil array that covers most of the cortical mantle and allows one to administer to the brain electronically-controlled spatiotemporal TMS sequences. We will develop machine-learning methods to optimize treatment paradigms and will translate mTMS methodology into physiological and clinical studies.
Disclosures:  **R.J. Ilmoniemi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nexstim Plc.  **F. Consulting Fees (e.g., advisory boards); Nexstim Plc.**  

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**U. Ziemann:** None.

**Poster**

341. Electrical Methods to Modulate Neural Activity I

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 341.08/DD8

**Topic:** I.08. Methods to Modulate Neural Activity

**Support:** DARPA TNT N66001-17-2-4010.

**Title:** Augmentation of transcutaneous electrical stimulation of the peripheral nervous system using the Injectrode, an injectable minimally-invasive electrode that cures in vivo

**Authors:**  

*J. K. TREVATHAN*1,5, I. W. BAUMGART1, E. N. NICOLAI5,1, B. A. GOSINK1, M. L. SETTELL5,1, B. E. KNUDSEN6,2, M. FRANKE7, A. J. SHOFFSTALL8,9,7, K. A. LUDWIG3,6,4,7;  

Abstract: Implanted neural stimulation devices, known as bioelectronic medicines, electroceuticals, or neuromodulation therapies, are successfully being used to treat a variety of disorders. Unfortunately, the complexity and invasiveness of the surgical procedures for implanting neural stimulation electrodes normally limits the availability of these therapies to patients that have failed conventional treatments. There is a critical need for electrodes that can be delivered through minimally-invasive procedures. To meet this need, we have developed the Injectrode, an electrode that can be injected as a flowable pre-polymer and cures in the body to form a conductive neural interface. Here, we also propose that the invasiveness of the procedure can be further reduced, compared to traditional implanted neural stimulation systems, by augmenting transcutaneous stimulation through the addition of an injected subcutaneous collector electrically coupled through intact skin to an external patch electrode. Experiments were performed with Injectrode made from silver particles embedded in a silicone elastomer. Scanning electron microscopy and electrochemical testing was performed to assess the neuromodulation relevant material properties of the Injectrode. Acute in vivo testing of the Injectrode in a swine model of vagus nerve stimulation, after correcting for differences in impedance between the electrodes, showed a similar dose-titration for evoking decreases in heart rate compared to a LivaNova stimulation electrode. Additionally, transcutaneous stimulation of the vagus nerve through an Injectrode with a subcutaneous collector evoked consistent decreases in heart rate that did not occur during application of the same stimulation without the collector. By reducing invasiveness of the electrode and complexity of the surgical procedure, the Injectrode fills an unmet need for less invasive neuromodulation electrodes that can stimulate complex neural targets. Additionally, the capability of the Injectrode to augment transcutaneous stimulation using a collector has the capacity to further reduce the invasiveness and potential failure points of the system. For these reasons, the adoption of this technology could lead to novel and more widely applicable neuromodulation therapies.


Poster

341. Electrical Methods to Modulate Neural Activity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 341.09/DD9

Topic: I.08. Methods to Modulate Neural Activity

Support: The Defense Advanced Research Projects Agency (DARPA) Biological Technologies Office (BTO) Targeted Neuroplasticity Training Program under the auspices of Doug Weber and Tristan McClure-Begley
Space and Naval Warfare Systems Command (SPAWAR) Systems Center with (SSC) Pacific grants no. N66001-17-2-4010
NIH SPARC Program Award 3OT2OD025340-01S2 Subaward A03-1423

Title: Connecting intrafascicular recordings, electromyography of the neck muscles, and functional magnetic resonance imaging during clinical vagus nerve stimulation in swine

Authors: E. N. NICOLAI1,2, M. L. SETTELL1,2, E. K. ROSS3, J. K. TREVATHAN1,2, B. E. KNUDSEN1, A. L. MCCONICO1, A. J. SUMINSKI2, J. C. WILLIAMS2, *K. A. LUDWIG2; 1Mayo Clin., Rochester, MN; 2Univ. of Wisconsin, Madison, WI; 3Cala Hlth., Burlingame, CA

Abstract: Vagal nerve stimulation (VNS) is FDA approved for epilepsy, depression, cluster headache, and migraine with over 100,000 patients having received VNS implants to date. However, clinical studies suggest that activation of vagus afferent pathways for therapeutic effect do not occur prior to reaching therapy-limiting side effects such as throat pain, voice alteration, and dyspnea putatively associated with neck muscle activation. One method to assess vagal afferent engagement during clinical VNS is functional MRI (fMRI), as it can be performed non-invasively. However, the dose response to VNS measured via fMRI has not been directly connected to both intrafascicular (LIFE) recordings of vagal fiber activation and electromyography (EMG) of the neck muscles in a human sized animal model.

To address this gap, we used a clinical VNS electrode and clinically relevant stimulation parameters in an anesthetized swine model to compare the dose response curves of EMG and vagal LIFE recordings with fMRI obtained in a partially overlapping cohort. We correlated these responses to VNS induced heart rate (HR) changes and activation of the Hering-Breuer (HB) reflex. ‘A-fiber’ activation and associated EMG response were evident at low levels of stimulation (~0.2 mA), without BOLD fMRI changes. At clinically tolerable levels (<1.5 mA), placement of the VNS electrode can dramatically change physiological outcome (no HR/HB, HR, HB, HR+HB). When a HR decrease was achieved, there were dose dependent increases in negative BOLD signal. When a HB reflex was achieved, global changes in BOLD signal were observed including changes in brainstem structures associated with activation of vagal afferents. During lower stimulation amplitudes, that did not elicit a HB response, there was little to no
change in BOLD signal. Neck muscle activation occurred at stimulus amplitudes lower than those required for afferent pathway activation. Putative B-fiber activation of preganglionic vagal efferents led to dose dependent increases in HR change and negative BOLD signal. Putative C-fiber afferent activation, which resulted in BOLD signal change consistent with those observed during clinical VNS, required stimulation amplitudes that caused visible neck muscle contractions AND specific electrode placement.


Poster 341. Electrical Methods to Modulate Neural Activity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 341.10/DD10

Topic: I.08. Methods to Modulate Neural Activity

Support: DARPA TNT N66001-17-2-4010

Title: Electrical stimulation of the cervical vagus nerve in mice modulates CSF penetrance into the brain parenchyma


Abstract: Vagal nerve stimulation (VNS) is an FDA approved neuromodulatory or ‘electroceutical’ strategy for the treatment of epilepsy, cluster headaches, and treatment resistant depression that is also currently being explored as a therapeutic option across a broad range of clinical indications such as obesity, anxiety, arthritis, and gastrointestinal disorders. Indeed, the VNS clinical population continues to grow with over 100,000 patients to date despite the actual therapeutic mechanisms behind VNS remaining poorly understood. Separately, the recently described brain waste clearance systems, including the meningeal lymphatic and glymphatic systems, have gained increasing research interest for their roles in the maintenance of a healthy brain homeostasis. These systems have been of especial interest in the realm of neurodegenerative diseases and traumatic brain injury as they are responsible for the proper removal of metabolic waste products and misfolded proteins from the brain interstitium. Of note, the glymphatic system, which revolves around the influx and interchange of cerebrospinal fluid
(CSF) with interstitial fluid (ISF), is partially driven by cerebral arterial pulsations and shown to be sensitive to noradrenergic signaling. Given the known effects of VNS on systemic and cerebral hemodynamics as well as brain noradrenergic levels we hypothesized that VNS, delivered at clinically derived parameters, could affect the influx of CSF to the brain parenchyma. To test this, we used an ex vivo slice model to quantify the spread of a lysine-fixable tracer injected into the CSF system through the cisterna magna. We found that VNS significantly increased the degree of CSF dye penetrance (18.60% ± 1.98%) relative to naïve controls (10.75% ± 1.26%) and sham (13.05% ± 2.29%) and that there was no difference between the naïve controls and sham VNS groups. These results demonstrate a novel and previously unappreciated effect of VNS with potential therapeutically beneficial applications across a broad range of central nervous system pathologies.


**Poster**

341. Electrical Methods to Modulate Neural Activity I

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #:Poster #:** 341.11/DD11

**Topic:** I.08. Methods to Modulate Neural Activity

**Support:** DARPA TNT N66001-17-2-4010

**Title:** Effects of electrical stimulation of the infraorbital branch of the trigeminal nerve on oscillatory activity in the barrel cortex

**Authors:** *J. P. NESS¹, W. ZENG², P. PARMETT¹, J. KEGAL¹, S. K. BRODNICK¹, K. P. CHENG¹, L. KRUGNER-HIGBY¹, W. B. LAKE³, K. A. LUDWIG⁴, J. C. WILLIAMS¹, A. J. SUMINSKI³;


**Abstract:** Over the past decade, electrical stimulation of various cranial nerves has been shown to be an effective adjunctive therapy for many neurodegenerative and neuropsychological diseases, and modulator of cognitive functions such as learning, memory and decision-making. This is especially true for the vagus and trigeminal nerves due to their direct access to neuromodulatory centers and arousal mechanisms. In particular, pairing stimulation of the vagus nerve with behavior is known to cause cortical reorganization that improves sensory discrimination, speeds the learning of motor tasks and drives functional rehabilitation after injury. It remains unclear, however, if trigeminal nerve stimulation (TNS) similarly modulates
cortical function. Here, we evaluate the hypothesis that electrical stimulation of the trigeminal nerve induces changes in cortical oscillations in the somatosensory cortex. Lewis rats (n = 5, all male) were chronically implanted with a custom nerve cuff electrode (1.5mm ID, 120μm wire diameter, 1.5mm spacing) on the infraorbital branch of the trigeminal nerve (IoN) and a 16 channel uECoG array (200um site diameter, 500um inter-electrode spacing) placed over barrel cortex. In 8 weekly sessions, we measured the cortical responses evoked by electrical stimulation (single cathode leading biphasic pulses, 200us phase duration, 50 - 300 uA current amplitude). We quantified the minimum stimulation current required to generate an evoked potential by comparing the peak to peak response magnitude to a baseline measured 20ms prior to the onset of stimulation. The threshold to evoke a cortical response decreased monotonically from week 1 to 4 and then remained stable in weeks 4 through 8. In parallel, we investigated the effects of TNS of oscillatory activity in the somatosensory cortex by measuring the local field potential (LFP) and computing the power spectra for separate 3 minute recordings before and after TNS and compared the total power in discrete frequency bands. Power in the gamma band (36-55Hz) decreased significantly following TNS, but we found no change in alpha/theta(6-12Hz) and beta (12-25Hz) power. This change in gamma power may reflect the mechanism by which stimulation of peripheral nerves modulates cognitive function.


Poster

341. Electrical Methods to Modulate Neural Activity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 341.12/DD12

Topic: I.08. Methods to Modulate Neural Activity

Support: MINECO-FEDER BFU2014-53820-P
MCIU-FEDER BFU2017-89615-P
NIH RF1MH114269
MECD FPU13/04858

Title: Transcranial direct-current stimulation on mice somatosensory cortex induce GABA-related asymmetric effects

Authors: C. SANCHEZ-LEON¹, I. CORDONES¹, M. GOMEZ-CLIMENT¹,², A. CARRETERO-GUILLEN¹,³, G. CHERON⁴,⁵, J. F. MEDINA⁶, *J. MARQUEZ-RUIZ¹; ¹Univ. Pablo de Olavide, Sevilla, Spain; ²Univ. Internacional de la Rioja, Logroño, Spain; ³Achucarro Basque Ctr. for Neurosci., Leioa, Spain; ⁴Univ. Libre De Bruxelles, Brussels,
Abstract: Transcranial direct current stimulation (tDCS) is a non-invasive technique that allows the modulation of cortical excitability. This modulation can be dissociated in immediate and long-term changes, with the first one referring to changes observed due to the interaction of the imposed electric field and the redistribution of intracellular ions in the neuron, and the later referring to changes that persist after electric current cessation and, therefore, mediated by protein, genetics and/or structural modifications. While immediate effects appear at the very moment of tDCS application, long-term effects need several minutes to take place. Because the mechanisms behind each one of these effects are partly unknown, the objective of this study was to deepen in such mechanisms. For this purpose, mice were prepared for chronic recording of sensory evoked potentials (SEPs) induced in the primary somatosensory cortex (S1) in response to whisker electrical stimulation before, during and after tDCS. S1-tDCS was performed at different current intensities for 5 s to test the immediate effects on SEPs, and during 20 min to analyze the long-term effects and expression of GAD65-67 and VGLUT1 after stimulation. 5 s of tDCS modulated the amplitude of the SEPs in a polarity and intensity manner. Anodal tDCS increased the amplitude of the SEPs up to a maximum of 41% for the maximal applied current, whereas cathodal tDCS decreased it up to a maximum of 26%. For 20 minutes of cathodal tDCS, a significant reduction in SEPs amplitude was induced together with a decrease in power spectrum between 10-100 Hz, and the effects were maintained for 40 minutes after tDCS cessation. For 20 minutes of anodal tDCS a significant increase in SEPs amplitude was observed together with an increase in power spectrum between 30-50 Hz, but these changes didn’t persist after tDCS cessation. The immunohistological analysis of the stimulated S1 brain regions showed a significant increase of GAD 65-67 immunoreactivity after cathodal stimulation but not after anodal or sham condition. No changes were observed in VGlut1 expression after anodal nor cathodal tDCS. The current results demonstrate a dissociation between immediate and long-term effects associated to tDCS of sensory cortex. Histological data suggest the implication of inhibitory mechanisms in the asymmetry of long-term effects consistent with electrophysiological results.


Poster

341. Electrical Methods to Modulate Neural Activity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 341.13/DD13

Topic: I.08. Methods to Modulate Neural Activity
Support: NIH Grant RF1 MH114269

Title: Impact of cerebellar tDCS on motor performance and Purkinje cell activity in mice

Authors: *A. SANCHEZ-LOPEZ*, S. OHMAE, J. MARQUEZ-RUIZ, J. F. MEDINA;  
1Dept. of Neurosci., Baylor Col. of Med., Houston, TX; 2División de Neurociencias. Univ. Pablo de Olavide, Seville, Spain

Abstract: Transcranial direct current stimulation of the cerebellum (CB-tDCS) has been used to alleviate motor impairments as well as to enhance different aspects of cognitive function. However, our understanding of the mechanisms of action of CB-tDCS is still in its infancy, particularly with regards to the neuromodulatory effects induced at the level of specific neural circuits in vivo. To address this question we have focused in a previously identified circuit in the mouse cerebellum, which is responsible for motor performance during an eyeblink conditioning task. Behavioral data indicates that application of cathodal CB-tDCS over lobule simplex of the cerebellar cortex causes an impairment of conditioned eyelid movements in a dose-dependent manner, whereas anodal CB-tDCS at the same site produces a subtle but very reliable enhancement at low density currents (<35.4 A/m²) and impairment at higher density currents (70.8 A/m²). Moreover, preliminary data obtained from Purkinje cells in this circuit indicates that CB-tDCS can be used to effectively modulate the resting state spontaneous firing rate in a polarity dependent manner. Altogether, our experiments provide the first glimpse at the local effects of CB-tDCS on the activity of Purkinje cells in a specific neural circuit of the cerebellar cortex, revealing how the polarity and intensity of the stimulation affect neural excitability to modulate behavior.


Poster

341. Electrical Methods to Modulate Neural Activity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 341.14/DD14

Topic: I.08. Methods to Modulate Neural Activity

Support: MINECO-FEDER BFU2014-53820-P  
MCIU-FEDER BFU2017-89615-P  
NIH RF1MH114269  
MECD FPU13/04858

Title: Impact of cerebellar tDCS on morphologically identified Purkinje cells and cerebellar sensory inputs
Authors: *C. SANCHEZ-LEON*¹, I. CORDONES¹, A. SANCHEZ-LOPEZ¹,², G. CHERON³,⁴, J. F. MEDINA², J. MARQUEZ-RUIZ¹;
¹Univ. Pablo de Olavide, Sevilla, Spain; ²Baylor Col. of Med., Houston, TX; ³Univ. Libre de Bruxelles, Brussels, Belgium; ⁴Univ. de Mons, Mons, Belgium

Abstract: Cerebellar transcranial direct current stimulation (cer-tDCS) is being used to modulate cerebellar function both in health and disease. Nevertheless, how external electric fields affect the different components in the cerebellar network remains to be elucidated. The aim of this study was to characterize the physiological mechanisms underlying short- and long-term effects associated to cerebellar tDCS in behaving mice. Mice were prepared for chronic recording of sensory evoked potentials (SEPs) in response to whiskers electrical stimulation, as well as single-cell activity in the CrusI/II region of the cerebellar cortex. Cer-tDCS in behaving mice was applied at different intensities for 10 s to test short-term effects, and during 20 min to characterize long-term effects on SEPs and expression of GAD65-67 and VGLUT1. In addition, neuronal juxtacellular recordings in vermis were made during cer-tDCS application in anesthetized mice and then, the recorded neurons were labeled with neurobiotin for morphological characterization. During the application of cer-tDCS, anodal and cathodal polarities increased and decreased, respectively, the amplitude of the trigeminal (T) component of SEPs, and slightly, yet reliably modulated the amplitude of the cortical (C) component in the opposite direction. Interestingly, Purkinje cell simple spike firing rate was modulated by cer-tDCS in a heterogeneous manner, observing an increase/decrease with anodal/cathodal stimulation in some of them, the opposite pattern in others, and no effects in the rest. Neurobiotin labeling showed that this diverse modulation largely depended on the axodendritic axis orientation of the Purkinje cells in the cerebellar layer with respect to the active electrode. However, despite the SEPs and Purkinje cells modulation during tDCS application, after cer-tDCS cessation no long-term effects were observed for the amplitude of SEP components, and accordingly no changes were observed in GAD65-67 nor VGLUT1 expression. Present results successfully demonstrate the immediate modulatory effects of cer-tDCS on cerebellar cortex activity in behaving mice. In addition, the experiments show the crucial importance of the Purkinje cells axodendritic axis orientation in the modulation of neuronal activity by cer-tDCS.


Poster

341. Electrical Methods to Modulate Neural Activity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 341.15/DD15

Topic: I.08. Methods to Modulate Neural Activity
Support: NIH R21-NS-085539  
Branfman Family Foundation  
Brain & Behavior Research Foundation

Title: Changes in evoked coherence in subregions of the striatum and substantia nigra pars reticulata in anesthetized rats

Authors: *H. LI¹, G. C. MCCONNELL²;  

Abstract: Introduction: Microelectrode recordings (MERs) during Deep Brain Stimulation (DBS) surgery are commonly used to verify and refine targeting of electrode placement. We hypothesized that changes in coherence spectra of stimulation evoked local field potentials (LFPs) between neighboring microelectrode recordings correlated with electrode position. We tested this hypothesis in two promising DBS targets in the Basal Ganglia: Substantia Nigra pars reticulata (SNr) and its subregions, medial SNr (mSNr) and lateral SNr (lSNr); striatum and its subregions, dorsal striatum (DS) and ventral striatum (VS). Methods: Fluorescently-coated single wire tungsten microelectrodes were lowered in anesthetized rat brain (step size of microdrive = 100μm). Stimulation was delivered 10s (amplitude = 100 μA; frequency = 0.5Hz; pulse width = 90 μs) at each depth and neural recordings were obtained during interpulse intervals. Coherence analysis of power spectrums (0-200Hz) of evoked LFPs was calculated between two adjacent depths after aligning the timestamps of stimulation. Borderlines of each brain region and its subregions were measured based on histology. Results: Electrodes penetrated through mSNr (n=6) and lSNr (n=6). Evoked coherence in mSNr showed a trend of low coherence dorsal to mSNr, high coherence within mSNr, and low coherence ventral to mSNr. Evoked coherence in lSNr showed a trend of high coherence dorsal to lSNr, low coherence within lSNr, and high coherence ventral to SNr. In total, 10 out of 12 trials (83%) showed significant difference between dorsal to SNr vs. within SNr; 9 out of 12 (75%) trials showed significant difference between within SNr vs. ventral to SNr. Overall, both mSNr and lSNr showed significant difference with the structures dorsal and ventral to them. Electrodes penetrated through DS (n=16) and VS (n=16). Evoked coherence showed a trend of high coherence in cortex, low coherence in DS, and high coherence in VS. In total, 14 out of 16 trials (87%) showed significant difference between cortex vs. DS, and 9 out of 16 trials (56%) showed significant difference between DS vs. VS. Overall, both DS and VS showed significant difference with cortex. DS and VS did not significantly differ, however, the same trend was observed in 14 out of 16 tracks. Conclusion: Our results suggest that coherence analysis of stimulation evoked LFPs can distinguish DBS targets from surrounding brain regions and lay the foundation for this technique to accelerate precise targeting during DBS implantation surgeries.

Disclosures: H. Li: None. G.C. McConnell: None.
Title: Probing the activity of neuronal networks using graphene-mediated optical stimulation

Authors: *E. MOLOKANOVA¹, V. CHERKAS², X. SHAN³, A. ALMENAR-QUERALT⁴, A. R. MUOTRI³, A. SAVTCHENKO¹;
¹Nanotools Biosci., La Jolla, CA; ²Bogomoletz Inst. for Physiol., Kiev, Ukraine; ³Pediatrics/Cellular Mol. Med., ⁴Neurosciences/Cellular and Mol. Med., UCSD, La Jolla, CA

Abstract: The ability to probe the activity of neuronal networks can allow deciphering the fundamental processes in the brain and, eventually, help with the diagnosis and treatment of neurological disorders. All-optical probing requires two components: a light-controlled cell stimulation method and an imaging-based method for recording of neuronal activity. Optogenetics is currently a leader among optical stimulation methods. However, optogenetic stimulation is a complex phenomenon that inevitably affects cells due to the need for high expression levels of exogenous light-sensitive ion channels, their gating kinetics, and the activity of specific ions conducted by optogenetic actuators. Therefore, in certain cell systems (e.g., stem cell-derived neurons), it is not desirable to express exogenous proteins that might affect physiology of differentiating and maturing cells. Our study offers an alternative optical stimulation method that enables fast and reversible optical stimulation of genetically intact neurons by taking advantage of optoelectronic properties of graphene. Previously, graphene materials have been successfully interfaced with various cell types in cell scaffolds, where graphene merely provides the passive structural support. In contrast, in this study, due to its ability to efficiently convert light to electricity, graphene can play an active role in interactions with live cells. Here we present a novel optoelectrical biointerface for graphene-mediated optical stimulation (GraMOS) of cells via external light-controlled electric field [1]. Our GraMOS biointerface does not interfere with either genetic make-up of cells or their structural integrity, thus providing truly non-invasive stimulation. By performing imaging and electrophysiological experiments on hiPSC-derived neurons, we demonstrated that the GraMOS biointerface exhibits the excellent biocompatibility and the ability to optically trigger action potentials in neurons. Using geometrically patterning of GraMOS biointerfaces, we are evaluating the connectivity of neuronal networks under various experimental conditions. Graphene-mediated optical stimulation
is a powerful new method that can be used 1) to probe the existing neuronal activity, decipher the fundamental processes in the brain and, eventually, help with the diagnosis and treatment of neurological disorders; and 2) to elevate the neuronal activity to the new level that could enable activity-dependent neurogenesis, restore vision, and assist with deep-brain stimulation.


Disclosures:  
E. Molokanova: None. V. Cherkas: None. X. Shan: None. A.R. Muotri: None. A. Savtchenko: None. A. Almenar-Queralt: None.

Poster

341. Electrical Methods to Modulate Neural Activity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 341.17/DD17

Topic: I.08. Methods to Modulate Neural Activity

Support: DARPA SUBNETS program under Cooperative Agreement Number W911NF-14-2-0043
Simons Collaboration for the Global Brain
NIH BRAIN Grant R01-NS104923

Title: A causal network analysis of neuromodulation in the cortico-subcortical mesolimbic network

Authors: *S. QIAO1, K. BROWN1, J. I. SEDILLO2, B. FERRENTINO1, B. PESARAN1;  
1Ctr. for Neural Sci., New York Univ., New York, NY; 2NYU, New York, NY

Abstract: Modern neural technologies offer new ways to study and control brain networks. Neuromodulation refers to a spectrum of technologies that seek to achieve therapeutic effects by intervening to alter neural activity. It has broad and wide-ranging potential to treat drug-resistant neurological and neuropsychiatric disorders. Despite the progress, whether and how electrical stimulation can be used to treat, for example, cognitive impairment remains controversial. These hurdles reflect limitations in the conceptual framework for neuromodulation-based treatment. Clinical targets for therapeutic stimulation are increasingly viewed within a circuit-based model of neural dysfunction, which highlights the need to assess the network mechanism of action - how the intervention alters activity not just focally but across the network. Network neuroscience describes networks as nodes connected by edges to form graphs and other generalizations. The edges can have different weights, reflecting different connection strengths. Network neuroscience thus provides a framework for testing the neuromodulation hypothesis. Neuromodulation may involve suppressing or facilitating the weight of the edge between two nodes - the edge modulation hypothesis. Alternatively, neuromodulation may modulate the nodes themselves, suppressing or facilitating activity at those sites - the node modulation hypothesis.
Here, we test the network mechanism of action of a primary intervention using the concept of neural excitability to disambiguate edge- and node-modulation hypotheses. Unlike correlation-based estimates, neural excitability estimates edge weight casually by measuring the neural response to a secondary intervention— an isolated stimulation pulse. Recording from many sites during the secondary intervention measures network excitability in a causal network analysis. Performing the causal network analysis before and after the primary therapeutic intervention, a stimulation pulse train, allowed us to test the edge- and node-modulation hypothesis. We performed a causal network analysis across a large-scale cortico-subcortical mesolimbic network before and after delivering a short-burst tetanic microstimulation (TetMS) at sites in either gray matter or white matter. Our results indicate that short-burst TetMS overwhelmingly acts to disrupt processing across edges while processing within nodes remains relatively undisturbed. Together, our results have implications for the rational design of therapeutic patterns of closed-loop stimulation based on a circuit-level understanding of the neuropsychiatric conditions.


Poster

341. Electrical Methods to Modulate Neural Activity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 341.18/DD18

Topic: I.08. Methods to Modulate Neural Activity

Support: MOST 1062410H182008MY2
CMRPD1F0502
CMRPD1H0461

Title: Neuromodulatory effects of transcranial direct current stimulation on motor excitability and inhibition in rats

Authors: *T.-H. HSIEH1,2, H.-H. LIU3, C.-H. JUAN4, A. ROTENBERG5, Y.-C. PEI6, Y.-Z. HUANG7,2;

Abstract: Transcranial direct current stimulation (tDCS) is one of non-invasive technique for modulating neural plasticity which is considered having therapeutic potentials in neurological
and neuropsychological disorders. For the purpose of translational neuroscience research, a suitable animal model could be the best way to provide a stable condition for identifying the mechanism insights that help to explore therapeutic strategies. Here, we developed a focused brain stimulation protocol using tDCS on the motor cortex for modulating the motor excitability in anesthetized rats. The change in motor excitability was evaluated by the changes of motor evoked potential (MEP) elicited by a single-pulse epidural cortical electrical stimulus. To examine tDCS-elicited plasticity responses, immediately MEP changes and MEP input-output (IO) curve were measured at baseline and for 30 min after anodal tDCS or cathode tDCS. In the results, analogous to those observed in humans, the present experiments demonstrate long-term potentiation (LTP) and long-term depression (LTD)-like plasticity can be induced by tDCS protocol in anesthetized rats. We found that the MEPs were significantly enhanced immediately after 0.1 mA and 0.8 mA anodal tDCS and remained enhanced for 30 min compared to the baseline MEP. Similarly, the MEPs were only suppressed immediately and lasted for 30 min or more after 0.8 mA cathodal tDCS. No effect was noted on the MEP magnitude under sham tDCS stimulation. Furthermore, the IO curve slope was elevated following anodal tDCS and showed a trend towards diminished slope after cathodal tDCS. These results indicate that the developed tDCS schemes can produce consistent, rapid, and controllable electrophysiological changes in corticomotor excitability in rats. Our studies provided a step toward extending human brain stimulation protocols to translational research where motor plasticity can be manipulated in rats. This newly developed tDCS animal model would be useful for further exploring the mechanical insights and may serve as a translational platform bridging human and animal studies for establishing new therapeutic strategies in neurological disorders.


Poster

341. Electrical Methods to Modulate Neural Activity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 341.19/DD19

Topic: I.08. Methods to Modulate Neural Activity


Title: Neural elements mediating side effects during cervical vagus nerve stimulation in the pig
Abstract: Stimulation of the vagus nerve (VNS) is FDA-approved for treatment of epilepsy and depression, as well as CE marked in Europe for multiple other conditions including heart failure. Despite widespread adoption and perceived efficacy, a post-hoc analysis of patients treated for heart failure using VNS showed that only 12% of patients experienced cardiac effects one year post-implant. This might be explained by evidence that 1) A-type nerve fibers responsible for muscle responses have lower stimulation amplitude thresholds than B-type nerve fibers mediating cardiac effects, and 2) stimulation amplitudes are limited in patients such that intolerable neck muscle responses do not occur. Therefore, lower threshold muscle responses may have prevented application of higher amplitudes required to achieve cardiac effects in clinical trials of VNS for heart failure. Understanding therapy-limiting side effects to VNS and finding ways to mitigate them are important challenges towards optimizing VNS for clinical practice.

To address this gap, we developed a swine model of VNS that utilizes the clinical LivaNova VNS electrode used in human patients. The swine model most closely matches the size of the vagus nerve and fascicular organization seen in humans. We hypothesized that neck muscle responses are mediated by somatic branches of the vagus—as opposed to direct electrical activation of the muscle fibers—and tested this using neuromuscular junction blocking agents, as well as systematic somatic vagus nerve branch transection. We measured compound nerve action potentials using intrafascicular electrodes placed within the vagus to identify activation of different nerve fiber types by conduction velocity. The downstream effects of nerve fiber activation, i.e. changes in heart rate and neck muscle responses (EMG), were measured using an arterial catheter and intramuscular electrodes, respectively.

Two pathways contribute to VNS-evoked neck muscle response: activation of nerve fibers in the recurrent laryngeal branch of the cervical vagus nerve at low amplitudes and in the superior laryngeal branch at higher amplitudes. This work represents a step towards mitigating intolerable side effects of VNS, thereby allowing maximization of intended target engagement.


Poster

341. Electrical Methods to Modulate Neural Activity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 341.20/DD20
Title: Evaluating the target engagement in focal transcranial magnetic stimulation (TMS) of the rat brain


Abstract: Transcranial magnetic stimulation (TMS) is emerging as a potential treatment for neuropsychiatric disorders, including drug addiction. However, the nature, extent, and mechanisms of TMS effects remain speculative, and clinical outcomes are variable. Creating an animal model that can mimic human TMS conditions is of great value in better understanding these neurobiological mechanisms. Our lab recently developed a rodent TMS system that can induce suprathreshold focal stimulation of the rat and mouse brains (Meng et al., Brain Stim 2018). The purpose of this study is to map brain regions activated by TMS using Fos immunochemistry. Rats were implanted with a head-post on the skull, directing the TMS coil E-field toward the medial prefrontal cortex (mPFC), +2.2mm from bregma. TMS parameters were as follows: 10 Hz pulses alternating at 2s on and 6s off with a total of 1800 pulses, at either high power (455V, n=7) or low power (220V, n=4). Each experimental animal was paired with a control animal receiving sham TMS at 25V(n=11). Animals were perfused 90 minutes post-TMS. Brain slices were evaluated under brightfield 10x magnification for Fos expression at three slices along the anterior-posterior axis: AP +3.2mm, AP +2.2mm, and AP+1.2mm. Brain regions were identified by tracing them from corresponding atlas images. The brain regions of the mPFC that were counted were: M2, IL, PRL, CG1, and CG2. A customized ImageJ script was used to quantify regions by setting an intensity threshold of the image to contrast the Fos labeled cells. The cells were automatically counted based on circularity and size and confirmed by visual inspection. Compared with the sham group, animals receiving high power TMS showed significantly higher level of Fos expression, which was particularly prominent in the center of the stimulation region ( A-P +2.2mm); within this region, all mPFC subregions (CG1, M2, IL, PRL) showed significantly higher Fos expression. One millimeter posterior to the stimulus focus (A-P +1.2mm), Fos activation was significantly higher only in CG1 of the high power TMS group compared to the sham group. One millimeter anterior to the stimulus focus (A-P +3.2), there was no difference in Fos expression between these two groups. There was no difference in Fos levels between the low power TMS group and the sham group. The higher level of Fos expression under the TMS focus region (A-P +2.2 mm) suggests that the coil is indeed focal in activating the mPFC. At lower power levels, stimulation may be insufficient to stimulate the target region. Ongoing work is to quantify appropriate TMS power levels that activate specific mPFC subregions. Acknowledgement: This work was supported by NIDA IRP, NIH.

Poster

341. Electrical Methods to Modulate Neural Activity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 341.21/DD21

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH F32 NS066694

Title: An algorithm for predicting cortical activation by transcranial magnetic stimulation

Authors: G. ARABKHERADMAND¹, F. SALINAS², P. FOX², *D. MOGUL¹;
¹Biomed. Engin., Illinois Inst. of Technol., Chicago, IL; ²Univ. of Texas, San Antonio, TX

Abstract: Transcranial magnetic stimulation (TMS) is a powerful technique to noninvasively activate neurons in the brain. However, the relationship between TMS-generated electric fields (E-fields) and specific cortical responses is not well understood. The goal of this study was to investigate the relationship between induced E-fields and neocortical activation measured by metabolic responses. Human subject-specific detailed finite element models (FEM) of the head were constructed to calculate the distribution of induced cortical E-field vectors. Positron emission tomography (PET) recordings were made during concurrent TMS application as a measure of cortical activation. A functional model of local circuit connections was developed to study the relationship between applied magnetic fields and neocortical activation and was fitted to experimental data. Induced E-fields were decomposed into vectors normal and tangential to the cortical surface under the assumption that cortical pyramidal neurons and interneurons were principally activated by the corresponding field vectors, respectively. Using published cortical network connectivity properties in a computational model, neuronal sensitivities to relevant E-fields were fitted to the experimental PET data. Sensitivity of interneurons to induced tangential E-fields were found to be over twice as strong as pyramidal neuron sensitivity to induced normal E-fields which may help explain why cortical electrophysiological responses to TMS have specific sensitivities to coil orientation. Furthermore, this study produced an algorithm for predicting near-field electrophysiological responses in human neocortex with high accuracy (>95%) and specificity (>99%) that could provide an invaluable tool for planning of specific regional cortical activation critical in both research and clinical applications.

Title: Modeling the cellular effects of transcranial electrical stimulation on the cerebellum

Authors: *X. ZHANG*¹,²,³, R. HANCOCK²,³,⁴, S. SANTANIELLO¹,²,³;  
¹Biomed. Engin. Dept., ²CT Inst. for the Brain and Cognitive Sci., ³UConn Brain Imaging Res. Ctr., ⁴Dept. of Psychology, Univ. of Connecticut, Storrs, CT

Abstract: Transcranial electrical stimulation of the cerebellum has raised increasing interest with a broad range of potential clinical applications, including Parkinson's disease, dystonia, essential tremor, and ataxia. Despite great promise, results have been inconsistent thus far, and an understanding of the cellular mechanisms of cerebellar transcranial electrical stimulation is still lacking. In this study, we leverage on the highly modular and homogeneous organization of the cerebellar cortex and a unique suite of computational modeling techniques to investigate the behavior of different cerebellar cell types under the electric field generated by transcranial electrical stimulation. Multi-compartment neuron models of granule cells, Golgi cells, Purkinje cells, and cerebellar interneurons, including stellate and basket cells, were paired with electric fields generated under several electrode montages using a realistic human head model and an established computational pipeline. For each cell type, changes in membrane potentials under the effects of the electric fields are simulated in NEURON and the resultant action potential initiation, propagation, and frequency are quantified. Finally, the cellular responses are integrated into a single-compartment network model of the olivo-cerebello-thalamic system to determine the effects of transcranial stimulation on the cerebellar output. Simulation results quantify the impact of the orientation and strength of the electric field onto the cerebellar output and pave the way towards the optimization of transcranial stimulation patterns for the treatment of movement disorders involving the cerebellum, such as essential tremor.

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Poster

341. Electrical Methods to Modulate Neural Activity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 341.23/DD23

Topic: I.08. Methods to Modulate Neural Activity

Support: NINDS 5R01NS030853-25
NICHHD 5T32HD057850-10

Title: Effects of tDCS on spontaneous spike activity in a healthy ambulatory rat model

Authors: S. MILIGHETTI¹, S. STERZI², F. FREGNI³, C. A. HANLON⁴, P. HAYLEY⁵, M. D. MURPHY⁷, R. J. NUDO⁶, *D. J. GUGGENMOS⁶;

Abstract: There has been increased usage in the clinical application of non-invasive forms of neurostimulation to treat a wide variety of clinical conditions, such as stroke, epilepsy, and pain. Some types of non-invasive stimulation, such as transcranial magnetic stimulation (TMS), can directly activate populations of cortical neurons and have well described physiological and physical properties. Other forms of non-invasive stimulation, such as transcranial direct current stimulation (tDCS) do not directly depolarize neurons, but instead are thought to act more nebulously though alterations in cortical excitability by shifting membrane potentials to change the likelihood of neurons firing. How populations of individual neurons respond to this type of stimulation has not been systematically studied in vivo. Here, we present a study to determine the effects of tDCS on single-unit and LFP activity in motor cortex of freely moving, healthy rats. A total of nine rats were implanted with a 16-shank microelectrode array over M1 for neural recordings. To administer tDCS, two screws were embedded bilaterally in the skull over M1 to act as anodal and cathodal poles for tDCS, and two screws in the interparietal bone to act as electrode and tDCS ground. Each rat underwent up to 12 recording sessions testing six different randomized stimulation conditions (48 hour wash out period between conditions) varying in current intensity (maximum current density = 90.5 A/m² at 0.4 mA) and polarity (anodal or cathodal). For each session, the rat was allowed to freely move about a behavioral box while neural activity was recorded before, during 20 minutes of continuous tDCS, and one hour after stimulation to look for changes in the patterns of neural activity. After analysis of 496 neural units across all rats, we found no effect of tDCS stimulation condition on firing rate or firing pattern. Restricting the analysis to the most responsive units, subtle, but statistically significant changes occurred only in the highest intensity anodal condition.

**Poster**

**341. Electrical Methods to Modulate Neural Activity I**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 341.24/DD24

**Topic:** I.08. Methods to Modulate Neural Activity

**Support:**  P41 GM103545-18  CAREER 1351112

**Title:** Optimization of transcranial temporal interference stimulation of targets of interest using realistic human head models

**Authors:**  *S. RAMPERSAD*¹, B. ROIG-SOLVAS¹, M. YAROSSI², E. SANTARNECCHI³, A. D. DORVAL⁴, D. H. BROOKS¹;

¹Dept. of Electrical and Computer Engin., ²Dept. of Physical Therapy, Movement and Rehabil. Sci., Northeastern Univ., Boston, MA; ³Cognitive Neurol., Harvard Med. Sch., Boston, MA; ⁴Dept. of Bioengineering, Univ. of Utah, Salt Lake City, UT

**Abstract:**  **Introduction** Transcranial current stimulation (tCS) can noninvasively and transiently modulate neural activity and affect behavior. Stimulation of superficial brain regions in both healthy subjects and patients has shown promising results. Transcranial temporal interference of two oscillating currents (tTIS) has been suggested as a new form of tCS that could reach deep brain areas selectively. In a recent simulation study, we showed that tTIS electric fields can create local maxima in deep brain regions. Here, we present methods to optimize tTIS and tCS current injection patterns and compare results for several brain areas that are often targeted with deep brain stimulation, and superficial areas commonly targeted with traditional tCS.

**Methods** Individual finite element head models were made using MRIs from 10 healthy adults following informed consent; a subset of models included anisotropic brain conductivities. We placed 88 electrodes on each model, plus a reference. We simulated injecting 1 mA current between each electrode and the reference. The resulting fields could then be combined into any desired current injection pattern. We searched for optimal current patterns to stimulate target regions in hippocampus, pallidum, thalamus, motor cortex, prefrontal cortex and cerebellum. Both an exhaustive search over 146M current patterns and mathematical optimizations were performed for tTIS and tCS to select patterns that produced the highest mean field strength in each target area. Additional optimizations also constrained the field strengths in regions outside the target area.

**Results** Our approach successfully found current patterns that maximized stimulation in each target area for each head model. We present optimized results for several cases, assess
intersubject variability, and pool all results to deduce general guidelines for electrode placement. **Conclusion** Our results suggest that most deep and superficial brain areas can be reached by tTIS with field strengths similar to tCS, but tTIS allows greater selectivity.

Using a realistic human head model (A; 10 models were made with 6 tissue types each) with 88 possible electrode positions (B; standard 10-10 locations plus additional locations to cover neck and cheeks), we found the optimal tTIS current pattern (C; on an extended 10-10 schematic (top view of the head with each electrode represented by a circle), electrodes marked 1 (or 2) represent one current loop injecting current at one frequency) to maximize the electric field along the direction of the neurons in a spherical target area (white circle) in the right pallidum (D).


**Poster**

341. Electrical Methods to Modulate Neural Activity I

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 341.25/DD25

**Topic:** I.08. Methods to Modulate Neural Activity

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**Title:** Transcranial direct current stimulation (tDCS) prevents chronic stress-induced hyperalgesia in rats

**Authors:** *I. L. S. TORRES*¹², I. C. MACEDO³, L. N. SPEZIA-ADACHI², V. L. SCARABELOT¹, G. LASTE⁴, A. SOUZA¹⁵, P. R. S. SANCHES², F. FREGNI⁶, W. CAUMO¹²; ¹UFRGS - Univ. Federal Do Rio Grande Do Sul, Porto Alegre, Brazil; ²HCPA - Hosp. de Clínicas de Porto Alegre, Porto Alegre, Brazil; ³Unipampa - Univ. Federal do Pampa, Uruguaiarna, Brazil; ⁴Ctr. Universitário Univates, Lajeado, Brazil; ⁵Unilasalle - Ctr. Universitário Unliasalle, Canoas, Brazil; ⁶Harvard Med. Sch., Boston, MA
Abstract: Background: Chronic stress (CS) is associated with a decrease in pain threshold caused by the changes in neural pain circuits. It can be associated to glucocorticoid imbalance with alterations in neural circuitry. Inhibition of stress-induced pain-related neural changes by using techniques that safely induce neuro-plasticity such as transcranial direct current stimulation (tDCS) may prevent hyperalgesia triggered by CS. Objective: This study aimed to verify the effect of tDCS performed prior to CS exposure on nociceptive response. Methods: Thirty-two rats were distributed in the following groups: control; stress; sham-tDCS þ stress; and tDCS þ stress. Bicephalic active tDCS was performed for 8 consecutive days before the CS exposure. The pain threshold was evaluated using a hot plate and tail flick latency (TFL) tests. Results: The tDCS exposure increased the pain threshold on stressed rats. Conclusion: The data obtained indicate that the treatment with bicephalic active tDCS before chronic stress exposure prevents stress-induced hyperalgesia.


Poster

341. Electrical Methods to Modulate Neural Activity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 341.26/DD26

Topic: I.08. Methods to Modulate Neural Activity

Support: NSF Award#1631820

Title: Exploring depth spread of the induced electric field in transcranial magnetic stimulation with small parallel coils

Authors: *H. Bagherzadeh\textsuperscript{1}, Q. Meng\textsuperscript{2}, J. Badjo\textsuperscript{1}, X. Du\textsuperscript{3}, L. Hong\textsuperscript{3}, F.-S. Choa\textsuperscript{1};\textsuperscript{1}Univ. of Maryland, Baltimore County, Baltimore, MD; \textsuperscript{2}NIH IRP, Baltimore, MD; \textsuperscript{3}Univ. of Maryland Sch. of Med., Catonsville, MD

Abstract: Recent years, noninvasive electrical stimulation-based treatments for neuropsychiatric disorders have been of high interest in both research and clinical studies. Among them, transcranial magnetic stimulation (TMS) is widely accepted as a safe and effective method. However, current TMS coils used for this purpose are not capable of stimulating targeted deeper brain regions due to the quick energy spread at deeper regions. Deep brain stimulation highly depends on the focality, intensity, and attenuation of the induced electric field at a distance away from the coils. In our previous works, we have shown that by increasing the diameter of a coil we can reduce the field strength decay rate of induced electrical field as a function of the depth away from the coil. In that work we have demonstrated that coils with different diameters will
produce different depth strength decay curves. However, increasing the coil diameter will require to use a larger magnetic core, which can be bulky and heavy. Furthermore, a larger diameter coil will have a larger inductance. If the coil inductance goes up, the induced electrical field power will drop when the bias voltage is kept constant. To obtain high electric field strength at deep brain region, we have to reduce the coil inductance. In this work, we show that using a group of parallel-connected coils that have smaller diameters we can greatly enhance the output power and at the same time enjoy the same field strength decay rate as a function of penetration depth as a large diameter coil when the combined diameter of the group of coils are about the same as that of the large diameter coil. In addition to the advantages of smaller size and lighter weight, such an array of smaller coils can produce much higher field strength compared with that of a single large diameter coil. For the purpose of analyzing and validating the behavior of the coils, Finite Element Method (FEM) calculations (COMSOL software) and experimental implementations and measurements were conducted.

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Poster

341. Electrical Methods to Modulate Neural Activity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 341.27/DD27

Topic: I.08. Methods to Modulate Neural Activity

Title: Transcranial focal electrical stimulation effect on neuronal activity mediated through early gene expression

Authors: L. A. BAUTISTA OROZCO¹, S. OROZCO-SUAREZ¹, L. L. ROCHA², *W. G. BESIO³;
¹Inst. Mexicano Del Seguro Social, Ciudad DE Mexico, Mexico; ²CINVESTAV, Mexico, Mexico; ³Electrical, Computer, and Biomed. Engineering/Interdisciplinary Neurosci. Program, Univ. of Rhode Island/CREmedical Corp., West Kingston, RI

Abstract: Experiments were conducted to evaluate the effects of transcranial focal electrical stimulation (TFS), through tripolar concentric ring electrodes, on the expression of early genes, related to brain activity and neuromodulation. Male Wistar rats received TFS (300Hz, 200-μs biphasic square charge-balanced 50-mA constant current pulses for 2 min and 5 min); 3.5 minutes later the brains were extracted by craniotomy, the cortex (located just below the electrode) of 6 rats for the control group, and 6 TFS. RNA was extracted by the method of Trizol reagent (Life technologies) to be used in microarrays of the complete transcriptome for rat GeneChip R Rat Gene 2.0 ST Array © of Thermo Fisher Affymetrix ©, which was processed in the microarray laboratory of the National Institute of Genomic Medicine (INMEGEN) SSA.
Microarray analysis was performed using: Transcriptome Analysis Console, PANTHER classification system and Cytoscape software, to determine the molecular functions that TFS exerts on genes.

The results with 2-minute stimulation demonstrated a significant difference in the expression of 39 genes, as compared with the control group. Of these 39 genes, 19 were overexpressed and 20 were under-expressed; the main pathways involved with overexpressed genes were: angiogenesis and the signaling pathway of VEGF, while the most under-expressed genes involved the signaling pathway of the nicotinic acetylcholine receptor and response to oxidative stress. For 5 minutes, the TFS group showed a significant difference in the expression of 1563 genes compared to the naïve rats not receiving TFS. The analysis of the possible signaling pathways in which the genes could be involved showed that there are 90 signaling pathways involved with the overexpressed genes and 53 pathways involved with the under-expressed genes. These pathways are related to inflammatory response, glutamatergic transmission, transport and metabolism of neurotransmitters, pathways related to neurogenesis and signal transduction, which accounted for the majority of genes involved. These results indicate that the 5-minute TSF affects more genes than the 2-minute stimulation and may be an alternative therapy in neurodegenerative processes, as epilepsy and Parkinson disease.


Poster

341. Electrical Methods to Modulate Neural Activity I

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 341.28/DD28

Topic: I.08. Methods to Modulate Neural Activity

Support: EU Grant LUMINOUS 686764

Title: Induced and sustained entrainment of oscillatory brain activity in the human EEG after combining repetitive transcranial magnetic stimulation (rTMS) with transcranial alternating current stimulation (tACS)

Authors: *A. JAMIL*¹, T. HOSSEINIAN², M.-F. KUO³, M. A. NITSCHÉ⁴;
¹Leibniz Res. Ctr. For Working Envrn. And Human Factors, Dortmund, Germany; ²Leiniz Res. Ctr. for Working Envrn. and Human Factors, Dortmund, Germany; ³Psychology and Neurosci., Leibniz Res. Ctr. For Working Envrn., Dortmund, Germany; ⁴Leibniz Res. Ctr. For Working Envrn. An, Dortmund, Germany
Abstract: Neural oscillations are associated with many cognitive functions, including consciousness-related processes such as sleep. Developing novel non-invasive brain stimulation techniques that can modulate specific oscillatory activity may be a viable approach for understanding functional causality of oscillatory rhythms in cognition, and developing novel treatment strategies for patients with disorders of consciousness. Among existing state-of-the-art techniques, repetitive transcranial magnetic stimulation (rTMS) induces frequency-specific but relatively short-lasting neuronal oscillations, whereas transcranial alternating current stimulation (tACS) primarily entrains endogenous oscillatory activity. In this study we investigate whether phase-synchronizing these two techniques may be a viable method in order to induce and entrain non-endogenous oscillatory rhythms in the human cortex. After assembling a control circuit that could precisely synchronize rTMS pulses with the phase of the tACS, we investigated whether combined alpha (10 Hz) rTMS and tACS to the prefrontal cortex (PFC) could induce sustained resting state PFC-alpha activity. 25 healthy participants took part in a single-blind, within-subject design. Resting state EEG was recorded at baseline before, immediately after, and 15 and 30 min after an 8 min stimulation block, in both eyes-open (EO) and eyes-closed (EC) conditions. 10 Hz tACS at 1 mA was applied through four bilateral electrodes over PFC and mastoid regions (EEG positions F3/F4, and TP9/TP10). 10 Hz rTMS at 70% AMT was delivered over positions F3/F4 with pulses aligned to the positive peaks of the tACS waveform. This combined protocol was compared to five control protocols: rTMS alone, tACS alone, sham rTMS combined w/sham tACS, combined rTMS+tACS during EC, and finally rTMS pulsed at the negative tACS peak. No effects were observed with sham rTMS+sham tACS. During both EO and EC states, PFC-alpha power increased the greatest (~30%) with the combined rTMS+tACS protocol (both during EO and EC) compared to the sham, with effects lasting up to 30 min. Slightly lesser increases were observed with the combined rTMS+tACS at the negative tACS peak, as well as short-duration effects of rTMS alone, while the weakest effects were observed for tACS alone. Our work demonstrates for the first time the feasibility of combined rTMS and tACS for enhancing non-endogenous oscillations. Further investigations are needed to unravel the mechanisms underlying these findings. Our study points towards the promising potential of integrating these protocols for functionally-relevant research and clinical applications.


Poster

342. Optogenetics II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:Poster #: 342.01/DD29

Topic: I.08. Methods to Modulate Neural Activity

Support: SNF MHV PMPDP3_171265
Title: Luminance and pattern discrimination in visual cortex (V1) of transgenic Opto-mGluR6 mice

Authors: *A. BHATTACHARYYA*¹, S. KLEINLOGEL²;
¹Inst. For Physiol., Bern, Switzerland; ²Physiol., Bern, Switzerland

Abstract: Opto-mGluR6 is a chimeric protein composed of the light sensing extracellular and transmembrane domains of melanopsin and the intracellular, G-protein coupling domains of the ON-bipolar cell-specific metabotropic glutamate receptor, mGluR6. Until now, Opto-mGluR6 is the most light sensitive construct that can elicit a response roughly equivalent to the amount of light at dusk/dawn. Although recovery of vision in Opto-mGluR6 transgenic mice (Opto-mice) has been shown by performing in vivo optical imaging of the primary visual cortex (V1) from both hemispheres, there is no direct measure of visual acuity at the cortical level in the Opto-mice. To understand what the brain is actually visualizing we conducted in vivo extracellular recordings from the V1 middle layers and looked at the multi-unit activity (MUA) and visual evoked potentials (VEP). Responses were recorded and averaged for 40-50 stimuli. We consistently found robust light responses from the middle layers of V1 in response to blue light stimulation (470nm). Next we assessed if the Opto-mice can discriminate full field grating stimuli from equiluminant gray and had any measurable acuity. An average response profile showed a transient increase in firing during change in luminance or a change from grey to equiluminant. These data imply that the chimeric protein expressed in the bipolar cells of the Opto-mice can reliably and reproducibly mediate responses to light pulses. In particular, by using an image discrimination paradigm, we could show that Opto-mice cannot only distinguish luminance levels, but also identify patterns. Thus gene therapy with Opto-mGluR6 can be used as a potential approach to impart light sensitivity and pattern discrimination for visual restoration.

Disclosures: A. Bhattacharyya: None. S. Kleinlogel: None.

Poster

342. Optogenetics II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #:/Poster #: 342.02/DD30

Topic: I.08. Methods to Modulate Neural Activity

Title: Optogenetic calcium sensor, voltage indicator and chemogenetic mouse models available from the Jackson Laboratory

Authors: *J. BECKWITH, S. ROCKWOOD, C. LUTZ;
The Jackson Lab., Bar Harbor, ME
Abstract: Understanding neural circuitry in both normal and disease states is a priority of the biomedical community. To facilitate this, The Jackson Laboratory (JAX) offers an impressive array of genetically-engineered tools enabling scientists to monitor the neural activity of intact mouse brain. Top most in this tool box are mouse lines using optogenetic and transient-sensing (calcium-, voltage-) technologies. Opsins are light-activated proteins that alter membrane potential in neurons, so that stimulation with light allows rapid control of neuronal activity. Several mouse lines express improved/optimized opsins fused to fluorescent proteins. These include mice with channelrhodopsin expression directed by specific promoters. Additional control is available in mice with Cre- or Tet-dependent expression of channelrhodopsin or halorhodopsin.

GCaMP fluorescence in response to calcium is an indicator of cell activation. These include Thy1-promoter driven GCaMP6 transgenic lines and Cre or Tet-dependent GCaMP6 variants. Both cytosolic- and membrane-targeted GCaMP6 mice are available. Furthermore, mice with GCaMP8 expression in capillaries allow studying the blood/brain barrier. Several intersectional strains utilize both Cre-lox and Tet-On/-Off functionality. Removal of a floxed-STOP allows Tet-dependent expression of channelrhodopsin (ReaChR/EYFP, ChR2*H134R/EYFP), GCaMP6s, GCaMP6f, RCAmp1.07, voltage-sensor (ASAP2s), bicistronic QuasAr voltage-indicator CheRiff channelrhodopsin (OptoPatch) or substrate-dependent reporters (APEX).

This set includes mice created by the Allen Institute for Brain Science, the Genetically-Encoded Neuronal Indicator and Effector (GENIE) Project (Janelia/HHMI), Duke/MIT and several others. Designer receptors exclusively activated by designer drugs (DREADDs) are mutant G-protein coupled receptors activated by the pharmacologically-inert molecule clozapine-N-oxide. Several chemogenetic strains have Cre- and/or FLP-inducible expression of DREADDs. As extensions of MMRRC-JAX 5xFAD mouse line utility, recently available are the AD-BXD panel of F1 hybrid mice - an isogenic resource useful for studying the effect of genetic background/diversity on the 5xFAD transgenic model of Alzheimer's disease. Offerings may be searched (jax.org/mouse-search). Researchers are encouraged to donate their mouse lines via a very short online form (jax.org/donate-a-mouse). Visit The Jackson Laboratory Resources for Optogenetics, Cre-dependent Optogenetic Tools and Cre Strains for Neurobiology (jax.org/optogenetics). JAX receives support from NIH, HHMI and private foundations.

Disclosures: J. Beckwith: None. S. Rockwood: None. C. Lutz: None.

Poster

342. Optogenetics II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 342.03/DD31

Topic: I.08. Methods to Modulate Neural Activity
Title: An open database for optogenetics in nonhuman primates

Authors: *S. TREMBLAY, C. REVSIONE, M. PLATT;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Optogenetics has revolutionized neuroscience in small laboratory animals, but its impact on animal models more similar to humans, such as nonhuman primates, has been mixed. The viral transfection methods optimized for rodents do not translate perfectly to primates, whom have a bigger brain and a more complex immunological system. Thus, when designing an optogenetic experiment, primate researchers are left with scant evidence to decide on viral vectors, genetic promoters, opsins and reporter genes. To make evidence-based decisions in primate optogenetics, our community would benefit from a centralized database listing all attempts, successful and unsuccessful, at expressing opsin genes in the primate brain. Over the last year, we emailed every member of our community who reportedly attempted optogenetics in primates to ask for their contribution to a central database. On the day of writing this abstract (May 1st, 2019), thirty-five laboratories around the world accepted to contribute their data to the repository. The database listed more than 500 viral injection attempts in nonhuman primates, and is still growing. The resource is free for everyone to consult on, and to contribute to on the Open Science Framework website. The “NHP Optogenetics Open Database” provides a portal for nonhuman primate researchers around the world to share their results using optogenetics with their community, including negative unpublished results. It contains a detailed list of viral transfection attempts and their associated methods (viral constructs, injection technique, outcome measures, etc.). The goal is to avoid duplication of efforts within our community and to maximize success in applying optogenetics in nonhuman primates, with hopes of future applications in human patients.

Disclosures: S. Tremblay: None. C. Revsine: None. M. Platt: None.

Poster

342. Optogenetics II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 342.04/DD32

Topic: I.08. Methods to Modulate Neural Activity

Title: Optogenetically driven electromyographic response characteristics and modulation strategies for peripheral motor stimulation

Authors: *J. J. WILLIAMS1, A. B. SCHWARTZ3, A. VAZQUEZ2;
1Systems Neurosci. Inst., 2Radiology, Univ. of Pittsburgh, Pittsburgh, PA; 3Dept Neurobiol, Univ. of Pittsburgh Dept. of Neurobio., Pittsburgh, PA
Abstract: Optogenetic stimulation of muscle activity via excitation of nerves genetically labeled with light-sensitive ion channels has recently emerged as a promising alternative to functional electrical stimulation for the purposes of reanimation of paralyzed muscle activity. While electrical stimulation has an extensive background of research data as well as clinical experience to guide the selection of stimulation patterns for muscle activation, the breadth of data available regarding muscle responses to peripheral optogenetic stimulation is comparatively basic. In this study, we sought to narrow this gap by examining electromyographic (EMG) and muscle force responses to single optical pulses, optical pulse trains of varying frequency, and pulse-width modulation of optical trains used to stimulate peripheral motor nerves in a Thy1-ChR2 transgenic mouse model. Normalized EMG responses to an optical pulse train demonstrated a consistent decay in magnitude with time constant proportional to optical pulse train frequency. Single electrical stimulus pulses could be tuned to elicit similar responses to optical stimuli, while responses to electrical pulse trains showed a slower decay rate with increasing frequency than matched optical stimuli. The impulse and frequency responses of EMG and force activity were then used to model an “effective optical stimulus” transformation in agreement with previous computational models of in vitro ChR2 kinetics. Finally, optical pulse trains utilizing either constant pulse width or pulse-width modulation were used to stimulate brief movements in anesthetized animals. Constant pulse-width trains initiated brief, jerky movements with a long refractory period, while modulating the optical pulse width in a sinusoidal fashion elicited smooth limb movements that could be repeated over a longer time period. These results from an animal model with “ideal” opsin expression highlight critical differences between peripheral optical and electrical stimulation that may make conventional electrical stimulation strategies incompatible with optical stimulation. Furthermore, we present an alternative optical stimulation approach aimed at producing smooth, functional movements in prosthetic applications.

Disclosures: J.J. Williams: None. A.B. Schwartz: None. A. Vazquez: None.

Poster

342. Optogenetics II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 342.05/DD33

Topic: I.08. Methods to Modulate Neural Activity

Support: SFB 1089 Synaptic Micronetworks in Health and Disease

Title: How does optogenetic modulation of the locus coeruleus affect the neuronal network activity in the cortex?

Authors: *D. MERCAN¹, M. POFAHL², N. NIKBAKHT², S. SCHWARTZ¹, H. W. BECK²,³, M. FUHRMANN³, M. T. HENEKA¹;¹Dept. of Neurodegenerative Dis. & Gerontopsychiatry, Univ. of Bonn Med. Ctr., Bonn,
Abstract: Norepinephrine (NE) is one of the major neurotransmitters in the central nervous system. The synthesis of NE is mainly achieved by the locus coeruleus (LC), which sends projections almost to all brain areas including the cortex and hippocampus. The LC plays a role in modulating general arousal, selective attention, stress, sleep architecture, synaptic plasticity, learning and memory. It has been demonstrated that the LC is one of the brain regions that is first affected at earliest in Alzheimer’s Disease (AD). Thus, the levels of NE are decreased in the LC projection areas during AD pathology. Importantly, the reduction in NE concentration strongly correlates with memory loss and cognitive impairment in humans and animal models.

Our aim was to investigate the activity of cortical circuits in response to transient silencing of the LC via optogenetic modulation in both wild type and APP/PS1 mice. For this we used mice that selectively expressed halorhodopsin (eNpHR3.0) in tyrosine hydroxylase positive LC neurons. The mice were subjected unilaterally to light of 593nm wavelength through an implanted cannula to achieve inhibition of the LC. The neuronal activity was measured in the cortex before, during and after the inhibition of LC by examining calcium (Ca$^{2+}$) currents using two-photon in vivo laser scanning microscopy. For visualization of the Ca$^{2+}$ currents GCaMP6, genetically-encoded Ca$^{2+}$ indicator, was used.

Our simultaneous recordings of cortical Ca$^{2+}$ transients revealed that photo-inhibition of the LC resulted in a significant decline in cortical neuronal activity for both wild type and APP/PS1 mice, while control animals did not show any changes in neuronal activity. These changes appear to be transient, as 1 hour after silencing we detected a slightly increased neuronal activity, which suggests a recovery of neurons after photo-inhibition.

In addition, using the same experimental strategy we also investigated whether optogenetic inhibition of the LC changes the structural plasticity of dendritic spines. In order to visualize dendritic spines Thy-1/YFP mice were used and spine density over time was imaged by 2-photon in vivo laser scanning microscopy. We observed that 2 days after the 1 hour inhibition, the number of lost spines increased and gained spines decreased, however control animals showed no change. These results suggest that LC silencing may affect the dynamics of dendritic spines in cortex.

Our findings shed light on the important modulatory function of the LC on cortical neuronal network activity, which might be modulated through the structural plasticity of dendritic spines.


Poster

342. Optogenetics II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 342.06/DD34
Abstract: Reversible inactivation techniques are valuable for elucidating links between neural activity and behavior. Pharmacological agents achieve robust inactivation but have limited spatiotemporal precision. In contrast, suppressive optogenetic tools achieve weak inactivation but have superior spatiotemporal precision.

To achieve robust and temporally precise inactivation of small (~1 mm³) cortical regions in macaques (three male Macaca mulatta), we used an AAV vector carrying the channelrhodopsin-2 (ChR2) gene along with a mDlx5/6 enhancer sequence which restricts gene expression to cortical GABAergic cells (Dimidschstein et al. 2016). We vetted this approach in the primary visual cortex (V1), a well understood area of the cerebral cortex and an ideal testbed for probing the effect of neural inactivation on behavior.

Histological analyses confirmed that ChR2 expression was largely restricted to cortical inhibitory interneurons. Extracellular recordings confirmed that laser stimulation modulated spiking activity of neurons at the AAV injection sites. Some units were activated at short latency, consistent with direct activation. Others were suppressed, consistent with them receiving synaptic input from directly activated ChR2+ inhibitory interneurons. We assessed the behavioral efficacy of the optogenetic perturbation using a visually guided saccade task and a contrast detection task. In the contrast detection task, a Gabor stimulus appeared on half of the trials, and the monkey was rewarded for reporting this event with a saccade to a remote target. In both tasks, laser simulation was pseudorandomly delivered on half of the trials. On laser trials, visual sensitivity was reduced within receptive fields of the illuminated neurons, consistent with illumination activating inhibitory circuits and suppressing V1 output. Overall, we demonstrate the utility of AAV-mDlx5/6-ChR2 as a robust and spatiotemporally precise inactivation tool for revealing the effects of neural activity on behavior in macaques.

Reference

Disclosures: A. De: None. Y. El-Shamayleh: None. G.D. Horwitz: None.
Poster

342. Optogenetics II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 342.07/DD35

Topic: I.08. Methods to Modulate Neural Activity

Title: Optogenetic manipulation of top-down feedback in mouse visual cortex

Authors: *A. BROGGINI, I. ONORATO, C. URAN, A. TZANOU, M. VINCK;
Ernst Strüngmann Inst., Frankfurt am Main, Germany

Abstract: Cortical feedback is thought to play an important role in many cortical processes such as attention and predictive coding. In this study we investigated how top down feedback (FB) projections from higher visual areas modulate neuronal activity in primary visual cortex (V1), and how this modulation depends on cortical layers and behavioral state. For this purpose, we recorded primary (V1) and secondary (V2) visual areas simultaneously with high-density silicon probes in awake, head-fixed mice placed on a running platform. In order to identify and later modulate connected visual areas, we used retrograde viral transfection in combination with microscopy. In a first experiment, we expressed the opsins Chronos or Channelrhodopsin (ChR2) in V2 FB neurons projecting to V1 through injection of the respective retrograde virus in V1. This led to EYFP fluorescence expression in both areas which we used to position our laminar probes. We observed that optogenetic light stimulation in area V2, even at relatively low light intensities, led to an increase of the activity of V1 neurons throughout supra and infra-granular layers. This effect may have been mediated by a direct activation of FB neurons, but could alternatively be explained by antidromic activation of V1 axons. In a second experiment, we therefore used another viral strategy in which expression of ChR2 or Chronos was restricted to area V2. Specifically, we injected AAV-Cre with a retro-helper in area V1 and combined this with Cre-dependent expression of ChR2 or Chronos in area V2. Using the described viral strategy, we obtained localized expression of opsins in area V2. We performed simultaneous recording of V1 and V2 throughout all cortical layers guided by fluorescence patterns, and found closely overlapping receptive fields. With the double virus strategy, we did not find a general increase in V1 activity due to optogenetic activation of FB neurons in area V2. This indicates that optogenetic activation of higher areas using AAV retro-helper can lead to strong antidromic activation in lower areas, even at low light intensities. By analyzing the data from the second experiment, we show that optogenetic stimulation of FB neurons in V2 leads to a layer-specific pattern of activity enhancement and suppression in area V1.

Disclosures: A. Broggiini: None. I. Onorato: None. C. Uran: None. A. Tzanou: None. M. Vinck: None.
Poster

342. Optogenetics II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 342.08/DD36

Topic: I.08. Methods to Modulate Neural Activity

Support: NHMRC Grant APP1156727

Title: Novel molecular tools for targeted subcellular neuronal transgene expression and neural circuit dissection

Authors: *A. D. WYKES*\(^1,2\), M. DEL ROSSO DE MELO\(^3\), S. L. GORDON\(^1\), L. M. PALMER\(^1\), A. M. ALLEN\(^3\), R. A. D. BATHGATE\(^1,2,4\);

\(^1\)The Florey Inst. of Neurosci. and Mental Hlth., Parkville, Australia; \(^2\)Florey Dept. of Neurosci. and Mental Hlth., \(^3\)Dept. of Physiol., \(^4\)Dept. of Biochem. and Mol. Biol., The Univ. of Melbourne, Parkville, Australia

Abstract: Neurons are highly polarized cells, with localised protein trafficking and expression providing dendrites, axon and soma with distinct structural, chemical and functional characteristics. Within the brain, densely interconnected networks formed by neurons give rise to further complexity. Data generated using transgenic approaches, which underlie much of modern neuroscience, can therefore be difficult to interpret without targeted subcellular expression of their protein actuators. One common method to direct transgene trafficking has been through fusion with targeting motifs derived from endogenously localised proteins. In studies presented here, we applied this approach to alter trafficking of the chloride-conducting ‘GtACR2’ opsin channel. Though having strong inhibitory effects at the soma and dendrites, differential ion flux through axonal GtACR2 has been found to elicit membrane depolarization rather than hyperpolarization. To avoid this axonal depolarization and achieve targeted somatic inhibition, we developed a GtACR2 variant fused with a trafficking motif from the potassium channel Kv2.1, previously shown to provide soma-restricted channelrhodopsin 2 (ChR2) expression. Initial studies expressing this construct from a recombinant adeno-associated viral (rAAV) vector in neurons of the rat pre-Bötzinger complex revealed greatly improved membrane trafficking and phenotypic effects of GtACR2-Kv2.1 compared to wild-type (WT) GtACR2 (n = 3 rats per construct). However, some axonal expression of GtACR2-Kv2.1 was still evident in brainstem and primary cultured hippocampal neurons. These findings are consistent with recent publications describing similar ‘somatic’ GtACR2-Kv2.1 fusion constructs, which despite reducing axonal expression relative to WT-GtACR2 under certain conditions do not provide exclusively somatic expression. Further rAAV vectors, expressing either GtACR2 or ChR2 fused with potential axonal or dendritic trafficking motifs, have been developed and are being characterised, particularly in ongoing studies of rat ventral respiratory column circuitry.
Successful identification of novel variants with targeted expression, along with GtACR2-Kv2.1-expressing vectors we have developed, will provide valuable tools in a range of neuroscience applications.


Poster

342. Optogenetics II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 342.09/DD37

Topic: I.08. Methods to Modulate Neural Activity

Support: ERC StG 337637 OptoNEUROMOD
Adelis Foundation
Human Frontier Science Program

Title: Optogenetic silencing of neurotransmitter release with a naturally-occurring invertebrate rhodopsin

Authors: M. MAHN\textsuperscript{1}, I. SARAF-SINIK\textsuperscript{1}, P. PATIL\textsuperscript{1}, M. PULIN\textsuperscript{2}, B. ROST\textsuperscript{3}, R. LEVY\textsuperscript{1}, E. BITTON\textsuperscript{1}, S. PALGI\textsuperscript{1}, I. DAVIDI\textsuperscript{1}, D. SCHMITZ\textsuperscript{2}, J. WIEGERT\textsuperscript{2}, *O. YIZHAR\textsuperscript{1}; \textsuperscript{1}Weizmann Inst. of Sci., Rehovot, Israel; \textsuperscript{2}Res. Group Synaptic Wiring and Information Processing, Ctr. for Mol. Neurobio., Hamburg, Germany; \textsuperscript{3}Charité-Universitätsmedizin, Berlin, Germany

Abstract: Understanding the role of defined neuronal pathways in cognitive and behavioral processes requires techniques for spatially selective and temporally-precise in vivo manipulation of synaptic transmission. Powerful optogenetic tools have been developed that allow light-driven excitation of long-range projecting axons. Although temporally-precise inhibition of presynaptic terminals has proven challenging with conventional optogenetic techniques, chemogenetic tools have been shown to efficiently suppress synaptic transmission through activation of the Gi/o signaling pathway. We examined the expression and function of several Gi/o-coupled rhodopsins in mammalian neurons. We found that a mosquito rhodopsin, homologous to the vertebrate encephalopsin (OPN3), can effectively recruit Gi/o signaling in mammalian neurons. Illumination of axonal terminals expressing an enhanced variant of this rhodopsin (eOPN3) led to robust, light-sensitive and stable suppression of presynaptic neurotransmitter release. Activation of eOPN3 in autaptic hippocampal neurons decreased the size of evoked excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) and increased the paired-pulse ratio, indicative of reduced neurotransmitter release. In organotypic hippocampal slices, paired whole-cell patch-clamp recordings showed that activation of eOPN3 at Schaffer collateral presynaptic
terminals reduces the amplitude of EPSCs without inhibiting CA3 cell action potential firing. We further validated eOPN3 in vivo using extracellular recordings in the barrel cortex, demonstrating temporally-precise inhibition of responses to whisker stimulation during activation of eOPN3 in thalamocortical axons. Our findings demonstrate that eOPN3 can be used to selectively suppress synaptic transmission through direct illumination of axonal terminals. This novel optogenetic approach will be useful for studies exploring the functional consequences of silencing specific long-range axonal projections.


Poster

342. Optogenetics II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 342.10/DD38

Topic: I.08. Methods to Modulate Neural Activity

Title: Optogenetic tool for spatiotemporal RNA visualization and manipulation

Authors: *Y. D. RIANI*¹, W. WANG²;
¹Life Sci. Inst., Ann Arbor, MI; ²Univ. of Michigan-Ann Arbor, Ann Arbor, MI

Abstract: Optogenetic tools allow the manipulation of biological function using light and have transformed the way neuroscience research is conducted in the last decade. Photoactivatable Cas9, which is composed of a light sensing protein and a DNA binding module - CRISPR/Cas9, has enabled in situ regulation of DNA in a spatiotemporal manner. Many biological processes rely on regulation of mRNA translation at specific cellular locales. Therefore, RNA regulation, and not just DNA, must be considered. However, there are few reports on optogenetic tools for manipulating RNA’s function. Here I introduce a rational design of light-switchable RNA binding protein for spatiotemporal visualization and manipulation of RNA. We choose Human PumHD (Pumilio Homology Domain) protein as the RNA recognition protein as PumHD is modular and can be designed to bind to any RNA sequences. For light regulation, we will incorporate the light sensing domain eLOV, an engineered version of the LOV2 domain that changes conformation effectively upon blue light irradiation. The goal is to design a light switchable PumHD that doesn’t bind to target RNA in dark and only binds when light is administered. This way, visualization or manipulation of RNA would be achieved with minimal perturbation to normal physiological function. In this poster, I will show the design and optimization of the light switchable PumHD.

Abstract: Glutamate, a major neurotransmitter in the brain, is an important signaling molecule in biological organisms. Glutamate dysregulation is associated with stroke and neurodegenerative disorders including Alzheimer’s disease. Genetically-encoded fluorescent protein (FP) indicators have emerged as powerful tools for studying neuronal circuits due to their specificity and high spatiotemporal resolution. Scientists from Janelia have developed an intensity-based glutamate sensing fluorescent reporter (iGluSnFR) constructed from Gltl domain and circularly permuted GFP. However, current variants are still hampered by several limitations including brightness, signal to noise ratio (SNR) for deep tissue imaging, and lack of spatial resolution. To address these fundamental challenges, efforts are being diverted to developing a new generation of iGluSnFR indicators that will enable deeper imaging into tissues using two-photon excitation with 1030-nm lasers. Recently, different variants of iGluSnFR with emission profiles ranging from blue to yellow were reported to improve upon the GFP based iGluSnFR. These new variants are brighter, have a higher spatial and temporal resolution, are more photostable, and allow imaging at kilohertz rates. The chromophore mutations from GFP iGluSnFR to Azurite FP lead to a blue emission profile, mTurquoise mutations lead to a cyan emission profile, and mVenus mutations on top of iGluSnFR lead to a yellow emission profile. Since the yellow Venus-based prototype also resulted in a red-shifted excitation that enables excitation with femtosecond fiber lasers, it is favorable to use Venus-iGluSnFR with 2P imaging due to its higher fluorescence change and brightness at 1030nm compared to the original iGluSnFR. We performed iterative directed evolution on top of Venus-iGluSnFR prototype and discovered nine unique variants with varying mutations that further red-shifted the current Venus-iGluSnFR excitation profile by 10nm. Two of these variants that have an excitation maximum at 518nm (1-photon) exhibit a glutamate-dependent response of 197% and 178%, at 1,030nm, and provide us with a starting point for a new line of red-shifted Venus-iGluSnFR’s. Using these new variants of red-shifted Venus-iGluSnFR’s, we hope to create a new iGluSnFR that researchers can use with 2-photon microscopy to investigate the physiological activity of organs, understand the signaling patterns deep within the tissue, and use to study the various disease states at the resolution and depth that is currently not possible.
Disclosures: A. Aggarwal: None. K. Podgorski: None.

Poster

342. Optogenetics II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 342.12/DD40

Topic: I.08. Methods to Modulate Neural Activity

Title: A novel optogenetic molecular probe for light-induced inhibition of vesicular release

Authors: *J. WON¹, C. J. LEE², W. HEO³; ¹KAIST/IBS, Daejeon, Korea, Republic of; ²IBS, Daejeon, Korea, Republic of; ³IBS/KAIST, Daejeon, Korea, Republic of

Abstract: Brain connectivity is composed of highly network which communicate each other: Neurons develop synapses, which is highly specialized structure and enabling exchange signals between each other. During these bidirectional communications between ‘neurons and neuron’, and ‘neurons and glia’, in a large percent of transmitters are released by secretory vesicles. Thus, manipulation of vesicle release technique is required to understand brain connectivity. The current optogenetic approaches, such as archaerhodopsin or halorhodopsin, for synaptic inhibition do not directly inhibit the vesicular release, but indirectly by hyperpolarizing the membrane potentials. Moreover, recent studies raise the issue of unexpected calcium increase and synaptic release during archaerhodopsin or halorhodopsin activation.

In this study, we have developed genetically-encoded optical inhibition system, Opto-vTrap, for direct inhibition of vesicular exocytosis. We used optogenetic dimers based on plant Arabidopsis cryptochrome2 (CRY2) and cryptochrome-interacting basic-helix-loop-helix (CIB1) interaction that oligomerize each other under blue light (~488nm) illumination. We co-expressed cytosolic N-terminal domain of CRY2 (PHR), and N-terminus of CIB1 (CIBN) fused with vesicle-associated membrane protein (VAMP2) to allow direct interaction with exocytotic vesicles. Under confocal microscopy, we confirmed the light-stimulated reversible clusterization of vesicle in Opto-vTrap transfected Cos-7 cells within 5 minutes. Under TIRF microscopy, we observed complete inhibition of exocytosis of NPY-containing vesicles in Opto-vTrap transfected NS-1 cells under blue light illuminations. In Opto-vTrap virus injected hippocampal slice, electrically-evoked synaptic currents were decreased upon blue light without paired-pulse ratio (PPR) changes.

Not only in the neurons, but also in GFAP-promoter-driven Opto-vTrap virus injected mice, the frequency of astrocytic vesicle-mediated fast inward NMDAR-mediated currents was selectively and reversibly decreased upon blue light illuminations within 5 minutes.

Our study provides a novel optogenetic molecular probe for direct inhibition of both vesicular synaptic- and glio-transmission upon light stimulations. This system will be applied to excitable
neuronal cells as well as non-excitatory cells, such as glial cells, without any need for hyperpolarization of the membrane potentials.

**Disclosures:** J. Won: None. C.J. Lee: None. W. Heo: None.

**Poster**

**342. Optogenetics II**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

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**Topic:** I.08. Methods to Modulate Neural Activity

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UK Engineering and Physical Sciences Research Council Grant EP/R00188X/1

**Title:** TRPswitch — A step function chemo-optogenetic ligand for the vertebrate TRPA1 channel

**Authors:** *P.-Y. LAM¹, A. R. THAWANI³, E. BALDERAS², D. CHAUDHURI², M. J. FUCHTER³, R. T. PETERSON¹;
¹Pharmacol. and Toxicology, ²Intrnl. Med., Univ. of Utah, Salt Lake City, UT; ³Chem., Imperial Col. London, London, United Kingdom

**Abstract:** Chemo-optogenetics has produced powerful tools for optical control of cell activity, but current tools suffer from a variety of limitations including low unitary conductance, the need to modify the target channel, or the inability to control both on and off switching. Using a zebrafish behavior-based screening strategy, we discovered “TRPswitch”, a photoswitchable non-electrophilic ligand for the transient receptor potential ankyrin 1 (TRPA1) channel. TRPA1 exhibits high unitary channel conductance, making it an ideal target for chemo-optogenetic tool development. Replacement of the TRPswitch azobenzene with azoheteroarene yielded TRPswitch-B, with improved photoswitching efficiency and longer thermal half-life. The TRPswitch compounds enable reversible and repeatable light-induced activation and deactivation of the vertebrate Trpa1b channel with violet and green light, respectively. The utility of TRPswitch compounds was demonstrated in larval zebrafish hearts exogenously expressing Trpa1b, where heartbeat could be controlled using TRPswitch and light. Therefore, TRPA1/TRPswitch represents a novel step-function chemo-optogenetic system with a unique combination of high conductance, high efficiency, activity against an unmodified vertebrate channel, and capacity for bidirectional optical switching. Overall, this TRPswitch/TRPA1
chemo-optogenetic system will be particularly applicable in systems where a large depolarization current is needed or sustained channel activation is desirable.

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**Poster**

342. Optogenetics II

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 342.14/DD42

**Topic:** I.08. Methods to Modulate Neural Activity

**Title:** Altering synaptic composition with a novel optogenetic approach

**Authors:** *A. BAREGHAMYAN*¹, G. G. GROSS¹², X. LU², W. ZHANG², R. E. CAMPBELL², D. B. ARNOLD¹;
¹USC, Los Angeles, CA; ²Chem., Univ. of Alberta, Edmonton, AB, Canada

**Abstract:** We have generated a novel, recombinant, photo-activatable protein complex, with extremely low background activity in the dark. This system is based on the photocleavable protein PhoCl, whose amino acid backbone is cleaved following absorption of 400 nm light, causing a 13-mer peptide to be released from its C-terminus. Using mRNA display we have generated a 10 amino acid peptide (PhoCl binding peptide, Pbp) that binds with high affinity and specificity to the pocket that is created by release of the native C-terminus of PhoCl, thus creating PhoCl-containing Light Inducible Complex, PhLIC. Because there is virtually no cleavage of PhoCl in the dark, and because Pbp does not bind to uncleaved PhoCl there is negligible interaction between PhoCl and Pbp in the absence of light. To demonstrate the efficacy of this system we have targeted PhoCl to different subcellular locations such as to the Golgi, and shown that we can inducibly target protein fused to PBP to those locations with light. Furthermore, we have shown that using this system we can ablate inhibitory synapses in a light-dependent manner using a split version of the protein GFE3, which uses an E3 ligase targeted to inhibitory synapses to degrade Gephyrin. In the dark, ablation of Gephyrin by the PhoCl/GFE3 complex is undetectable, even when it has been expressed for over one week. Because of its low background activation, even over long periods of time, we believe that this system will be practical for applications requiring photo-activation in vivo. We are also extending the application of PhLIC to generate a light-inducible system for eliminating excitatory synapses.

**Disclosures:**  A. Bareghamyan: None. G.G. Gross: None. X. Lu: None. W. Zhang: None. R.E. Campbell: None. D.B. Arnold: None.
Title: Photosensitive regulation of voltage-gated potassium currents

Authors: H. H. JERNG¹, J. PATEL¹, B. R. ARENKIEL², *P. J. PFAFFINGER¹;
¹Neurosci., ²Mol. & Human Genet. and Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Optogenetics provides exciting tools to activate or silence specific neuronal populations; however, what is lacking are good optogenetic tools to explore the roles that specific neuronal currents, such as voltage-gated potassium (Kv) channels play in shaping neuron and circuit function, and behavior. To create such a tool we engineered voltage-gated potassium (Kv) channels with the light-sensitive LOV domain of Vaucheria frigida Aureochrome 1 (VfAu1-LOV) fused to the N-terminus of the Kv channel T1 domain, to create Opto-Kv channels. Using electrophysiological methods, we then tested these Opto-Kv channels in Xenopus oocytes and mammalian cells to characterize the light-dependence of channel expression. Our studies found strong induction of current with blue light for all tested mammalian and invertebrate Opto-Kv channels (aKv1, mKv1.1, mKv2.1, mKv3.1) of between 3.5-10x depending on the construct and expression system. Consistent with a regulation of channel assembly and trafficking, the induction and reversal kinetics were 5-10x faster in mammalian cells at 37 °C than Xenopus oocytes at 16 °C, with maximal current changes occurring within 1-3 hrs of the initiation or termination of blue light (A). We also found that a dominant negative construct Opto-Kv1(V400D) dramatically suppressed currents by 60-90% in a light dependent manner with a similar time course (B). Finally we tested if Opto-Kv1(V400D) expressed in mitral cells could produce light dependent olfactory behavioral effects similar to the Supersmeller phenotype seen with Kv1.3 knockout. Using a FLEx AAV to restrict expression of Opto-Kv1(V400D) plus P2A-FP to targeted CRE-expressing mitral cells (C), we found that 2-3 hrs of blue light exposure to the bulb was sufficient to produce a significantly increased sensitivity to a novel odor after desensitization to a structurally similar odor(D). In conclusion, Opto-Kv constructs provide a new way to examine the roles of normal Kv channels and disease-causing mutations in brain function and behavior.

Poster

342. Optogenetics II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 342.16/DD44

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant NS099429

Title: Wide-field multi-scale areal parcellation of neural circuits in mice

Authors:  *L. M. BRIER, J. R. BUMSTEAD, H. B. BANKS, A. R. BICE, J. P. CULVER; Radiology, Washington Univ. In St. Louis, Saint Louis, MO

Abstract: Improved parcellation of neural networks has been a goal spanning multiple decades of research in the functional MRI community in order to navigate the amazing complexity of the human brain. While advances in this realm [1] have led to higher-level organizational structures, increased statistical rigor, and reduced data complexity, the same mathematical development in the mouse brain, which is frequently studied to understand human conditions, has been lagging. To this end, we used functional concurrent optical intrinsic signal (fcOIS) and fluorescence
imaging combined with genetically encoded calcium indicators (GECIs, e.g. GCaMP6) to study mouse brain circuits in a cell-specific manner. Here, sequential wide-field illumination supplied by four LEDs (470nm, 530nm, 590nm, 625nm) was captured by a high-powered sCMOS sensor (Andor Zyla) coupled with a 1X objective lens (Olympus MVX10, NA=0.25), which allowed for concurrent calcium and hemoglobin imaging with high spatial resolution across the whole cortex at 15 frames per second (fps). A large field-of-view craniotomy spanning from the anterior suture to lambda and 8mm laterally allowed for direct brain imaging and increased spatial resolution due to less light scattering medium in the imaging path. The unique design of the optical path allows for targeting various cell types using transgenic Thy1-GCaMP6 or virally injected Aldh1l1-RGECO mice for direct excitatory neuron or astrocyte recording, respectively. Specifically in the present work, wide field Thy1-GCaMP6 transients were recorded either during rest or various types of sensory/motor stimulation. During rest, functional connectivity (FC) maps were calculated and evaluated against commonly used mesoscopic metrics of recording whole-cortex brain activity [2]. The robustness of commonly used parcellation techniques, including a k-means clustering approach [3], across multiple spatial scales was evaluated. Further developments will allow for more sophisticated mathematical techniques to parse out more accurate cortical networks and how these networks deteriorate in different disease states.

References:


Poster

342. Optogenetics II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #/Poster #: 342.17/DD45

Topic: I.08. Methods to Modulate Neural Activity

Support: BrainBIT - ERC Grant 692943

Title: Mesoscopic imaging of optogenetically-evoked motor maps reveals patterns of cortical activation specific for movement category

Authors: *E. MONTAGNI, F. RESTA, G. DE VITO, A. SCAGLIONE, A. ALLEGRA MASCARO, F. S. PAVONE; LENS - European Lab. for Non-linear Spectroscopy, Univ. of Florence, Sesto Fiorentino, Italy
Abstract: Neuronal networks in living organisms are highly interconnected. Usually, to study their functional roles in healthy conditions, task-evoked neuronal responses are correlated with the behavioral readout in freely moving or head-fixed animals. Mapping of motor representation has been classically performed by inducing reproducible movement via serial electrical stimulation of targeted cortical sites. Recently, optogenetics proved to be a useful tool to manipulate targeted neuronal circuits using light. In parallel, the development of red-shifted genetically encoded calcium indicators (red-GECIs) like jRCaMP1a allowed to reduce the spectral overlap with the most common optogenetic actuator, ChR2. Combining these optical tools is possible to develop all-optical systems, which are smart approaches for long-term low-invasive studies of neuronal patterns. Here we developed a new all-optical system for motor mapping by combining simultaneous wide-field calcium imaging, optogenetic stimulation of neuronal activity and recording of behaviour in awake mice. Animals were double transfected with jRCaMP1a and ChR2 over one cortical hemisphere by local AAV injections. The laser beam for stimulation was displaced using Acousto-Optic Deflector (AOD). In this way, we were able to create temporal patterns of light delivered to the motor cortex. First, we characterized the functional response by performing single pulse optogenetic stimulation and we observed that evoked calcium signals increase at increasing laser power. Furthermore, we reproducibly induced two distinct complex behaviours: (i) grasping like movements and (ii) tap like movements by applying trains of optogenetic stimulation on the rostral forelimb area (RFA) and caudal forelimb area (CFA) respectively. The analysis of the calcium response induced by single site optogenetic stimulation showed that the spatio-temporal patterns of neuronal activity are specific and well separated for movement category. Preliminary experiment using complex optical patterns interference confirmed these results. We believe that our combination of techniques represent a fundamental tool to unravel the interplay of brain activity and motor control in healthy and pathological settings.

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Poster

342. Optogenetics II

Location: Hall A

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Program #/Poster #: 342.18/DD46

Topic: I.08. Methods to Modulate Neural Activity

Support: CIHR Grant FDN-143209
Canadian Partnership for Stroke Recovery
Brain Canada for the Canadian Neurophotonics Platform to THM
Brain Canada Multi-Investigator Research Initiative program that THM was part of
Title: Real-time neural feedback of mesoscale cortical gcamp6 signals using raspberry-pi

Authors: *P. K. GUPTA, T. H. MURPHY;
Dept. of Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Previous work has found that mice can learn to control specific neuronal ensembles using sensory (eg. auditory) cues (Clancy et al. 2014) or even artificial optogenetic stimulation (Prsa et al. 2017). Real-time neurofeedback of cortical mesoscale brain activity can be used in goal-directed training and learning. In the present work, we measure mesoscale cortical activity with GCaMP6s and provide graded auditory feedback (within ~100 ms after GCaMP fluorescence) based on changes in neuronal activation within a specified region of interest (ROI). We define a compact, low-cost optical brain-machine interface capable of image acquisition, processing, and conducting closed-loop auditory feedback for water reward experiments, using a multithreaded program on a single Raspberry Pi. The ROI activation level, calculated as the fraction of pixels that exceed their running baselines, determines the pitch of the audio feedback (1-24 kHz in quarter-octave steps). Water rewards are delivered if the activation level surpasses a preset threshold. To investigate learning in this context, water-deprived tetO-GCaMP6s mice (N=4) were trained for about 45-minute sessions per day for six days. Results across daily training sessions indicate that mice increase the number of brain-activity mediated water reward deliveries (p<0.001, over 6 days) even at increasing thresholds (25-80% activation of ROI pixels). Analysis of the reward-triggered brain activity (dF/Fo) over time indicated that mice progressively learned to activate the cortical ROI to a greater extent (p=0.002) than surrounding areas. In this regard, training increased the number of rewards(p<0.001) and allowed all the mice to perform at a higher threshold (p=0.002). In contrast, randomizing the reward delivery led to a decline in % pixel activation in the ROI (p=0.0015). To determine the impact of auditory feedback on this type of cortical modulation, we trained mice with auditory feedback (AF, N=2) and with no audio feedback (NAF, N=2). We found that AF mice who received auditory feedback were able to increasingly modulate cortical activity and perform better than NAF mice (p=0.009). Additionally, AF mice showed persistently increased cortical activity in ROI over six days of recording (p<0.0001 daily; p<0.0001 overall) while NAF mice exhibited increase within the daily tasks (p<0.01) but it did not last over the days (p=0.08). In conclusion, we developed an open-source system for closed-loop feedback that can be added to experimental scenarios for brain activity training and could be possibly effective in inducing neuroplasticity.

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Poster

342. Optogenetics II

Location: Hall A

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**Topic:** I.08. Methods to Modulate Neural Activity

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NVKP_16-1-2016-0043  
2017-1.2.1-NKP-2017-00001

**Title:** Random access 3D photostimulation and calcium imaging for in vivo cortical measurements using acousto-optical two-photon microscopy

**Authors:** *C. J. CSUPERNYÁK*¹², G. SZALAY¹, M. MAROSI¹, G. KATONA³¹, K. ÓCSAI³, R. BOLLA², A. FEHÉR², M. VERESS², Á. SZEPESI¹, B. ROZSA¹;  
¹IEM HAS, Budapest, Hungary; ²Budapest Univ. of Technol. and Econ., Budapest, Hungary; ³Pázmány Péter Catholic Univ., Budapest, Hungary

**Abstract:** The major direction of developments in two-photon microscopy is to exploit the widening toolset of optogenetics to allow simultaneous stimulation and imaging in 3D which is an excellent approach to further understand mechanisms in the living brain. Efficacy of two-photon optogenetics is usually limited by single site stimulation. We sought to overrun this limitation using cutting-edge quasi-simultaneous two-photon photostimulation and Ca^{2+}-imaging.

We used a novel acousto-optical scanning solution which is controlling two laser lines with different wavelengths in three dimensions. Switching between the two lasers and scanning patterns happens almost instantaneously, making it possible to perform photostimulation and Ca^{2+}-imaging in an interleaved fashion. We used Thy1-Cre mouse line expressing soma-targeted ChrimsonR-mRuby2 and GCaMP6f.

By fine-tuning the scanning parameters, we were able to record every second frame for imaging during stimulation, which let us follow activity even during photostimulation. With this technique it is possible to affect and monitor at least 80 neurons in a desired 3D network. Accuracy of acousto-optical focusing also enables precise dendritic stimulation at even more sites than for cells. As a result, since interlaminar connection and dendritic computation are crucial features of cortical processing, with this method we can select, image and stimulate neuronal ensembles in a more sophisticated way than with conventional methods, taking us closer to dynamic cortical connection mapping and understanding of the main features of information processing. These results demonstrate for the first time the possibilities and effectiveness of acousto-optical focusing to perform in vivo quasi-simultaneous imaging and photostimulation of a desired neural network in large cortical volume with micrometer resolution.

**Poster**

**342. Optogenetics II**

**Location:** Hall A

**Time:** Monday, October 21, 2019, 8:00 AM - 12:00 PM

**Program #/Poster #:** 342.20/DD48

**Topic:** I.08. Methods to Modulate Neural Activity

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Human Frontiers Science Program- Grant RGP0015/2016
Fondation Bettencourt Schueller
Axa Research Fund

**Title:** Characterization and modelling of temporally precise two-photon optogenetics experiments

**Authors:** *D. TANESE*, V. ZAMPINI, I. BENDIFALLAH, A. PICOT, F. BUI, K. KILBORN, O. ASSAYAG, E. BAMBERG, B. FORGET, V. EMILIANI;
¹Photonics Dept., Vision Inst., Paris, France; ²3i, Denver, CO; ³Dept. of Biophysical Chem., Max Planck Inst. of Biophysics, Frankfurt, Germany

**Abstract:** Over the past years, progress in opsin engineering and light delivering approaches have enabled neuroscientists to drive and read neural circuits with single action potential precision and cellular resolution, opening fascinating perspectives in both fundamental and medical neuroscience. Precisely, a large number of variants in microbial opsins have been recently screened or engineered in the laboratory, to fasten their kinetics, improve their conductance, confine their expression and shift their absorption peak. On the other hand, advanced wave front shaping approaches have been developed to optimize temporal resolution and precisely guide light through tissues using either scanning or parallel two-photon(2P) excitation. If joint progresses in these two fields have significantly widened the possible experimental configurations for 2P optogenetics, they have also made it more difficult to choose the combination of opsin and illumination configuration that better matches the experimental requirements. In this work, we investigated, experimentally and theoretically, the temporal dynamics of photoevoked currents using opsins with different kinetics and different 2P-illumination conditions. Specifically, by using fast (f-Chrimson), intermediate (CoChR) and slow (ReachR) opsins, we studied, in cell culture and brain slices, the role of opsin kinetics on the photostimulation under 2P-scanning and -parallel illumination. We then used 3- and 4-states models to reproduce the experimentally photoevoked currents under both illumination conditions. This characterization and the prediction of the model can provide crucial information to guide the opsin choice and development and to optimize the design of 2P optogenetics experiments.
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Poster

342. Optogenetics II

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Program #/Poster #: 342.21/DD49

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH OD023852
NIH NS099577
NSF CBET1631912

Title: In vivo holographic photo-stimulation and two photon GCaMP6 imaging of vagus nerve axons using a GRIN lens integrated nerve cuff

Authors: A. K. FONTAINE¹, G. L. FUTIA¹, S. LITTICH¹, C. MCCULLOUGH¹, D. RESTREPO², R. F. WEIR¹, J. H. CALDWELL², *E. A. GIBSON¹;
¹Bioengineering, ²Cell and Developmental Biol., Univ. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: Vagus nerve interfacing is of interest due to its central role in parasympathetic regulation of the visceral organs, as well as its modulatory effects on the brain, which have been shown to influence epilepsy, depression and migraines. Electrical vagus nerve stimulation (VNS) has shown therapeutic effect in humans, yet it lacks the specificity for controlling and studying targeted pathways. In contrast, optical techniques may enable axon-specific neuromodulation using genetically targeted opsin expression and spatial patterning of the photo-stimulus. In addition to light-activated stimulation, calcium-sensitive fluorescent reporters such as GCaMP6 present a pathway for axon-specific optical recording of activity. We demonstrate in vivo photo-stimulation and two-photon GCaMP6 fluorescence imaging in the vagus nerve using a novel GRIN lens-coupled nerve cuff in the anesthetized mouse. A pulsed near-IR laser (1040 nm, 300 fs) was modified by a spatial light modulator (SLM) in the Fourier plane and focused by the microscope objective through a GRIN relay lens to the cervical vagus nerve. By actuating the SLM, spatially selected regions of axons could be differentially stimulated within the nerve. Mouse vitals were monitored with a MouseOx suite and used to detect physiological changes in response to photo-stimulation. We were able to induce differential modulations in heart rate, respiratory rate, and blood-oxygen saturation upon photo-stimulation of selective spatial regions
of the nerve. Additionally, we recorded two-photon GCaMP6 Ca^{2+} transients in vagal axons in response to both photo-stimulation and electrical stimulation.


Poster

342. Optogenetics II

Location: Hall A

Time: Monday, October 21, 2019, 8:00 AM - 12:00 PM

Program #: Poster #: 342.22/DD50

Topic: I.08. Methods to Modulate Neural Activity

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NIH Grant K25NS083754

Title: Cerebral hemodynamics are differentially regulated by excitatory and inhibitory neural circuits

Authors: J. LEE^1, A. BICE^2, Z. ROSENTHAL^2, J.-M. LEE^2, *A. Q. B. BAUER^1;^1 Washington Univ. in St. Louis, Saint Louis, MO; ^2 Washington Univ., Saint Louis, MO

Abstract: Neurovascular coupling relies on different populations of brain cells to regulate blood flow via vasoactive messengers[1]. Recent studies using optogenetics have shown that targeting all GABAergic neurons results in dilation followed by constriction[2] and increased blood flow despite suppression of neural activity[3,4]. However, it is currently unclear if specific subpopulations of GABAergic neurons independently regulate cerebral perfusion. Parvalbumin (PV)-expressing interneurons are the largest subpopulation of GABAergic interneurons[5], but the role of PV-interneurons in NVC has been less thoroughly examined. We combined optogenetics, laser speckle contrast imaging (LSCI), and optical intrinsic signal (OIS) imaging in awake mice to investigate how PV-circuits regulate local hemodynamics. Hemodynamic changes following photostimulation (PS) of the left barrel cortex in PV- (n=5) and Thy1-ChR2 (n=5) mice were measured using OIS-LSCI (Figs.1A,B). Thy1-circuit stimulation elicited robust local hemodynamic responses consistent with those following peripheral stimulation. However, activation of PV-circuits resulted in opposite responses, characterized by negative Δ[HbO], positive Δ[HbR], and negative Δ[HbT] changes (Figs.1C,D). Interestingly, we also observed reductions in CBF and CMRO2 following PV-ChR2 stimulation in contrast to that of Thy1-ChR2 (Figs.1C,D). We next tested how activation of PV circuits affected evoked responses due to whisker stimulation(WS). Simultaneous excitation and inhibition of left barrel cortex (WS+PS) resulted in a significant reduction of Δ[HbO] compared to that of WS only (Figs.1E,F). Our results demonstrate a role of PV-expressing interneurons in regulating the brain’s blood supply. The flipped local hemodynamic activity following PV-circuit activation is consistent
with local oxygen consumption occurring simultaneously with vasoconstriction; an effect emphasized with concurrent WS and PS of left barrel cortex. Future work will investigate specific molecular mechanisms mediating these responses.

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